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Key points in evaluating immunogenicity of pandemic influenza vaccines: A lesson from immunogenicity studies of influenza A(H1N1) pdm09 vaccine

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ABSTRACT

Introduction: Immunogenicity studies on pandemic influenza vaccine are necessary to inform rapid development and implementation of a vaccine during a pandemic. Thus, strategies for immunogenicity assessment are required.
Objective: To identify essential factors to consider when evaluating the immunogenicity of pandemic influenza vaccines using the experience in Japan with the influenza A(H1N1)pdm09 vaccine.
Methods: We conducted a search of observational studies using PubMed and IchushiWeb. Search terms included "influenza vaccine AND (immunogenicity OR immune response) AND Japan AND (2009 OR pdm09) NOT review," and was limited to studies conducted in humans.
Results: A total of 33 articles were identified, of which 16 articles met the inclusion criteria. Immunogenicity of the commercially available influenza A(H1N1)pdm09 vaccine satisfied the international criteria for influenza vaccine immunogenicity in all study populations. The most remarkable immune response was observed in junior high school students, while the lowest immune response was observed in hematological malignancy patients. Similar to immunogenicity studies on seasonal influenza vaccines, factors such as patient background (e.g., age, underlying condition, pre-vaccination titer, body mass index, etc.) and study procedure (e.g., concurrent measurement of pre- and post-vaccination antibody titer, effects of infection during the study period) may have affected the assessment of immunogenicity to the influenza A(H1N1)pdm09 vaccine. In addition, prior vaccination with the seasonal influenza vaccine may inhibit antibody induction by the influenza A(H1N1)pdm09 vaccine.
Conclusions: This review discusses factors and strategies that must be considered and addressed during immunogenicity assessments of pandemic influenza vaccines, which may provide useful information for future influenza pandemics.

1. Introduction

In the Northern Hemisphere, seasonal influenza viruses typically circulate from late fall through early spring. Such characteristics of seasonal influenza enable us to prepare influenza vaccines in advance to prevent influenza illnesses. In addition, we can advise populations at high risk for severe influenza to receive influenza vaccination early [1].

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HI, hemagglutination inhibition; MFR, mean fold rise; OR, odds ratio; SCR, seroconversion proportion; SRP, seroresponse proportion; SPP, seroprotection proportion.

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However, the situation is quite different for pandemic influenza. In April 2009, swine-origin influenza A(H1N1) virus was first identified in the United States; it rapidly spread throughout the world, resulting in the first influenza pandemic of the 21st century [2]. Since this was a new strain of influenza, no vaccine was available at the early stage of this pandemic. In addition, there was no data on high-risk populations of this virus. To control this influenza pandemic, various information regarding the epidemiology of influenza A(H1N1)pdm09 virus was necessary, and an effective influenza vaccine had to be produced as soon as possible.

The general procedure for an immunogenicity study of influenza vaccines includes the following processes: (1) measure the antibody titer for paired serum samples (i.e., before and several weeks after vaccination), and (2) analyze data of antibody titers. In these analyses, most studies calculate the following markers as outcome indices: (1) the geometric mean titer (GMT), (2) mean fold rise (MFR), (3) seroconversion proportion (SCR), (4) seroresponse proportion (SRP), and (5) seroprotection proportion (SPP) in all study subjects. The immunogenicity of influenza vaccines in the target population is also assessed according to the international licensing criteria of the European Medicines Evaluation Agency and the United States Food and Drug Administration (Table 1) [3,4]. However, several factors can affect vaccine immunogenicity.

Here, we present a summary of the results from immunogenicity studies of influenza A(H1N1)pdm09 vaccine conducted in Japan. The main objective was to discuss key points to consider when evaluating the immunogenicity of pandemic influenza vaccines.

2. Immunogenicity studies of influenza A(H1N1)pdm09 vaccine in Japan

We conducted a comprehensive search using PubMed and IchushiWeb provided by Japan Medical Abstracts Society with the search terms "influenza vaccine AND (immunogenicity OR immune response) AND Japan AND (2009 OR pdm09) NOT review." Only studies conducted in humans were included. The literature search was conducted on June 7, 2017 and yielded a total of 33 articles, of which 17 articles were excluded. Reasons for exclusion included investigation of immune responses after influenza A (H1N1)pdm09 virus infection (n = 2), investigation of viral characteristics of influenza A(H1N1)pdm09 virus (n = 1), investigation of immunogenicity of influenza vaccine in another season (n = 6), experimental studies conducted in mice (n = 2), and clinical trials for a non-commercial vaccine (n = 6). Finally, the results from 16 articles [5–20] are summarized in Table 2.

