



The Immunogenicity and Safety of Influenza Vaccines among Indonesian Older adult

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Abstract

BACKGROUND: Immunosenescence, frailty, sarcopenia, and Vitamin D deficiency were thought of having a negative influence on adequate immune response following vaccination. Epidemiological data related to influenza vaccination and its immunogenicity and safety in Indonesia are still scarce.

AIM: This study aimed to assess the immunogenicity and safety of the influenza vaccine in older adults in Indonesia and common related conditions.

METHODS: This study was a one-group, pre-test, post-test, quasi-experimental study. The vaccine used was a trivalent type. Immunogenicity was assessed according to rate of seropositivity and seroconversion based on hemagglutination inhibition antibody titer. Immunization safety was assessed according to adverse events following immunization (AEFI).

RESULTS: A total of 227 subjects were involved in this study. Antibody titers were significantly increased after 28-day influenza vaccination for all strains ($p < 0.001$). Seropositive rate in 28-day post-vaccination for A/H1N1, A/H3N2, and influenza B was 98.7%, 99.1%, and 97.4%, respectively. Meanwhile, the seroconversion rate for A/H1N1, A/H3N2, and influenza B strains was 54.2%, 66.1%, and 60.4%, respectively. The conditions of the frailty status, sarcopenia, and Vitamin D level were not associated with either seroconversion or seropositive status, both at baseline and endpoint. AEFI was occurred on seven subjects (3.08%) with pain at the injection site and one subject (0.44%) with arm tenderness.

CONCLUSION: Influenza vaccination showed adequate immune response and safety regardless of the frailty status, sarcopenia, or Vitamin D level. This result strengthened the importance of influenza vaccine administration in Indonesia older adults.

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Introduction

Influenza is an infectious disease that is often found in Indonesia. A surveillance study conducted in 2003–2007 on patients in the hospital by Kosasih *et al.* reported that 20.1% of patients with influenza-like illness (ILI) were infected with influenza virus [1]. Previous studies showed that 37.1% of virus subtype A H3 and 45.5% of virus subtype B were found in influenza virus culture samples. Virus A (H1N1) in Indonesia was found in 11.8%–29.0% in 2010–2011 [2]. Influenza and its complications have a major effect on the health of the older adult population. A previous study showed seasonal influenza increased morbidity and mortality in older adults [3]. Based on data from the World Health

Organization (WHO), 5%–15% of the world's population were infected with influenza annually. Every year, nearly 1 billion individuals were infected with influenza, and 3–5 million of them are serious cases resulting in 250,000–500,000 deaths. There was an increase in the global older adult population including in Indonesia due to the increase in life expectancy and this resulted in increase of influenza-related deaths in developed countries is found in the older adult population aged 65 years or over [4]. Influenza in general is one of the most common causes of pneumonia in older adults (24.5%) [5]. Previous study has shown that hospitalization rates due to influenza infection were highest at ≥ 65 years of age (58%) [6].

Previous studies have shown influenza vaccines to be effective in reducing the burden of disease due to influenza in the older adult population [7], [8]. Older

adult people are more susceptible to severe infections and have a lower immune response to vaccination responses due to the presence of immunosenescence and inflammaging, which pro-inflammatory factors were increased due to the aging process and consequently blunting the immune response to vaccination.[9] Both frailty and sarcopenia were previously known as factors associated to inflammaging conditions in older adults [10]. Previous studies showed supplementation of Vitamin D would be effective in preventing and treating sarcopenia in older adults, as Vitamin D deficiency is very common in older people.[11] Therefore, a good and a more thorough understanding of the immunological response to vaccination is needed, especially in the older adult population.

The influenza vaccine has a short immunity coverage and thus requires revaccination annually.[12] The protective effectiveness of the influenza vaccine in the older adult population aged 65 years and over was approximately in the range of 30%–50%.[13], [14] Influenza vaccine coverage in Indonesia is still low, namely below 1%. There are few data on the immunogenicity of influenza vaccines in the older adult population.[15] Another condition associated with a decreased immune response to vaccines in the older adult population is frailty syndrome. In Indonesia, the prevalence of outpatient frailty syndrome in the 13 main referral hospitals was 25.2%.[16], [17], [18].

