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Original Article

Immunogenicity of influenza A(H1N1)pdm09 vaccine and the associated factors on lowered immune response in patients with hepatitis C

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Background Patients with underlying disease represent a high-risk group for influenza-associated complications and hospitalization. However, few studies investigated the immunogenicity of influenza vaccine in patients with liver disease.

Objective To examine immunogenicity of influenza A(H1N1)pdm09 vaccine in patients with liver disease and to explore the associated factors on lowered immune response.

Patients/Methods A single subcutaneous dose of monovalent inactivated unadjuvanted split-virus influenza A(H1N1)pdm09 vaccination was performed in 80 patients with chronic hepatitis C virus infection at Osaka City University Hospital in Japan. To measure the hemagglutination inhibition antibody titer, serum samples were collected before and 3 weeks after vaccination.

Results No serious adverse events were observed. After vaccination, antibody titers ≥1:40 were observed in 56 patients (71%). The corresponding seroconversion proportion was 72%, and the mean fold rise was 10·3. Immune responses were robust regardless of severity of liver disease or existence of probable cirrhosis. However, patients with older age, lower body mass index, or receiving Stronger Neo-Minophagen C tended to show lower antibody responses to A(H1N1)pdm09 vaccine. In addition, reduced immune responses were observed in patients who had received the 2009/10 seasonal vaccination prior to A(H1N1)pdm09 vaccination.

Conclusions Single dose of A(H1N1)pdm09 vaccine achieved a sufficient level of immunity among patients with chronic hepatitis C. Antibody response may be affected by age, body mass index, Stronger Neo-Minophagen C administration, and recent seasonal influenza vaccination.

Keywords Influenza A(H1N1)pdm09 vaccine, lowered immunity, patients with liver disease.

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Introduction

Influenza-related morbidity and mortality rates are increased among patients with underlying illness.[1,2] One case report suggested that influenza infection can cause hepatic decompensation and hospitalization in patients with advanced liver disease.[3] As influenza vaccination is the most effective method for preventing influenza illness and its complications, the Advisory Committee on Immunization Practices in the United States has recommended annual influenza vaccination for patients with underlying illnesses, including chronic liver disease.[4] In Japan, however, no recommendations about influenza vaccination for these patients had been proposed prior to the 2009 influenza A (H1N1) pandemic.

One of the reasons for this lack of recommendations might have been little scientific evidence regarding the immunogenicity and reactogenicity of influenza vaccine among patients with liver disease. To the best of our knowledge, only three studies have reported that seasonal influenza vaccine was immunogenic in patients with liver cirrhosis.[5–7] Most previous studies, however, did not determine the effects of treatments for liver disease such as interferon and Stronger Neo-Minophagen C. Interferon treatment, which is currently the most effective antiviral therapy for hepatitis C, acts as an activator of innate and

humoral immune response.[8] On the other hands, Stronger Neo-Minophagen C, which is often used for patients with hepatitis mainly in Japan, has a steroid-like structure[9] and thus may affect the immune response to influenza vaccine.

This study investigated immunogenicity of the monovalent influenza A(H1N1)pdm09 vaccine in patients with chronic hepatitis C virus infection. Induction of serum hemagglutination inhibition (HAI) antibody was assessed using conventional parameters (i.e., mean fold rise, seroresponse proportion, seroconversion proportion, and seroprotection proportion), and then several stratified and multivariate analyses were performed to consider the effects of potential predictors including liver disease severity and its treatment.

Materials and methods

Study subjects

In Japan, monovalent inactivated unadjuvanted split-virus influenza A(H1N1)pdm09 vaccine was available for tiered use in October 16, 2009. Vaccination was scheduled first for healthcare workers, pregnant women, and then provided to patients with underlying illness from November 2009, according to the order of priority of the groups. The present observational study was performed in this vaccination schedule.

In November 2009, patients with chronic hepatitis C virus infection who visited the Department of Hepatology at Osaka City University Hospital for clinical follow-up were invited to participate in the study on immunogenicity of influenza A(H1N1)pdm09 vaccine. Exclusion criteria were as follows: patients who had no detectable plasma HCV RNA levels at the time of recruitment; patients with a prior episode of influenza A(H1N1)pdm09 virus infection; acute febrile illness or signs of severe acute illness at the time of vaccination; history of anaphylaxis because of vaccine components; or other inappropriate condition for vaccination. The first 80 eligible patients who agreed on the participation were recruited. All subjects provided written informed consent after the nature and possible consequences of the study had been explained. The study protocol was approved by the ethics committee at the Osaka City University Faculty of Medicine and was implemented in accordance with the Declaration of Helsinki.