When the results of these 16 studies were evaluated against the international criteria for influenza vaccine immunogenicity as shown in Table 1, the immunogenicity of the commercially available influenza A(H1N1)pdm09 vaccine satisfied to meet the criteria in all study populations. However, GMT after 1 dose of vaccination (S1) ranged from 13 to 162, whereas SPP at S1 ranged from 25% to 92% (Table 2). Therefore, specific factors may be involved which affected immune responses to influenza A(H1N1)pdm09 vaccine. We reviewed these studies, paying careful attention to the study procedure and the effect of subject background characteristics.

Table 1
International criteria for influenza vaccine immunogenicity.

EMEA criteria (satisfies 1 or more of the following 3 items)		
	Age: 18–60 years	Age: ≥61 years
(1) SCP*	>40%	>30%
(2) MFR	>2.5	>2.0
(3) SPP†	>70%	>60%
FDA criteria		
	Age: ≤64 years	Age: ≥65 years
(1) Lower limit of 95% CI of SCP*	>40%	>30%
(2) Lower limit of 95% CI of SPP†	>70%	>60%

Cited and reconstructed from Refs. [3,4].
Abbreviations: EMA, European Medicines Evaluation Agency; SCP, seroconversion proportion; MFR, mean fold rise; SPP, seroprotection proportion; FDA, Food and Drug Administration.
* SCP: the proportion of persons with pre-vaccination HI antibody titer of <1:10 and post-vaccination titer of ≥1:40 or ≥4-fold post-vaccination rise in antibody titer.
† SPP: the proportion of persons satisfying the protective level of antibody titer (HI antibody titer of ≥1:40).

3. Factors to consider in immunogenicity studies of pandemic influenza vaccines

3.1. At the study procedure

In general, the first key point to consider in the study procedure is existed in the measurement of pre- and post-vaccination antibody titers. These measurements should be performed concurrently. Even if test accuracy has recently improved, a 1-tube (i.e., 2-fold) difference in an influenza hemagglutination inhibition (HI) antibody titer could occur as a result of measurement error. For example, if pre- and post-vaccination antibody titers are measured at separate time points, the pre-vaccination antibody titer could be 1-tube lower than the true value, whereas the post-vaccination antibody titer could be 1-tube higher than the true value due to measurement error. This measurement error would result in a 4-fold increase from pre- to post-vaccination titers although the patient's antibody titer did not actually increase. Therefore, to minimize the effects of measurement errors, the test environment must be standardized as much as possible.

According to the descriptions in the papers we reviewed, approximately 60% of studies on influenza A(H1N1)pdm09 vaccine in Japan performed concurrent measurement of pre- and post-vaccination antibody titers [5,8,10–16,18]. Thus, in these studies, the study procedure for the measurement of antibody titer did not seem to explain the variation in antibody response. To provide proper interpretation of vaccine immunogenicity, concurrent measurement of paired serum samples would be needed, and the description would help readers to interpret the results appropriately.

The second key point to consider in the study procedure concerns analysis and interpretation of results. During the study period, some subjects may develop influenza. If the effects of subjects who develop influenza (including subclinical infection) during the study period are included, then post-vaccination antibody titers will be increased because of influenza infection, which can lead to an overestimation of the immunogenicity of the influenza vaccine. Thus, in immunogenicity studies of influenza vaccines, we should collect information regarding the development of influenza during the study period, and infected subjects should be excluded from the analyses.

Among published papers from Japan, approximately 60% of papers disclosed the inclusion/exclusion of subjects who developed influenza during the study period and the management methods of such infected subjects [5,7,8,11–14,16,18,19]. Especially in the case of the pandemic influenza vaccine, the spread of influenza preceded the development of the vaccine. Thus, even in the relatively short study period of an immunogenicity study (generally 3–4 weeks), subjects can develop pandemic influenza. In fact, in our study of influenza A(H1N1)pdm09 vaccine, 9 of 111 study subjects experienced a confirmed influenza A virus infection (as determined by the rapid test) between the first dose

Table 2
Immunogenicity of influenza A(H1N1)pdm09 vaccine in Japan.