The lack of epidemiological data related to influenza infection, especially in the older adult population, could be one of the causes of the lack of universal coverage of influenza vaccines in Indonesia. Hence, in the end, it can influence strategic policy-making and infection prevention in older adults. This study aims to assess the immunogenicity and safety of the influenza vaccine available in the older adult population in Indonesia and in addition, we also analyze common conditions that may influence immunosenescence such as frailty syndrome, sarcopenia, and level of Vitamin D.

Material and Methods

This study is part of “The dynamics of serologic titer and safety of trivalent influenza vaccine (TIV) in Indonesian Older adult Population Study,” involving patients from the Integrated Geriatric Clinic, General Hospital Dr. Hasan Sadikin Bandung. This was a one-group, pre-test, post-test, quasi-experimental study. The study was conducted for a year from August 2019 to August 2020. All protocols in this study have received Ethical Approval from the Ethics Committee of Dr. Hasan Sadikin General Hospital/Faculty of Medicine Universitas Padjadjaran (No.88/UN6.KEP/EC/2019). Training of the surveyor team and testing of the questionnaire were carried out. REDCAP (Research

Electronic Data Capture) Software version 8.6.0 which was developed and distributed by Vanderbilt University, Nashville, Tennessee, USA was used for processing data entry. Data were obtained and recorded from the questionnaires, control cards, and blood samples of the subjects.

Research subject

The subjects of this study were taken from the community-based primary health care and outpatients of Geriatric Clinic of Dr. Hasan Sadikin General Hospital Bandung through a consecutive (purposive) sampling, who was declared healthy, agreed with the investigator’s explanation, and signed a statement of consent to participate in this study. All subjects were included in the study regardless of prevaccination antibody titer status. The exclusion criteria from this study were subjects who have been included or will be included in other studies; subjects reporting symptoms suggestive of influenza, ILI, or respiratory tract disorders; had moderate or severe acute illness (according to the investigators’ judgment) on the day of vaccination, or had a fever (axilla temperature $>37.5^{\circ}\text{C}$). Patients with a history of allergies to components contained in vaccines such as protein from eggs or chicken protein or with a history of adverse events associated with the influenza vaccine were not included in this study. This study also did not include patients with a history of use of anticoagulant drugs or a history of blood clotting disorders (including cases of thrombocytopenia $<50,000$) that contraindicated intramuscular injections (note: consumption of antiplatelet drugs such as aspirin and clopidogrel were allowed). We also excluded those with a history of Guillain–Barré syndrome, or receiving medication in the last 4 weeks that may alter the immune response such as intravenous immunoglobulin, blood-derived products, or long-term (>2 weeks) corticosteroids), or those who suffer from chronic diseases which may influence the assessment of the final objectives of the study, or those with the history of prior influenza vaccine in the last previous year.

Research and measurement procedures

Recording of the questionnaire

Some data were obtained from recording forms and questionnaires. The assessment of frailty status was conducted using the INA-FRAIL scale questionnaire (RAPUH). The INA-FRAIL questionnaire is a questionnaire consisting of five components of the frailty syndrome phenotype consisting of resistance, physical activity, disease of more than 4, walking effort, and weight loss of more than 5% in the last 1 year. This questionnaire has gone through a process of translation, adapted, and validation.[18]

Anthropometric measurements

Subjects in this study were weighed using minimum clothing according to standard procedures using measuring instruments that were calibrated and assisted by trained research assistants.[17] The subject's body weight was measured using an electronic scale (Tanita MC 980 MA) and recorded to approx. 0.1 kg. The subject's height was measured using an estimate based on the patient's knee height. Body mass index is categorized using WHO recommendations for the Asian population.[19]

Assessment of sarcopenia and frailty syndrome

Sarcopenia was defined based on the criteria of the Asian Working Group for Sarcopenia which includes decreased skeletal muscle mass (measured by multiple segmental frequency of bioelectrical impedance analysis: men <7 kg/m² and women 5.7 kg/m²) accompanied by at least one decline in function (measured by walking speed <0.8 m/s) or decreased muscle strength (measured by handgrip test, in men <26 kg, and in women <18 kg). The FS assessment was carried out using the INA-FRAIL questionnaire. A score greater than or equal to three is stated as having frailty.[18]