Vaccination

Subjects received a single subcutaneous dose of a monovalent inactivated unadjuvanted split-virus influenza A(H1N1)pdm09 vaccine (Lot. HP01A; BIKEN) into the arm at the time of recruitment. In Japan, subcutaneous administration is the routinely way of influenza vaccination. Some of the subjects had received the commercially available inactivated unadjuvanted split-virus trivalent influenza vaccine for the 2009/10 season before the recruitment, as annual influenza vaccination have been recommended for subjects aged 65 years or more in Japan. For subjects with 2009/10 seasonal influenza vaccination before the recruitment, A(H1N1)pdm09 vaccine was administered into the other arm. Vaccine dose was 0·5 ml, containing 15 μg of hemagglutinin antigen. The seed virus was prepared from reassortant vaccine virus A/California/7/2009, distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs using standard methods for the production of seasonal trivalent inactivated vaccine.

Data collection

At the time of recruitment, the following information was obtained from the patients using a self-administered questionnaire: age at vaccination; height and body weight; underlying illnesses other than liver disease (i.e., heart disease, respiratory disease, renal disease, atopic dermatitis, asthma, diabetes mellitus, etc.); 2009/10 seasonal influenza vaccination before recruitment; and date of vaccination if vaccinated. In addition, the physician in-charge completed a structured questionnaire to collect the following clinical information: interferon treatment; Stronger Neo-Minophagen C treatment; hepatocellular carcinoma; ascites; hepatic encephalopathy; and laboratory data such as platelet count, total bilirubin, albumin, prothrombin activity. Using these data, Child-Pugh score was calculated according to the conventional method.[10] Child-Pugh score of 5 or more was considered as liver cirrhosis.

Serum collection and antibody titer measurement

Serum samples were collected before vaccination (S0) and 3 weeks after vaccination (S1). All serum specimens were stored at −80°C until assayed, with all specimens assayed at the same time. Antibody titers against the vaccine strain were measured using the HAI assay with chicken erythrocytes according to standard methods.[11] Serum samples were treated with receptor-destroying enzyme (RDE, Vibrio cholera filtrate; Denka Seiken, Tokyo, Japan) to inactivate non-specific inhibitors. All samples were assayed in the laboratory at the Surveillance Center, Research Institute for Microbial Disease at Osaka University at April 2010.

Statistical analysis

The following outcomes were calculated for assessing the immunogenicity of influenza vaccine: geometric mean titer (GMT); mean fold rise; seroresponse proportion (≥4-fold rise from pre- to post-vaccination samples); seroprotection proportion (post-vaccination titer ≥ 1:40); seroconversion proportion (pre-vaccination titer < 1:10 and post-vaccination titer ≥ 1:40, or pre-vaccination titer ≥1:10 and ≥4-fold rise). For data processing, titers < 1:10 were

regarded as 1:5, and reciprocal antibody titers were handled after logarithmic transformation. Calculated values were converted back to the original scale by exponential transformation and shown as results. To consider the effect of potential confounders, the following stratified analyses were conducted: age (tertile); gender; body mass index (tertile); 2009/10 seasonal influenza vaccination (unvaccinated or vaccinated); time elapsed between seasonal vaccination and H1N1 vaccination (unvaccinated, ≥21 or ≤20 days); pre-vaccination titer (<1:10, 1:10–1:20, or ≥1:40); current treatment with Stronger Neo-Minophagen C (no or receive); current treatment with interferon (no or receive); platelet count (<10 or ≥10 × $10^4/\mu$l); albumin (<3·5 or ≥3·5 g/dl); prothrombin activity (<80% or ≥80%); Child-Pugh score (<5 or ≥5); and hepatocellular carcinoma (absent or present). The significance of fold rise within a category was assessed using the Wilcoxon signed-rank-sum test, while an intercategory comparison was made using either the Wilcoxon rank-sum test or the Kruskal–Wallis test. The *t*-test, chi-square test, or Mantel extension test for trend was also employed where appropriate.

Based on the results of stratified analyses, we extracted the variables that were significantly associated with at least one of the immunogenicity outcomes (i.e., GMT after vaccination, fold rise, seroresponse, and seroprotection). The independent effect of each variable on antibody induction was evaluated by multivariate logistic regression models. Models were constructed using either seroresponse or seroprotection as the dependent variable, and odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated.

All tests were two-sided, and *P* value of <0·05 was considered statistically significant. All analyses were performed using sas version 9.1.3 software (SAS Institute, Cary, NC, USA).