Ref.	Study subjects	N	Age (years)	No. of doses	GMT			MFR		SCP* (95%CI) at S1	SPP† (95%CI) at S1
					S0‡	S1‡	S2‡	S1/S0	S2/S0		
[5]	Junior high school	60	12–15	2	10	162	158	15.6	15.4	83% (73–93%)	92% (84–106%)
	High school	46	15–18	2	15	126	136	8.3	8.3	72% (58–85%)	89% (80–98%)
[6]	Health-care workers	389	20–65	1	6	22	–	3.5	–	35% (30–40%)	38% (33–43%)
[7]	Pediatricians	16	27–49	1	10	27	–	5.4	–	44% (17–71%)	44% (16–71%)
[8]	Pregnant women	149	17–41	2	8	139	114	17.1	14.1	91% (86–96%)	89% (84–94%)
[9]	Pregnant women	128	34.8 ± 4.1	2	Not applicable					Not applicable	90% at S2
[10]	Hematological malignancy patients	50	21–83	2	6	13	22	2.3	3.9	32% (19–45%)	27% (14–40%)
[11]	Subjects with severe motor and intellectual disability	104	40.1 ± 12.9	2	7	39	41	5.4	5.6	54% (44–64%)	56% (46–66%)
[12]	Diabetes mellitus patients	48	26–75	1	6	53	–	9.0	–	73% (60–86%)	73% (60–86%)
[13]	Diabetes mellitus patients	48	28–78	2	6	33	34	5.3	5.6	46% (32–60%)	25% (38–66%)
[14]	Hepatitis C patients	79	64.5 ± 10.6	1	8	82	–	10.3	–	72% (62–82%)	71% (61–81%)
[15]	Duchenne muscular dystrophy patients	44	17–47	1	7	75	–	10.5	–	SRP§: 84% (73–95%)	70% (57–83%)
[16]	HIV-infected patients	104	34.3–53.0	2	8	31	39	Not applicable		44% (31–58%)	52% (38–66%)
[17]	HIV-infected patients	182	46.6 ± 12.7	1	10	35	–	Not applicable		39% (32–46%)	50% (43–57%)
[18]	Children with renal diseases under immunosuppressive therapy	15	11.8 ± 4.0	2	6	104	50	16.9	9.0	69% (39–91%)	77% (46–95%)
[19]	Pediatric liver transplant recipients	13	1–18	2: <13 years; 1: ≥13 years	6	32		5.2		46% (19–75%)	54% (25–81%)
[20]	Healthy adults with prior seasonal influenza vaccination	51	22–61	2	8	45	41	6.4	5.9	59% (44–72%)	61% (46–74%)
	Healthy adults without prior seasonal influenza vaccination	59	23–62	2	8	100	107	14.6	15.6	80% (67–89%)	80% (67–89%)

Abbreviations: CI, confidence interval; GMT, geometric mean titer; MFR, mean fold rise; SCP, seroconversion proportion; SPP, seroprotection proportion, SRP, seroresponse proportion.
* SCP: the proportion of persons with pre-vaccination HI antibody titer of <1:10 and post-vaccination titer of ≥1:40 or ≥4-fold post-vaccination rise in antibody titer.
† SPP: the proportion of persons satisfying the protective level of antibody titer (HI antibody titer of ≥1:40).
‡ S0, before vaccination; S1, 3–4 weeks after 1st dose of vaccination; S2, 4 weeks after 2nd dose of vaccination.
§ SRP: the proportion of persons with ≥4-fold post-vaccination rise in antibody titer.

and serum sampling after the second dose and thus were excluded from the analyses [5]. Therefore, to accurately determine vaccine immunogenicity, it is essential to compile data on the presence/absence of disease development during the study period, and infected individuals must be rigorously excluded from the analysis.

3.2. The effect of subject background characteristics

When we reviewed immunogenicity studies on influenza A (H1N1)pdm09 vaccine in Japan (Table 2), the most remarkable immune response was observed in junior high school students [5], while the lowest immune response was observed in hematological malignancy patients [10]. This difference is likely due to differences in patient characteristics, as patients with underlying illnesses and/or receiving immunosuppressive therapy are known to demonstrate lower immune responses to vaccines [1].