Administration of vaccines and measurement of antibody titer

The influenza HA vaccine (Flubio[®]) is produced by Bio Farma Company, an Indonesia state-owned enterprise. Each of 0.5ml Flubio[®] vaccine consists of 15 lg of HA with three influenza strains and 4 lg of thimerosal. Those three strains consist of influenza A/H1N1 (A/Singapore/GP1908/2015[IVR180]), influenza A/H3N2 (A/Singapore/INFIMH-16-0019/2016[IVR-186]), and influenza B/Maryland/15/2016[NYMC BX-69A]). It is recommended to be given 0.5 mL per dose, with repetitions once per year. Flubio[®] vaccine is only given to healthy people aged 12 years and over by injection through the intramuscular route of administration in the upper arm. The injection cannot be administered the injection by the intravenous, subcutaneous, or intradermal route of administration. And should not be used by means of reconstitution in one syringe with another vaccine.

Antibody titer was measured using the inhibitory hemagglutination method and carried out at the Immunology Laboratory, Bio Farma Clinical Trials Department. This study assessed the immunogenicity of the influenza vaccine by calculating the percentage of the anti-influenza titer seroconversion rate of 1:40 units of hemagglutinin inhibition, postvaccination seropositive. Seropositive was defined as each antibody titer $\geq 1:40$. Meanwhile, seroconversion was defined as four-fold increase of antibody titer and/or transition

from the seronegative to seropositive status in period of before to 28-day post-vaccination [20].

Assessment of Vitamin D level

The Vitamin D levels measurement was conducted in the Dr. Hasan Sadikin Pathological Clinic Laboratory, using the method of ELFA, an enzyme immunoassay competitive method with fluorescent detection; from Vidas (Bioemeriex-France). The sample was in the form of serum of 100 ul and stored at the temperature of -70°C. The status of Vitamin D adequacy was divided into Vitamin D deficiency for Vitamin D levels <20 ng/mL, insufficiency of 20–30 ng/mL, and sufficiency >30 ng/mL [21].

Vaccine safety assessment

Vaccination safety was assessed as according to the number and percentage of subjects and along with its frequency based on those who experienced rapid reactions during 30 minutes after vaccination, those with least 1 local reaction (28 days after vaccination) and 1 systemic reaction (28 days post vaccination).

Statistical analysis

Descriptive data such as demographic characteristics, sarcopenia, frailty status, Vitamin D level categories, and vaccination effectiveness were presented in the form of a frequency distribution table with proportion (percentage). Data on a numerical scale were presented with mean \pm standard deviation or median (minimum-maximum). An assessment was made of the characteristics of the independent variables in each group, and descriptive data were presented in percentage units.

Pairwise numerical comparisons for the assessment of antibody titers before vaccination and after 28 days of vaccine administration were analyzed by the Wilcoxon signed-rank test. Categorical variables associations were analyzed using Chi-square with Fisher's exact test or linear-by-linear association as its alternatives. Pre-test–post-test seropositive status was compared by McNemar test. All statistical calculations in this study were performed using IBM[®] SPSS[®] Statistics software (SPSS, Inc, Chicago, Illinois, USA), under the license name of Universitas Padjadjaran. P <0.05 was considered statistically significant.

Results

A total of 227 subjects were involved in this study. The subjects were dominantly women (72.69%), with a

median age of 68.5 (72.1–64.5) years, and a median body mass index (BMI) status of 24.9 kg/m² (17.4–39.6). Majority of subjects already had seropositive for all strains at baseline (96.50% for A/H1N1, 74.4% for A/H3N2, and 74.0% for influenza B). The majority of our study subjects were robust individual (94.3%) and did not have sarcopenia (90.75%). Both sarcopenia and frail status did not show a significant difference in each baseline seropositive status ($p > 0.05$). The majority of subjects had Vitamin D deficiency. However, Vitamin D status in each baseline seropositive status was not significantly different ($p > 0.05$). Age, sex, BMI, and comorbidity were similar between seropositive and seronegative subjects ($p > 0.05$). Baseline characteristic comparison between seropositive and seronegative subjects at baseline is detailed in Table 1.