Results

Eighty patients with chronic hepatitis C received a single-dose vaccination between November 9, 2009 and December 4, 2009. None of the patients received both the 2009/10 seasonal influenza vaccine and the monovalent influenza A(H1N1)pdm09 vaccine at the same time. No serious adverse events were observed after A(H1N1)pdm09 vaccination. No patients developed physician-diagnosed influenza during the study period. However, one serum sample was not able to be collected at 3 weeks after vaccination. Eventually, data from 79 patients were employed for immunogenicity analyses.

Patient characteristics are shown in Table 1. Mean age was 64·5 years, and 19% of patients were men. One-third of patients had underlying diseases other than liver disease, such as diabetes mellitus (10%) and asthma (6%), but only three patients had received steroid therapy for more than 2 weeks during the last 6 months. A total of 39% of patients had received the 2009/10 seasonal influenza vaccine prior to A(H1N1)pdm09 vaccination. Regarding clinical information, 19% of patients were receiving Stronger Neo-Minophagen C treatment, whereas 39% were receiving interferon therapy at the time of recruitment. Patients with probable cirrhosis (Child-Pugh score ≥ 5) or hepatocellular carcinoma comprised 29% and 8% of patients, respectively.

Table 2 summarizes antibody responses to A(H1N1)pdm09 vaccine. Single-dose vaccination induced a rise of about 10-fold in the average level of HAI antibody ($P < 0{\cdot}01$). The seroresponse proportion was 72% (95% CI, 62–82%), and the seroprotection proportion was 71% (61–81%).

Table 1. Selected characteristics among patients with chronic hepatitis C (*n* = 79)

Characteristics		*n* (%)
Age (years)	Mean ± standard deviation	64·5 ± 10·6
Gender	Male	15 (19)
Body mass index (kg/m^2)	Mean ± standard deviation	21·5 ± 3·3
Other underlying illness	Present	26 (33)
Diabetes mellitus	Present	8 (10)
Asthma	Present	5 (6)
Atopic dermatitis	Present	5 (6)
Heart disease	Present	4 (5)
Renal disease	Present	3 (4)
2009/10 seasonal influenza vaccination	Vaccinated	31 (39)
Clinical condition at A(H1N1)pdm09 vaccination		
Duration from diagnosis (years)	Mean ± standard deviation	14·7 ± 10·3
	Data missing	2
Current treatment for liver disease		
Stronger Neo-Minophagen C	Receive	15 (19)
Interferon	Receive	31 (39)
Laboratory data		
Platelet count (×10^4/mm^3)	<10·0	20 (25)
Albumin level (g/dl)	<3·5	10 (13)
	Data missing	1
Prothrombin activity (%)	<80	11 (15)
	Data missing	9
Child-Pugh Score	5+	20 (29)
	Data missing	9
Hepatocellular carcinoma	Present	6 (8)

Data are expressed as *n* (%) unless otherwise indicated.

Table 2. Immune responses to monovalent influenza A(H1N1) pdm09 vaccine among patients with chronic hepatitis C