Age is another important factor that can affect immune responses to vaccines. In general, elderly subjects are likely to exhibit lower immune responses to vaccines, whereas young children require two doses of influenza vaccine to achieve a sufficient immune response [1]. In fact, the international criteria of vaccine immunogenicity take into consideration the effect of age (Table 1) [3,4]. According to these criteria, most studies about influenza A (H1N1)pdm09 vaccine conducted in Japan also considered the effect of age by using stratified or multivariate analyses [5–8,10–14,16,17]. In the study of hematological malignancy patients, half of the subjects were ≥60 years of age, which might explain the observed lower immune responses to the vaccine [10]. In addition, other studies also indicated that higher aged subjects had lower GMT and lower SPP after vaccination among diabetes mellitus patients [12,13] and hepatitis C patients [14]. It is therefore considered that higher age also affects the immunogenicity of influenza A(H1N1)pdm09 vaccine.

As for other subject characteristics which potentially affect to vaccine immunogenicity, some studies showed that subjects with a lower body mass index exhibited lower immune responses to the vaccine, regardless of the effect of age, disease condition, medication and pre-vaccination titer [12,13]. Another study indicated that a lower serum protein level was associated with a lower immune response, after adjusting for potential confounders including body mass index [14]. Although the precise mechanisms remain unclear, these results suggest that malnutrition might account for the decreased immune response, since malnutrition is related to a lower body mass index.

3.3. The effect of pre-vaccination titer

An inverse association between the pre-vaccination titer and MFR and SRP has been shown, referred to as the "law of initial value" or "negative feedback," in an immunogenicity study of seasonal influenza vaccine [21]. In general, persons with high pre-vaccination titers (i.e., an influenza HI antibody titer of ≥1:40) are likely to show lower MFR or lower SRP values. Inclusion of these immunized subjects may lead to underestimation of vaccine immunogenicity unless the effect of these immunized subjects is appropriately considered in the analyses and interpretation of results. The pre-vaccination titer is a significant concern in immunogenicity assessment of seasonal influenza vaccines because many people have some level of antibody due to a previous infection or vaccination with a similar strain as the relevant vaccine. On the other hand, for pandemic influenza vaccines, clinicians may presume few subjects have antibody to pandemic influenza before vaccination and thus consider the effect of pre-

vaccination titer negligible. However, our review of immunogenicity studies of influenza A(H1N1)pdm09 vaccine demonstrated some subjects had pre-vaccination titers of ≥1:40, despite that all studies excluded subjects with a history of confirmed or suspected infection of influenza A(H1N1)pdm09 at the study recruitment. As one example, we present below the results of an immunogenicity study of the influenza A(H1N1)pdm09 vaccine in adolescents [5].

We conducted a study to provide information for a national decision regarding the recommended number of doses of influenza A(H1N1)pdm09 vaccine for adolescents. We recruited 106 subjects without any history of influenza A(H1N1)pdm09 infection. In the analysis, however, approximately 28% of high school students demonstrated pre-vaccination titers [5], which may have resulted from asymptomatic infection of influenza A(H1N1)pdm09 because the influenza A(H1N1)pdm09 pandemic in Japan started among high school students. In addition, immunogenicity markers as the study outcome (i.e., GMT, MFR, SCR, and SPP after 1 dose of vaccination) were lower in high school students than junior high school students (Table 2) [5]. Unless we considered the effect of subjects with pre-vaccination titer, the results could suggest that the immune response to this influenza vaccine was lower in high school students than junior high school students, which would mislead the decision about the number of doses for adolescents. To avoid such misinformation, we conducted additional analyses using stratified or multivariate analyses.

As shown in Table 3, a lower SRP value was also observed among high school students compared with junior high school students, which resulted in a lower (approximately half) odds ratio for SRP in high school students compared to junior high school students in the univariate analysis. However, subjects with higher pre-vaccination titers also had lower SRP values, so-called "law of initial value." Thus, when we considered the effect of pre-vaccination titer in the multivariate analysis, the odds ratio of high school students approached the null value, indicating no difference in the antibody response to this vaccine observed between junior high school students and high school students. It became clear that the results obtained from Table 2 (i.e., high school students had a lower MFR in antibody titer and a lower SCP than junior high school students) were merely due to the effect of pre-vaccination antibody titer. The results of this study emphasize the importance of considering the effect of pre-vaccination titer even in the study of pandemic influenza vaccines.