Table 2 shows the increasement of antibody titers between before and after 28-day post-vaccination for each strain. Antibody titers for all strains were significantly increased ($p < 0.001$). Table 3 shows the seroconversion and seropositivity 28-day post-vaccination based on the status of seropositivity at baseline. For H3N2 and B strains, subjects who were both seropositive and seronegative at baseline showed significant seroconversion and seropositivity rates ($p < 0.001$). Meanwhile, these were not found in the H1N1 strains where the seroconversion and seropositivity rate were not significantly different for neither of those with seropositive or not. The description of 28-day post-vaccination seroprotection rate is presented in Table 4. The seropositivity rate in 28-day post-vaccination for A/H1N1, A/H3N2, and influenza B was 98.7%, 99.1%, and 97.4%, respectively. Table 5 shows the seroconversion rate for A/H1N1, A/H3N2, and influenza B strains was 54.2%, 66.1%, and 60.4%, respectively.

We also compared age, sex, BMI, frailty status, sarcopenia, Vitamin D, and ≥ 4 -comorbidity status

according to 28-day post-vaccination seroprotection [Table 4] and seroconversion status [Table 5] in each strain. All subject characteristics including frailty, sarcopenia, Vitamin D, and comorbidity status were not associated with seroprotection and seroconversion status in 28-day post-vaccination (all $p > 0.05$). Based on data on local and systemic reactions experienced by study subjects after influenza vaccine administration, influenza vaccines were generally quite safe, and only a few subjects reported experiencing local reactions, which are pain at the injection site (3,08%) and arm tenderness (0.44%).

Discussion

Through vaccination, most of the subjects in this study showed significant seroconversion and seropositivity (also called as seroprotection in other studies) against influenza. The assessment of estimated seroconversion and seropositivity of vaccines would reflect the state of immunogenicity in older adult subjects, which is hypothetically lower than that of the young adult population. In our study, subjects who received influenza vaccination experienced seroconversion rates of 54.4 to 64.8%, higher than the previous study (17%–53%) by Goodwin *et al.*[22] In a previous study, an increase in serum titer against influenza occurred 2 to 6 weeks after vaccination. In our study, we examined the influenza antigen titer at week 4 after vaccination.[23]

The TIV consists of two subtypes of influenza A vaccine (A/H1N1 and A/H3N2) and one influenza B subtype (either Victoria or Yamagata strain). Our study revealed the baseline seropositivity (seroprotection)