Characteristics	Category	*n*	Geometric mean titer (95%CI)*				Post vac titer**	
							≥fourfold rise	≥1:40
			Pre	Post	Fold rise	*P*	*n* (%, 95%CI)	*n* (%, 95%CI)
Entire sample		79	8 (7–9)	82 (58–116)	10·3 (7·2–14·9)	<0·01	57 (72, 62–82)	56 (71, 61–81)
Age (years)	<62	24	7 (6–8)	113 (62–205)	16·5 (8·5–31·8)	<0·01	20 (83, 68–98)	20 (83, 68–98)
	62–69	28	8 (6–10)	131 (73–235)	16·8 (9·1–31·2)	<0·01	23 (82, 68–96)	23 (82, 68–96)
	70+	27	9 (7–12)	38 (23–64)	4·1 (2·5–6·7)	<0·01	14 (52, 33–71)	13 (48, 29–67)
			P = 0·35	*P* < 0·01	*P* < 0·01		*P* = 0·01	*P* < 0·01
Gender	Male	15	8 (6–10)	80 (37–171)	10·6 (4·7–23·6)	<0·01	11 (73, 51–95)	11 (73, 51–95)
	Female	64	8 (7–9)	83 (56–122)	10·3 (6·8–15·5)	<0·01	46 (72, 61–83)	45 (70, 59–81)
			P = 1·00	*P* = 0·98	*P* = 1·00		*P* = 1·00	*P* = 1·00
Body mass index(kg/m^2)	<20·2	26	10 (7–13)	68 (34–135)	7·0 (3·5–13·9)	<0·01	16 (62, 43–81)	17 (65, 47–83)
	20·2–22·5	28	8 (6–10)	59 (34–104)	7·4 (4·3–13·0)	<0·01	19 (68, 51–85)	17 (61, 43–79)
	22·6+	25	6 (5–8)	143 (86–239)	22·3 (12·4–40·2)	<0·01	22 (88, 75–100)	22 (88, 75–100)
			P = 0·06	*P* = 0·06	*P* = 0·02		*P* = 0·04	*P* =0·08
2009/10 seasonal influenza vaccination	Unvaccinated	48	7 (6–8)	137 (87–213)	20·7 (13·2–32·6)	<0·01	41 (85, 75–95)	39 (81, 70–92)
	Vaccinated	31	11 (9–13)	37 (24–57)	3·5 (2·4–5·1)	<0·01	16 (52, 34–70)	17 (55, 37–73)
			P < 0·01	*P* < 0·01	*P* < 0·01		*P* < 0·01	*P* =0·01
Time elapsed between seasonal vaccination and A(H1N1)pdm09 vaccination								
	Unvaccinated	48	7 (6–8)	137 (87–213)	20·7 (13·2–32·6)	<0·01	41 (85, 75–95)	39 (81, 70–92)
	21days ormore	17	12 (9–15)	53 (31–92)	4·5 (2·6–7·7)	<0·01	10 (59, 36–82)	11 (65, 42–88)
	Within 20 days	14	10 (7–14)	24 (13–45)	2·6 (1·5–4·3)	<0·01	6 (43, 17–69)	6 (43, 17–69)
			P < 0·01	*P* < 0·01	*P* < 0·01		*P* < 0·01	*P* < 0·01
Prevaccination titer	<1:10	44	5 (5–5)	84 (51–139)	16·8 (10·1–27·8)	<0·01	36 (82, 71–93)	30 (68, 54–82)
	1:10–1:20	31	12 (11–14)	73 (46–117)	6·0 (3·7–9·6)	<0·01	20 (65, 48–82)	22 (71, 55–87)
	≥1:40	4	48 (34–67)	160 (23–1093)	3·4 (0·5–23·7)	0·50	1 (25, 0–67)	4 (100, 100–100)
			P < 0·01	*P* = 0·75	*P* < 0·01		*P* = 0·01	*P* = 0·33
Clinical condition at A(H1N1)pdm09 vaccination								
Current treatment for liver disease								
Stronger Neo-Minophagen C	No	64	8 (7–9)	98 (68–141)	12·7 (8·5–19·1)	<0·01	50 (78, 68–88)	48 (75, 64–86)
	Receive	15	9 (6–13)	38 (16–92)	4·2 (2·0–8·8)	<0·01	7 (47, 22–72)	8 (53, 28–78)
			P = 0·41	*P* = 0·03	*P* = 0·02		*P* = 0·01	*P* = 0·10
Interferon	No	48	8 (7–10)	60 (38–95)	7·1 (4·5–11·3)	<0·01	30 (63, 49–77)	30 (63, 49–77)
	Receive	31	7 (6–9)	134 (83–215)	18·3 (10·4–32·1)	<0·01	27 (87, 75–99)	26 (84, 71–97)
			P = 0·29	*P* = 0·03	*P* = 0·01		*P* = 0·02	*P* = 0·04

Table 2. (Continued)

Characteristics	Category	*n*	Geometric mean titer (95%CI)*				Post vac titer**	
							≥fourfold rise	≥1:40
			Pre	Post	Fold rise	*P*	*n* (%, 95%CI)	*n* (%, 95%CI)
Laboratory data								
Platelet count (*10^4/mm^3)	<10·0	20	8 (6–10)	80 (41–157)	10·6 (5·0–22·3)	<0·01	14 (70, 50–90)	14 (70, 50–90)
	10·0+	59	8 (7–10)	83 (55–124)	10·2 (6·7–15·6)	<0·01	43 (73, 62–84)	42 (71, 59–83)
			P = 0·77	*P* = 0·97	*P* = 0·98		*P* = 0·80	*P* = 0·92
Albumin level (g/dl)	<3·5	10	11 (7–16)	92 (36–237)	8·6 (3·6–20·5)	<0·01	8 (80, 55–100)	8 (80, 55–100)
	3·5 +	68	8 (7–9)	80 (55–117)	10·5 (7·0–15·8)	<0·01	48 (71, 60–82)	47 (69, 58–80)
			P = 0·07	*P* = 0·90	*P* = 0·69		*P* = 0·72	*P* = 0·71
Prothrombin activity (%)	<80	11	8 (6–11)	43 (20–90)	5·5 (2·4–12·3)	<0·01	7 (64, 36–92)	6 (55, 25–85)
	80+	59	8 (7–10)	79 (53–119)	9·7 (6·4–14·7)	<0·01	42 (71, 59–83)	41 (69, 57–81)
			P = 0·96	*P* = 0·22	*P* = 0·32		*P* = 0·72	*P* = 0·33
Child-Pugh Score	<5	50	8 (7–10)	73 (48–110)	9·1 (5·8–14·2)	<0·01	35 (70, 57–83)	33 (66, 53–79)
	5+	20	8 (6–11)	70 (33–146)	8·3 (4·1–16·7)	<0·01	14 (70, 50–90)	14 (70, 50–90)
			P = 0·66	*P* = 0·91	*P* = 0·84		*P* = 1·00	*P* = 0·75
Hepatocellular carcinoma	Absent	73	8 (7–9)	85 (59–122)	11·0 (7·5–16·3)	<0·01	54 (74, 64–84)	52 (71, 61–81)
	Present	6	13 (5–29)	57 (23–141)	4·5 (2·1–9·4)	0·03	3 (50, 10–90)	4 (67, 29–100)
			P = 0·18	*P* = 0·55	*P* = 0·21		*P* = 0·34	*P* = 1·00