The effect of the pre-vaccination antibody titer on vaccine immunogenicity has been recognized in immunogenicity studies of seasonal influenza vaccines [22]. Moreover, factors suggested from immunogenicity studies of seasonal influenza vaccines (e.g., age, pre-vaccination antibody titer, underlying illness, use of an immunosuppressant) should similarly be considered in the assessment of vaccine immunogenicity of pandemic influenza vaccines. Taking these factors into consideration by adjusting for subject characteristics and pre-vaccination titer in stratified and multivariate analyses or by using strict inclusion criteria will lead to proper assessment of vaccine immunogenicity.

3.4. Time interval between seasonal influenza vaccination and vaccination with a relevant pandemic influenza vaccine

In the assessment of pandemic influenza vaccine immunogenicity, effects resulting from the time interval between seasonal influenza vaccination and pandemic influenza vaccination should be considered. As one example, we present the results of an immunogenicity study of the influenza A(H1N1)pdm09 vaccine in pregnant women [8]. Single vaccination of influenza A(H1N1)pdm09 vaccine led to a sufficient antibody response in pregnant women, which satisfied the international criteria for the immunogenicity of the pandemic influenza vaccine (Tables 2 and 4). However, the antibody response to influenza A(H1N1)pdm09 vaccination was lower in pregnant women who had received seasonal influenza vaccination prior to the influenza A(H1N1)pdm09 vaccine (Table 4). The lower antibody response was particularly remarkable in subjects who had a vaccination interval of <20 days. Moreover, when an additional analysis was performed by changing the cut-off value for the vaccination interval from 20 days to 14 days, subjects who had been vaccinated with the seasonal vaccine within 14 days demonstrated even lower antibody responses to the influenza A (H1N1)pdm09 vaccine (post-vaccination GMT = 49, MFR = 4.9, SCP = 60%, and SPP = 50%) [8]. Similar observations were also reported in patients with hepatitis C [13] and healthy children [23]. Lower immune responses were also observed in the study of health-care workers, in which 85% of study subjects had received the seasonal influenza vaccine 7–10 days before influenza A(H1N1)pdm09 vaccine [6]. In addition, one randomized controlled trial among healthy adults also showed that subjects with prior vaccination with the seasonal influenza vaccine had lower SPP and SCP to influenza A(H1N1)pdm09 vaccine than those without prior vaccination with seasonal influenza vaccine, as shown in Table 2 [20]. These findings suggest the possibility that, when the interval after vaccination with the seasonal vaccine is short, interference between the two vaccines can occur, and the antibody response to the influenza A(H1N1)pdm09 vaccine might decrease.

For pandemic influenza, two main influenza vaccines (monovalent influenza vaccine against the pandemic influenza strain and seasonal influenza vaccine) would be available. However, supply

Table 3
Effects of pre-vaccination titer on seroresponse proportion.

Category	N	SRP* (95%CI)	Univariate		Multivariate†	
			OR (95%CI)	P	OR (95%CI)	P
School type						
Junior high school	60	87% (78–96%)	1.00		1.00	
High school	46	78% (66–90%)	0.55 (0.20–1.54)	0.26	0.86 (0.25–3.03)	0.82
Pre-vaccination titer						
<1:10	48	93% (86–100%)	1.00		1.00	
1:10–1:20	36	94% (86–102%)	1.13 (0.18–7.16)	0.89	1.14 (0.18–7.22)	0.89
≥1:40	22	41% (20–62%)	0.05 (0.01–0.20)	<0.01	0.05 (0.01–0.21)	<0.01
			Trend P < 0.01		Trend P < 0.01	

Cited and reconstructed from Ref. [5].
Abbreviations: OR, odds ratio; CI, confidence interval; SRP, seroresponse proportion.
* SRP: the proportion of persons with ≥4-fold post-vaccination rise in antibody titer.
† Model includes school type and pre-vaccination titer.

Table 4
Immunogenicity in pregnant women after 1 dose of vaccination with influenza A(HIN1)pdm09 vaccine.