Table 1: Baseline characteristics according to baseline seroprotection status

Characteristics	Baseline seroprotection								
	A/H1N1			A/H3N2			Influenza B		
	Yes, n (%)	No, n (%)	p	Yes, n (%)	No, n (%)	p	Yes, n (%)	No, n (%)	p
Baseline seroprotection	219 (96.5)	8 (3.5)		169 (74.4)	58 (25.6)		168 (74.0)	59 (26.0)	
Age (year old)									
60–69	127 (96.2)	5 (1.5)	0.653 ^a	99 (98.5)	33 (25.0)	0.780 ^a	103 (78.0)	29 (22.0)	0.098 ^a
70–79	81 (96.4)	3 (1.2)		60 (71.4)	24 (28.6)		58 (69.0)	26 (31.0)	
≥ 80	11 (100)	0		10 (90.9)	1 (9.1)		7 (63.6)	4 (36.4)	
Sex									
Male	57 (91.9)	5 (8.1)	0.037 ^b	49 (79.0)	13 (21.0)	0.213 ^b	41 (66.1)	21 (33.9)	0.070 ^b
Female	162 (98.2)	3 (1.8)		120 (72.7)	2 (27.3)		127 (77.0)	38 (23.0)	
BMI									
Underweight	6 (100)	0	0.315 ^a	5 (83.3)	1 (16.7)	0.452 ^a	5 (83.3)	1 (16.7)	0.914 ^a
Normal	59 (100)	1 (1.7)		47 (78.3)	13 (21.7)		44 (73.3)	16 (26.7)	
Overweight	46 (95.8)	2 (4.2)		34 (70.8)	14 (29.2)		34 (70.8)	14 (29.2)	
Obese	108 (95.6)	5 (4.4)		83 (73.5)	30 (26.5)		85 (75.2)	28 (24.8)	
Frailty									
Yes	14 (100)	1 (0.0)	0.426 ^b	11 (73.3)	4 (26.7)	0.999 ^b	9 (60.0)	6 (40.0)	0.226 ^b
No	205 (96.7)	7 (3.3)		158 (74.5)	54 (25.5)		159 (75.0)	53 (25.0)	
Sarcopenia									
Yes	20 (95.2)	1 (4.8)	0.546 ^b	19 (90.5)	2 (9.5)	0.113 ^b	14 (66.7)	7 (33.3)	0.438 ^b
No	199 (96.5)	7 (3.4)		150 (72.8)	56 (27.2)		154 (74.8)	52 (25.2)	
Vitamin D status									
Sufficient	43 (95.6)	2 (4.4)	0.608 ^a	36 (80.0)	9 (20.0)	0.534 ^a	30 (66.7)	15 (33.3)	0.245 ^a
Insufficient	46 (95.8)	2 (4.2)		34 (70.8)	14 (29.2)		36 (75.0)	12 (25.0)	
Deficient	130 (97.0)	4 (3.0)		99 (73.9)	35 (26.1)		102 (76.1)	32 (23.9)	
Comorbidity									
≥ 4	37 (92.5)	3 (7.5)	0.151 ^b	31 (77.5)	9 (22.5)	0.693 ^b	27 (67.5)	13 (32.5)	0.319 ^b
< 4	181 (97.3)	5 (2.7)		137 (73.7)	49 (26.3)		141 (75.8)	45 (24.2)	

Data were presented in the form of frequency and percentage. p value was obtained by ^aChi-square test, ^bFischer exact's test, significant if $p < 0.05$. BMI: Body mass index.

Table 2: Trivalent influenza vaccination serum antibody titer between baseline and 1 month

Influenza	Serologic titer, median (IQR)		p
	Baseline	1 month	
Strain A/H1N1	160 (80–320)	640 (320–1280)	< 0.001*
Strain A/H3N2	40 (20–160)	320 (160–640)	< 0.001*
Strain B	40 (20–80)	160 (80–320)	< 0.001*

Data presented by median and IQR, p value using Wilcoxon test, significant if p < 0.05. IQR: Inter Quartile Range.

rate for all strains is quite high, especially with H1N1. This result may be due to the fact that most of the participants are outpatients of Geriatric Clinics, which might have prior influenza infection; thus provide them with seroprotection.[24] Although we were aware this could affect the result of the study, the reason why in the methods, we decided to involve all patients regardless of the prior infection is that because of the fact according to our findings, the positive titer antibody from the prevaccination condition clearly does not derive from previous vaccination but could be from a previous infection. Indonesia is the largest nation in Southeast Asia and is importance for influenza surveillance, as it is endemic for some influenza viruses. The virus pattern in Indonesia is different each year due to the fact Indonesia is located in the equator between the North and South Hemispheres. Adding to that, the clinicians are not accustomed to regular virus identification procedures in those showing the symptoms of influenza or ILI.[25] In this study, the seroconversion rate also was lower and not statistically significant in the influenza A/H1N1 strain compared to the other two strains. Suggestively, we assumed these results are caused that in fact most of the subjects had already high seropositivity status at prevaccination thus it could reduce the positivity rate post-vaccination statistically. However, it is worthy to be noted that the seropositivity status is occurred to most of the patients, out of 6 from 8 subjects whose not seroprotected at baseline. This may indicate that seroconversion did occur on these patients even though it did not reach significance statistically. Frailty syndrome in Indonesia is a quite significant health problem in older adults with a high prevalence rate.[16] Physical frailty and sarcopenia shared similar pathological aspects, which both could affect overall functional capacity in a single older adult. The pathomechanism of sarcopenia involving progressive loss of muscle mass resulting in increased production of pro-inflammatory factors (Interleukin [IL-6], IL-7, IL-15) which could interfere the immune cells function.[26] Both biological processes are associated with immunosenescence which consequently increases the susceptibility of the older adults to become infected