*Wilcoxon signed-rank test for intra-category comparisons, and either the Wilcoxon ranksumtest or Kruskal–Wallis test for inter-category comparisons.
**Sero-response proportion (>fourfold rise) and sero-protection proportion (post vaccination titer >1:40).
Chi-square test between two categories and the Mantel-extension method for trend test among three categories.

Table 3. Association between selected characteristics and sero-response proportion (>fourfold-rise) after vaccination

Category	*n*	*n* (%, 95%CI)	Univariate analysis		Mutivariate model*	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age (years)						
<62	24	20 (83, 68–98)	1·00		1·00	
62–69	28	23 (82, 68–96)	0·92 (0·22–3·90)	0·91	1·12 (0·18–6·76)	0·91
70+	27	14 (52, 33–71)	0·22 (0·06–0·80)	0·02	0·46 (0·09–2·43)	0·36
			Trend $P = 0·01$		Trend $P = 0·25$	
Body mass index (kg/m^2)						
<20·2	26	16 (62, 43–81)	0·22 (0·05–0·92)	0·04	0·20 (0·03–1·18)	0·07
20·2–22·5	28	19 (68, 51–85)	0·29 (0·07–1·22)	0·09	0·36 (0·06–2·10)	0·26
22·6+	25	22 (88, 75–100)	1·00		1·00	
			Trend $P = 0·04$		Trend $P = 0·07$	
2009/10 seasonal influenza vaccination						
Unvaccinated	48	41 (85, 75–95)	1·00		1·00	
Vaccinated	31	16 (52, 34–70)	0·18 (0·06–0·53)	<0·01	0·21 (0·04–1·07)	0·06
Time elapsed between seasonal vaccination and A(H1N1)pdm09 vaccination						
Unvaccinated	48	41 (85, 75–95)	1·00		1·00**	
21 days or more	17	10 (59, 36–82)	0·24 (0·07–0·86)	0·03	0·64 (0·08–5·18)	0·68
Within 20 days	14	6 (43, 17–69)	0·13 (0·03–0·48)	<0·01	0·10 (0·02–0·67)	0·02
			Trend $P < 0·01$		Trend $P = 0·01$	
Prevaccination titer						
<1:10	44	36 (82, 71–93)	1·00		1·00	
1:10–1:20	31	20 (65, 48–82)	0·40 (0·14– 1·17)	0·10	1·04 (0·25–4·35)	0·95
>1:40	4	1 (25, 0–67)	0·07 (0·01–0·81)	0·03	0·21 (0·02–2·80)	0·24
			Trend $P = 0·01$		Trend $P = 0·41$	
Current treatment for liver disease						
Stronger Neo-Minophagen C						
No	64	50 (78, 68–88)	1·00		1·00	
Receive	15	7 (47, 22–72)	0·25 (0·08–0·79)	0·02	0·35 (0·07–1·64)	0·18
Interferon						
No	48	30 (63, 49–77)	1·00		1·00	
Receive	31	27 (87, 75–99)	4·05 (1·22–13·5)	0·02	1·29 (0·28–6·06)	0·75

OR, odds ratio; CI, confidence interval.
*Model included all variables in the table.
**The ORs were obtained from the model in which 2009/10 seasonal influenza vaccination was replaced by time elapsed between seasonal vaccination and A(H1N1)pdm09 vaccination.