Category	N	GMT		MFR	SCP* (95%CI) at S1	SPP† (95%CI) at S1
		S0‡	S1‡			
All subjects	149	8	139	17.1	91% (86–96%)	89% (84–94%)
Vaccination with current seasonal vaccine						
Not vaccinated	114	8	159	20.3	95% (91–99%)	92% (87–97%)
Vaccinated	35	9	90	9.8	80% (67–93%)	77% (63–91%)
		P = 0.41	P = 0.03	P = 0.008	P = 0.007	P = 0.02
Vaccination interval from seasonal vaccine						
Not vaccinated	114	8	159	20.3	95% (91–99%)	92% (87–97%)
≥20 days	17	8	120	15.4	100% (100%)	88% (73–100%)
<20 days	17	10	68	6.8	65% (42–88%)	65% (42–88%)
		P = 0.69	P = 0.08	P = 0.02	Trend P < 0.01	Trend P < 0.01

Cited and reconstructed from Ref. [8].
Abbreviations: CI, confidence interval; GMT, geometric mean titer; MFR, mean fold rise; SCP, seroconversion proportion; SPP, seroprotection proportion.
* SCP: the proportion of persons with pre-vaccination HI antibody titer of <1:10 and post-vaccination titer of ≥1:40 or ≥4-fold post-vaccination rise in antibody titer.
† SPP: the proportion of persons satisfying the protective level of antibody titer (HI antibody titer of ≥1:40).
‡ S0, before vaccination; S1, 3–4 weeks after 1st dose of vaccination.

of the pandemic influenza vaccine is limited, rendering it difficult to obtain the pandemic vaccine. In this situation, many people may choose to receive seasonal influenza vaccination first, and there is no clear standard concerning the duration between seasonal influenza vaccination and pandemic influenza vaccination. In general, a vaccination interval of 4 weeks after vaccination with a live vaccine and an interval of 1 week after vaccination with an inactivated vaccine are recommended to avoid mutual interference between vaccines. Moreover, findings concerning the influenza A(H1N1) pdm09 vaccine suggest that a vaccination interval of at least 3 weeks may be necessary. However, this vaccination interval may only be applicable to the influenza A(H1N1)pdm09 vaccine, since vaccines greatly differ from each other. Therefore, for future pandemic influenza vaccines, the effect of the vaccination interval between seasonal influenza vaccine and the newly developed pandemic influenza vaccine should be considered in the immunogenicity assessment of the pandemic vaccine. This can be performed using similar methods described for other factors, such as adjusting for the vaccination interval in stratified and multivariate analyses.

4. Conclusion

We encountered an influenza pandemic in 2009, which gave us an opportunity to study the immunogenicity of the influenza A (H1N1)pdm09 vaccine. This review describes the lessons our experiences have taught us, which may provide useful information for future influenza pandemics. However, studies on pandemic influenza will most certainly succeed studies on seasonal influenza. Therefore, appropriate procedures suggested by seasonal influenza studies and factors affecting the immunogenicity of seasonal influenza vaccines may be applied to similar studies on pandemic influenza vaccines. In addition, factors and strategies described herein might be applicable to immunogenicity studies of vaccines for other infectious diseases, as they share the basic principles of immunogenicity assessments.

Conflict of interest

None.