and the response is not optimal to vaccination, also resulting in a decrease in the possibility of older adults to cope with infection or sepsis.[10], [27], [28] The majority of the population were robust individuals. The good seroconversion and seropositivity rates in this study may be due to the fact that the majority of subjects in this study were robust older adults not frail. Multi-comorbidity status (≥ 4 comorbidities) and frailty status were not significantly associated with either seroconversion or influenza vaccine seropositivity. This result was similar with previous study done by Koesno *et al.* that also presented that frailty did not significantly influence the immune response to influenza vaccine.[29] In our study, we assessed frailty status by Indonesia adapted FRAIL questionnaire, a phenotype approach method, a different method used in the study conducted by Koesno *et al.* using 40-item frailty index, a deficit accumulation approach.[29], [30] Although both of these studies using different methods in detecting frailty, previous diagnostic studies showed similar comparable results in detecting frailty using either method. One fundamental difference in these two methods is the severity of the frailty, different with deficit accumulation which besides determining also assess the severity of the frailty, the phenotype approach could not. In this study, the participants were the outpatients of the Geriatric Clinics and community-based primary health care, even though some of them were considered frail or having multiple comorbidities but they were individuals with a fairly good health status and awareness who were also managed under multidisciplinary geriatric team, thus seroprotection value achieved in this study was very good for all frailty groups. [31] Nevertheless, we have not known exactly for how long does the seroprotection for influenza last in the frail older adult. Therefore, a complete cohort longitudinal observational study is needed to clarify this issue.

Based on clinical and epidemiological data, Vitamin D insufficiency is considered to increase the risk of influenza infection although the precise mechanism explaining this relationship between Vitamin D and the immunogenicity of influenza vaccine is still unclear, Vitamin D is considered to have an effect on the immune system by reducing the production of cytokines in the inflammatory response hence it may have a certain effect on the immune response to vaccination.[30] Our study showed there were more subjects with Vitamin D deficiency status (59%) compared to the similar study of female older adult subjects in Jakarta

Table 3: After 28 days of vaccination immunogenicity outcomes comparison according to baseline seropositivity status

After 28 days vaccination immunogenicity outcomes	Baseline Seropositivity Status								
	A/H1N1			A/H3N2			Influenza B		
	Yes (n = 219)	No (n = 8)	p	Yes (n = 169)	No (n = 58)	p	Yes (n = 168)	No (n = 59)	p
Seroconversion, n (%)									
Yes	119 (54.3)	4 (50.0)	0.809 ^a	85 (50.3)	38 (65.5)	< 0.045 ^a	89 (53.0)	48 (81.4)	< 0.001 ^a
No	100 (45.7)	4 (50.0)		84 (49.7)	6 (34.5)		79 (47.0)	11 (18.6)	
Seropositive, n (%)									
Yes	218 (99.5)	6 (75.0)	0.125 ^b	168 (99.4)	57 (98.3)	< 0.001 ^b	164 (97.6)	57 (96.6)	< 0.001 ^b
No	1 (0.5)	2 (25.0)		1 (0.6)	1 (1.7)		4 (2.4)	2 (3.4)	

Data were presented in the form of frequency and percentage. p value was obtained by ^aChi-square test, ^bMcNemar test, significant if p < 0.05

Table 4: After 28 days seropositive and its association with other characteristics