Corresponding seroconversion proportion was at the same level as the seroresponse proportion 72% (62–82%). Immune responses were robust regardless of gender, severity of liver disease (e.g., platelet count, albumin level, or prothrombin activity), or existence of probable cirrhosis (Child-Pugh score ≥ 5). On the other hand, older patients and those with lower body mass index revealed lower antibody responses to A(H1N1)pdm09 vaccine. In addition, reduced immune responses were observed in patients who had received the 2009/10 seasonal vaccine prior to A(H1N1)pdm09 vaccination (particularly with a shorter interval between vaccinations). Patients with higher pre-vaccination titers also indicated lower fold rise and seroresponse proportions with clear dose–response relationships ($P < 0·01$ and $P = 0·01$, respectively). Regarding current treatment for liver disease, patients with Stronger Neo-Minophagen C treatment showed a decreased antibody response to A(H1N1)pdm09 vaccine (P for seroresponse = 0·01), whereas those with interferon treatment exhibited higher GMT and seroresponse and seroprotection proportions ($P = 0·03$, $P = 0·02$, and $P = 0·04$, respectively).

After considering the effects of potential confounders in multivariate analysis, patients with lower body mass index tended to have decreased ORs for seroresponse to A(H1N1)pdm09 vaccination with a marginal significance (Table 3). However, patients who had received the 2009/10 seasonal vaccine prior to A(H1N1)pdm09 vaccination,

particularly within a short period (≤20 days) between vaccinations, showed significantly lower seroresponse proportions (OR, 0·10; 95% CI, 0·02–0·67). There were no obvious significant associations with other variables.

Table 4 shows associations with seroprotection following A(H1N1)pdm09 vaccination. In multivariate analyses, ORs for seroprotection were significantly decreased in older patients and patients with lower body mass index (Trend $P = 0·05$ and 0·01, respectively). Patients with 2009/10 seasonal vaccine (particularly shorter interval between vaccinations) also had a significantly lower OR (OR, 0·07; 95% CI, 0·01–0·65). On the other hand, patients with higher prevaccination titers showed a significantly increased OR for seroprotection (OR, 6·37; 95% CI, 1·12–36·3). Regarding current treatment for liver disease, patients with Stronger Neo-Minophagen C treatment tended to show a decreased OR, although significant relationship could not be observed.

Additional analyses were conducted when the cutoff point of time elapsed between seasonal influenza vaccination and influenza A(H1N1)pdm09 vaccination was changed from 21 days to 14 days. Among seven subjects with

Table 4. Association between selected characteristics and sero-protection proportion (titer > 1:40) after vaccination*

Category	*n*	*n* (%, 95%CI)	Univariate analysis		Mutivariate model**	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age (years)						
<62	23	19 (83, 68–98)	1·00		1·00	
62–69	27	22 (81, 66–96)	0·93 (0·22–3·95)	0·92	0·70 (0·11–4·35)	0·70
70+	25	11 (44, 25–63)	0·17 (0·04–0·63)	<0·01	0·21 (0·04–1·16)	0·07
			Trend P <0·01		Trend $P = 0·05$	
Body mass index(kg/m^2)						
<20·2	23	14 (61, 41–81)	0·21 (0·05–0·92)	0·04	0·09 (0·01–0·59)	0·01
20·2–22·5	27	16 (59, 40–78)	0·20 (0·05–0·83)	0·03	0·14 (0·02–0·85)	0·03
22·6+	25	22 (88, 75–100)	1·00		1·00	
			Trend $P = 0·04$		Trend $P = 0·01$	
2009/10 seasonal influenza vaccination						
Unvaccinated	46	37 (80, 68–92)	1·00		1·00	
Vaccinated	29	15 (52, 34–70)	0·26 (0·09–0·73)	0·01	0·14 (0·02–0·98)	0·04
Time elapsed between seasonal vaccination and A(H1N1)pdm09 vaccination						
Unvaccinated	46	37 (80, 68–92)	1·00		1·00***	
21 days or more	16	10 (63, 39–87)	0·41 (0·12–1·41)	0·16	0·32 (0·04–2·89)	0·31
Within 20 days	13	5 (38, 12–64)	0·15 (0·04–0·58)	<0·01	0·07 (0·01–0·65)	0·02
			Trend P <0·01		Trend $P = 0·02$	
Prevaccination titer						
<1:10	44	30 (68, 54–82)	1·00		1·00	
1:10–1:20	31	22 (71, 55–87)	1·14 (0·42–3·11)	0·80	6·37 (1·12–36·3)	0·04
Current treatment for liver disease						
Stronger Neo-Minophagen C						
No	62	46 (74, 63–85)	1·00		1·00	
Receive	13	6 (46, 19–73)	0·30 (0·09–1·02)	0·05	0·26 (0·05–1·50)	0·13
Interferon						
No	45	27 (60, 46–74)	1·00		1·00	
Receive	30	25 (83, 70–96)	3·33 (1·08–10·3)	0·04	0·77 (0·16–3·70)	0·75