References

[1] Recommendations of the Advisory Committee on Immunization Practices (ACIP). Prevention and control of seasonal influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practice (ACIP) — United States, 2016–2017 Influenza Season. MMWR 2016; 65 (5): 1–52.
[2] World Health Organization. New influenza A/H1N1 virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009;84:249–57.
[3] European Committee for Proprietary Medical Products. Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). London: European Agency for the Evaluation of Medical Products; 1997.
[4] Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. Bethesda, MD: Food and Drug Administration; May 2007.
[5] Kobayashi M, Ohfuji S, Fukushima W, Maeda A, Maeda K, Fujioka M, et al. Immunogenicity and reactogenicity of a monovalent inactivated 2009 Influenza a vaccine in adolescents: with special reference to pre-existing antibody. J Pediatr 2012;160:632–7.
[6] Igari H, Segawa S, Watanabe A, Suzuki A, Watanabe M, Sakurai T, et al. Immunogenicity of a monovalent pandemic influenza A H1N1 vaccine in health-care workers of a university hospital in Japan. Microbiol Immunol 2010;54:618–24.
[7] Hata A, Mano C, Nakamura Y, Nishida H, Kumakura A, Mizumoto H, et al. Low response to a monovalent inactivated unadjuvanted influenza A (H1N1) pdm09 vaccine in pediatricians of a general hospital in Japan. Hum Vaccin Immunother 2012;8:587–91.
[8] Ohfuji S, Fukushima W, Deguchi M, Kawabata K, Yoshida H, Hatayama H, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine among pregnant women: lowered antibody response by prior seasonal vaccination. J Infect Dis 2011;203:1301–8.
[9] Horiya M, Hisano M, Iwasaki Y, Hanaoka M, Watanabe N, Ito Y, et al. Efficacy of double vaccination with the 2009 pandemic influenza A (H1N1) vaccine during pregnancy. Obstet Gynecol 2011;118:887–94.
[10] Ide Y, Imamura Y, Ohfuji S, Fukushima W, Ide S, Tsutsumi C, et al. Immunogenicity of a monovalent influenza A(H1N1)pdm09 vaccine in patients with hematological malignancies. Hum Vaccin Immunother 2014;10:2387–94.
[11] Hara M, Hanaoka T, Mizushima T, Honma J, Maeda K, Ohfuji S, et al. Diminished immunogenicity to pandemic H1N1 2009 influenza vaccine in subjects with severe motor and intellectual disability. Vaccine 2011;29:8323–9.
[12] Egawa Y, Ohfuji S, Fukushima W, Yamazaki Y, Morioka T, Emoto M, et al. Immunogenicity of influenza A(H1N1)pdm09 vaccine in patients with diabetes mellitus: with special reference to age, body mass index, and HbA1c. Hum Vaccin Immunother 2014;10:1187–94.
[13] Eto T, Matsubara M, Nagamizu M, Ishibashi M, Tsuru T, Ito K, et al. Immunogenicity of an inactivated vaccine against influenza A(H1N1)pdm09 virus in diabetic patients. J Japan Diab Soc 2013;56:219–26.
[14] Ohfuji S, Fukushima W, Tamori A, Maeda K, Maeda A, Hirota Y. Immunogenicity of influenza A(H1N1)pdm09 vaccine and the associated factors on lowered immune response in patients with hepatitis C. Influenza Other Respir Viruses 2013;7:456–65.
[15] Saito T, Ohfuji S, Matsumura T, Saito T, Maeda K, Maeda A, et al. Safety of a pandemic influenza vaccine and the immune response in patients with duchenne muscular dystrophy. Intern Med 2015;54:1199–205.
[16] Hatakeyama S, Iwatsuki-Horimoto K, Okamoto K, Nukui Y, Yata N, Fujita A, et al. Unadjuvanted pandemic H1N1 influenza vaccine in HIV-1-infected adults. Vaccine 2011;29:9224–8.
[17] Yanagisawa N, Maeda K, Ajisawa A, Imamura A, Suganuma A, Ando M, et al. Reduced immune response to influenza A (H1N1) 2009 monovalent vaccine in HIV-infected Japanese subjects. Vaccine 2011;29:5694–8.
[18] Tanaka S, Saikusa T, Katafuchi Y, Ushijima K, Ohtsu Y, Tsumura N, et al. Serologic response after vaccination against influenza (A/H1N1)pdm09 in

children with renal disease receiving oral immunosuppressive drugs. Vaccine 2015;33:5000–4.
[19] Torii Y, Kimura H, Ochi N, Kaneko K, Ando H, Kiuchi T, et al. Immunogenicity of inactivated 2009 H1N1 influenza vaccine in pediatric liver transplant recipients. Vaccine 2011;29:4187–9.
[20] Uno S, Kimachi K, Kei J, Miyazaki K, Oohama A, Nishimura T, et al. Effect of prior vaccination with a seasonal trivalent influenza vaccine on the antibody response to the influenza pandemic H1N1 2009 vaccine: randomized controlled trial. Microbiol Immunol 2011;55:783–9.
[21] Hobson D, Baker FA, Curry RL. Effects of influenza vaccines in stimulating antibody in volunteers with prior immunity. Lancet 1973;2:155–6.
[22] Hirota Y, Kaji M, Goto S, Oka T. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. Vaccine 1996;14:1597–602.
[23] Nolan T, McVernon J, Skeljo M, et al. Immunogenicity of a monovalent 2009 influenza A (H1N1) vaccine in infants and children. JAMA 2010;303:37–46.