Characteristics	A/H1N1			A/H3N2			Influenza B		
	Yes, n (%)	No, n (%)	p	Yes, n (%)	No, n (%)	p	Yes, n (%)	No, n (%)	p
Seropositive (28 days postvaccination)	224 (98.7)	3 (1.3)		225 (99.1)	2 (0.9)		221 (97.4)	6 (2.6)	
Age (year old)									
60–69	130 (98.5)	5 (1.5)	0.693 ^a	130 (98.5)	36 (27.30)	0.260 ^a	129 (97.7)	3 (2.3)	0.400 ^a
70–79	83 (98.0)	3 (1.2)		84 (100)	0		82 (97.6)	2 (2.4)	
≥ 80	11 (100)	0		11 (100)	0		10 (90.9)	1 (9.1)	
Sex									
Male	61 (98.4)	1 (1.6)	0.618 ^b	62 (100)	0	0.527 ^b	60 (96.8)	2 (3.2)	0.522 ^b
Female	163 (98.8)	2 (1.2)		163 (98.8)	2 (1.2)		161 (97.6)	4 (2.4)	
BMI									
Underweight	6 (100)	0	0.731 ^a	6 (100)	0	0.620 ^a	6 (100)	0	0.642 ^a
Normal	60 (100)	0		60 (100)	0		58 (96.7)	2 (3.3)	
Overweight	45 (93.8)	3 (6.3)		47 (97.9)	1 (2.1)		46 (95.8)	2 (4.2)	
Obese	113 (100)	0		112 (99.1)	1 (0.9)		111 (98.2)	2 (1.8)	
Frailty									
Yes	15 (100)	0	0.814 ^b	15 (100.0)	0	0.872 ^b	15 (100)	0	0.660 ^b
No	209 (98.6)	7 (1.4)		210 (99.1)	2 (0.9)		206 (97.2)	6 (2.98)	
Sarcopenia									
Yes	21 (100)	0	0.746 ^b	21	0	0.823 ^b	20 (95.2)	1 (4.8)	0.445 ^b
No	203 (98.5)	3 (1.5)		204 (99.0)	2 (1.0)		201 (97.6)	5 (2.4)	
Vitamin D status									
Sufficient	44 (97.8)	1 (2.2)	0.898 ^a	45 (100)	0	0.280 ^a	43 (95.6)	2 (4.4)	0.855 ^a
Insufficient	48 (100)	0		48 (100)	0		48 (100)	0	
Deficient	132 (98.5)	2 (1.5)		132 (98.5)	2 (1.5)		130 (97.0)	4 (3.0)	
Comorbidity									
≥ 4	39 (97.5)	1 (2.5)	0.444 ^b	39 (97.5)	1 (2.5)	0.323 ^b	38 (95.0)	2 (5.0)	0.288 ^b
< 4	184 (98.9)	2 (1.1)		185 (99.5)	1 (0.5)		182 (97.8)	4 (2.2)	

Data were presented in the form of frequency and percentage. p value was obtained by ^aLinear-by-linear association, ^bFischer's exact test, significant if p < 0.05. BMI: Body mass index.

(35.1%).[32] The older adults including those who are frequently exposed to sunlight produces less Vitamin D (25%) than the younger population.[33] Our study did not show a significant association between Vitamin D adequacy status and influenza vaccine seroprotection and seroconversion. This might be due to the fact that the majority of the subjects already had seroprotection against influenza in the beginning of the study, therefore, the level of Vitamin D has nonsignificant association. There is still a lack of studies and contradictory results on seroconversion. The study by Sundaram *et al.* also showed a similar result to our study with no significant association with seroconversion after vaccination.[34] Meanwhile, study conducted by Chadha *et al.* demonstrated a significant association between adequate Vitamin D levels and serological response to influenza vaccination. However, there was a fundamental

difference in the study subjects which consisted of male population with comorbid prostate cancer, while the majority of our study subjects were fit older adult people.[35] The study conducted by Goncalves-Mendes *et al.* showed no significant difference in seroconversion between the group receiving Vitamin D supplementation and placebo. This study also reported that Vitamin D supplementation for 3 months before the subject received influenza vaccination could increase plasma transforming growth factor β (TGF β) levels, but did not increase antibody production.[36] We also assume there might be possibility of different normal value reference on Vitamin D level on different population, which might be lower than a standardized international reference and it might be necessary to do an epidemiological study to determine the normal value in Indonesian population.