OR, odds ratio; CI, confidence interval.
*75 study subjects were included for the analyses because four subjects with a prevaccination titer of 1:40 or more were excluded.
**Model included all variables in the table.
***The ORs were obtained from the model in which 2009/10 seasonal influenza vaccination was replaced by time elapsed between seasonal vaccination and A(H1N1)pdm09 vaccination.

seasonal vaccination within 14 days, GMT levels at S0 and S1 were 8 and 16, respectively, resulting in 2·0-fold rises after H1N1 vaccination. The seroresponse proportion was 28%, and the seroprotection proportion was 29%. Multivariate analyses showed that ORs of subjects with seasonal vaccination within 14 days were lower for both seroresponse and seroprotection as outcome indices (seroresponse, OR = 0·03, 95% CI = 0·00–0·42; seroprotection, OR = 0·03, 95% CI = 0·00–0·48).

Besides, another four patients received 2009/10 seasonal influenza vaccine between A(H1N1)pdm09 vaccination and serum sampling at 3 weeks after vaccination. However, immune responses to A(H1N1)pdm09 vaccine among these intercurrent vaccinated patients were almost similar levels to those among the rest 44 unvaccinated patients (data not shown).

Discussion

This study shows that single dose of A(H1N1)pdm09 vaccination produced sufficient antibody response among patients with chronic hepatitis C. The immunity was sufficient to meet the international criteria of the European Agency for the Evaluation of Medical Products and the US Food and Drug Administration.[12,13] However, the seroprotection proportion among patients with chronic hepatitis C (71%; 95% CI, 61–81%) was slightly lower than the reported proportions in age-matched healthy adults (79–94%).[14–16] While the three previous studies used the same type of vaccines (i.e., inactivated unadjuvanted split-virus vaccine containing 15 μg of hemagglutinin antigen), they used a different injection route (i.e., intramuscular) compared with our study. According to a study on trivalent influenza vaccine, seroprotection proportion with a subcutaneous injection was reported to be approximately 10% lower than that with an intramuscular injection for both A strains, especially in elderly women.[17] It is therefore considered that the discrepancy in immunogenicity across studies would not be beyond the range expected by the variation of the injection route. Another Japanese study, which used the same vaccine and injection route as ours, reported the seroprotection proportion of 80% (95% CI, 73–86%) in healthcare workers aged 20–60 years.[18] The proportion was comparable to that of the youngest age group (<62 years) in the present study. Taken together, immunogenicity of influenza vaccine in patients with chronic hepatitis C would not be lower than that of healthy adults.

In this study, the following factors might have affected the lowered seroprotection after A(H1N1)pdm09 vaccination: older age; lower body mass index; and 2009/10 seasonal vaccination prior to A(H1N1)pdm09 vaccination. Reduced immune response to vaccines in the elderly has been shown in previous studies of seasonal influenza vaccine.[19] The mechanisms have not been fully elucidated, but decreased T-cell activity[20–22]and the effects of malnutrition associated with aging have been suggested.[23,24] Conversely, no studies have reported the decreasing effect of lower body mass index on immune response. However, malnutrition might also account for such decreased immune response,[23,24] as this can be considered strongly related to lower body mass index.

Immune response to A(H1N1)pdm09 vaccine was affected by recently received seasonal vaccine, suggesting potential interference in immune responses between seasonal vaccination and A(H1N1)pdm09 vaccination. Most of the patients who had received seasonal vaccination prior to A(H1N1)pdm09 vaccination were aged 65 years or more, as annual influenza vaccination was recommended for subjects aged 65 years or more in Japan. However, lower immunogenicity in patients with recently received seasonal vaccine was independently observed even after adjusted not only for the categorical age groups, but also for continuous age (data not shown). Thus, the association between recent seasonal vaccination and lower immunogenicity of A(H1N1)pdm09 vaccine would be free from the effects of age. According to previous reports, simultaneous administration of seasonal and A(H1N1)pdm09 vaccine could induce sufficient levels of antibody to both seasonal and A(H1N1)pdm09 vaccine strains.[25] However, an immunogenicity study of A(H1N1)pdm09 vaccine in pregnant women reported the same result as the present study. In that study, pregnant women who had received the 2009/10 seasonal influenza vaccine prior to influenza A(H1N1)pdm09 vaccination, particularly with a shorter time elapsed between vaccinations, exhibited lower immune responses to A(H1N1)pdm09 vaccine.[26] Another study showed that when seasonal and A(H1N1)pdm09 vaccines were administered separately, the GMT to A(H1N1)pdm09 vaccine strain tended to be lower among the seasonal-vaccinated group than among the unvaccinated group, although the difference was not significant.[27] As several studies have reported similar findings, the decreased immune response in the seasonal-vaccinated group seems unlikely to be attributable to chance. In addition, decreases in immune response show a dose–response relationship with time intervals between vaccinations. To prepare for future influenza pandemics, further studies are required to examine potential interference across influenza vaccines.