Table 5: After 28 days seroconversion and its association with other characteristics

Characteristics	A/H1N1			A/H2N3			Influenza B		
	Yes, n (%)	No, n (%)	P	Yes, n (%)	No, n (%)	p	Yes, n (%)	No, n (%)	p
Seroconversion (28 days postvaccination)	123 (54.2)	104 (45.8)		150 (66.1)	77 (33.9)		137 (60.4)	90 (39.6)	
Age (year old), median (years)									
60–69	72 (54.5)	60 (45.50)	0.774 ^a	91 (68.90)	41 (31.10)	0.272 ^a	81 (61.40)	51 (38.60)	0.582 ^a
70–79	44 (52.4)	40 (47.60)		54 (64.30)	30 (35.70)		51 (60.70)	33 (39.30)	
≥ 80	7 (63.6)	4 (36.40)		5 (45.50)	6 (54.50)		5 (45.50)	6 (54.50)	
Sex									
Male	37 (59.7)	25 (40.30)	0.309 ^b	44 (71.00)	18 (29.00)	0.340 ^b	35 (56.50)	27 (43.50)	0.461 ^b
Female	86 (52.1)	79 (47.90)		106 (64.20)	59 (35.80)		102 (61.80)	63 (38.20)	
BMI									
Underweight	2 (33.3)	4 (66.7)	0.582 ^a	4 (66.7)	2 (33.3)	0.985 ^a	3 (50.0)	3 (50.0)	0.613 ^a
Normal	34 (56.7)	26 (43.3)		39 (65.0)	21 (35.0)		33 (55.5)	27 (45.0)	
Overweight	23 (47.9)	25 (52.1)		31 (64.6)	17 (35.4)		32 (66.7)	16 (33.3)	
Obese	64 (56.6)	49 (43.4)		76 (67.3)	37 (32.7)		69 (61.1)	44 (38.9)	
Frailty									
Yes	7 (46.7)	8 (53.3)	0.545 ^b	11 (73.30)	4 (26.70)	0.539 ^b	9 (60.00)	6 (40.00)	0.977 ^b
No	116 (54.7)	96 (45.3)		139 (65.60)	73 (34.40)		128 (60.40)	84 (39.60)	
Sarcopenia									
Yes	12 (57.1)	9 (42.9)	0.775 ^b	16 (76.20)	5 (23.80)	0.304 ^b	14 (66.70)	7 (33.30)	0.535 ^b
No	111 (53.9)	95 (46.1)		134 (65.00)	72 (35.00)		128 (59.70)	83 (40.30)	
Vitamin D level									
Sufficient	22 (48.9)	23 (51.1)	0.530 ^a	30 (66.67)	15 (33.33)	0.702 ^a	27 (60.00)	18 (40.00)	0.942 ^a
Insufficient	29 (60.4)	19 (39.6)		34 (70.83)	14 (29.07)		30 (62.50)	17 (37.50)	
Deficient	72 (53.7)	62 (46.3)		86 (64.18)	48 (35.82)		80 (59.70)	54 (40.30)	
Comorbidity									
≥ 4	18 (45.0)	22 (55.0)	0.209 ^b	28 (70.00)	12 (30.00)	0.549 ^b	22 (55.00)	18 (45.00)	0.461 ^b
< 4	104 (55.9)	82 (44.1)		121 (65.05)	65 (34.95)		114 (61.30)	72 (38.70)	

Data was presented in the form of frequency and percentage. p value was obtained by ^aLinear-by-linear association, ^bFischer's exact test, significant if p < 0.05. BMI: Body mass index.

Influenza vaccine is also considered safe to be given for older adults. Our study shows that there is no serious adverse event and only few subjects were having mild side effects such as pain symptoms at the injection site accompanied by pain in the arm.

Conclusion

Influenza vaccination showed an adequate immune response based on the status of seroconversion, seroprotection, and safety regardless of the condition of frailty status, sarcopenia, or Vitamin D level. This result strengthened the importance of influenza vaccine administration in Indonesia's older adults.

Declaration of interest

The author declares no conflict of interest. This study was sponsored by the Medical Faculty of Universitas Padjadjaran through Academic Leadership Grant (1427/UN6.3.1/LT2020). Biofarma, a state-owned enterprise, as a sponsor by providing the trivalent influenza vaccine (Flubio[®]) for this study also conducting the measurement of antibody titer.

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