An inverse association between pre-vaccination titer and both fold rise and seroresponse proportion is known as the ''law of initial value'' or ''negative feedback''.[28] This phenomenon was also clearly demonstrated in the present study (Table 2). The immunogenicity of a pandemic influenza vaccine is inevitably investigated during the epidemic period. Therefore, even if patients in whom influenza A(H1N1)pdm09 virus infection had already confirmed

were excluded on recruitment into the study, the possibility remained that patients with asymptomatic infection may have been included as study subjects. The effect of pre-vaccination antibody titers must therefore be appropriately considered in evaluating immunogenicity of pandemic influenza vaccines, as shown in previous studies.[29,30] In the present study, however, older age, lower body mass index, and 2009/10 seasonal vaccination prior to A(H1N1)pdm09 vaccination were independently associated with lowered seroprotection, even if the effect of pre-vaccination titer was also considered.

Regarding clinical characteristics, immune responses to influenza A(H1N1)pdm09 vaccine were robust regardless of severity of liver disease (e.g., platelet count, albumin level, or prothrombin activity) or existence of probable cirrhosis (Child-Pugh score ≥ 5). These results agreed with those of previous studies among patients with liver cirrhosis.[5–7] In the present study, however, patients with hepatocellular carcinoma were too limited to perform the further analyses including the assessment of anticancer agent. Further studies would be needed to confirm the immunogenicity in patients with hepatocellular carcinoma.

As for interferon treatment at the time of vaccination, multivariate ORs for seroresponse or for seroprotection were relatively fluctuated, and both of these associations were not significant. Thus, we considered that interferon treatment was unlikely to affect the antibody induction of influenza vaccine. Previous study also indicated that cirrhosis with interferon treatment had a comparable immunogenicity of influenza vaccine with those without interferon treatment.[5]

On the other hands, patients with Stronger Neo-Minophagen C treatment showed lower ORs for both seroresponse and seroprotection. Lack of statistical significance in multivariate analyses might be attributed by insufficient sample size of this category, as only 19% of patients received Stronger Neo-Minophagen C treatment. To date, no other study has reported on the association between Stronger Neo-Minophagen C and the immune response to any vaccines. However, Stronger Neo-Minophagen C has a steroid-like structure and directly leads to down-regulated T-cell activity.[9] Thus, it may be speculated that Stronger Neo-Minophagen C suppresses T-cell activity, and consequently, lowers the antibody induction. Besides, Stronger Neo-Minophagen C has been found to interfere with replication and cytopathogenic effect induction of many viruses including influenza viruses,[31,32] and thus may affect the immune response to the live-attenuated influenza vaccine. Further studies are needed to confirm the present finding and to clarify the mechanisms.

In the present study, however, the following limitations must be considered. First, the sample size was limited in the consideration of some associated factors on lowered immune response, although that might be enough to assess the immunogenicity of influenza vaccine in patients with chronic liver disease. Second, as this study targeted patients with chronic hepatitis C virus infection in a relatively stable condition and in mostly women, caution is needed when generalizing the present results. However, the results are consistent with previous studies conducted in patients with liver cirrhosis or liver transplantation with different causes.[5–7] Third, as body mass index was calculated using self-reported height and weight, it may be inaccurate compared with the measured values. However, previous study indicated that self-reported height and weight were precise and accurate in adult Japanese women.[33] Besides, the observed association between lower body mass index and decreased seroprotection could be free from the inaccuracy, as the inaccuracy was considered to be non-differential in the study subjects. Finally, in the 2009 influenza A (H1N1) pandemic, about one-third of subjects ≥65 years old were reported to have pre-existing antibody before the epidemic, as many had been exposed to antigen similar to influenza A(H1N1)pdm09 virus during childhood.[34] In the present study, however, despite the fact that about half of patients were ≥65 years old, proportions of patients with pre-existing antibody were lower than in previous studies. Although the reason remains unclear, this situation made it easier to evaluate immunogenicity of influenza A(H1N1)pdm09 vaccine.

Conclusions

Single dose of A(H1N1)pdm09 vaccination achieved a sufficient level of immunity, meeting international criteria among patients with chronic hepatitis C. Immune responses were robust regardless of severity of liver disease or existence of probable cirrhosis. However, immune responses may be reduced by older age, lower body mass index, seasonal vaccination prior to A(H1N1)pdm09 vaccination, or Stronger Neo-Minophagen C treatment. The potential interference between A(H1N1)pdm09 vaccination and seasonal vaccination needs to be investigated more thoroughly in a different study setting to prepare for future influenza pandemics.

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