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Original Article

Effectiveness of influenza vaccine in children in day-care centers of Sapporo

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Abstract ***Background***: We conducted a retrospective cohort study for evaluating the effectiveness of the trivalent inactivated influenza vaccine (TIV) among children aged 0–6 years in the 2011–2012 season in Sapporo City, Japan, because of scarce evidence.

Methods: From 10 day-care centers in Sapporo City, Japan, 629 parents participated in the study. Each parent of the subjects described whether a subject received TIV once or twice in the 2011–2012 season, as well as the exact dates of receiving TIV from records in a maternal and child health handbook marked by a pediatrician. The incidence of influenza was defined as being affected with influenza as diagnosed by a pediatrician. Cox's proportional model was used for calculating a hazard ratio (HR) and its 95% confidence interval (95%CI) of TIV on an influenza incidence.

Results: After adjusting potential confounding variables, such as the day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in the home in addition to the age and sex of the child, HR was significantly reduced in the subjects aged 1 year (HR = 0.22, 95%CI 0.09–0.54) as well as in the total subjects (HR = 0.72, 95%CI 0.52–0.99). Consequently, the effectiveness of TIV was calculated as 78% for the subjects aged 1 year and 28% for the total subjects.

Conclusion: Our study suggests that TIV is effective, especially in subjects aged 1 year. Further studies are necessary in different seasons, places, and populations to clarify the effectiveness of the influenza vaccine in children.

Key words children, effectiveness, influenza vaccine, retrospective cohort studies.

The influenza virus causes annual epidemics in the winter season in Japan, and it has been stated that vaccination against influenza in children should be promoted to prevent influenza-associated encephalitis-encephalopathy.[1] Increased awareness of the importance of influenza infection in children has led to an increase in the use of the influenza vaccine in Japan.[2] Trivalent inactivated vaccine (TIV) is now used every year for children in Japan.

According to the recent definition of vaccine efficacy and effectiveness,[3,4] efficacy is best measured by randomized controlled trials (RCT), and effectiveness is usually measured by observational studies. Efficacy or effectiveness of the live attenuated vaccine,[5–9] as well as the inactivated vaccine,[10–14] has been reported around the world. An RCT of the influenza vaccine in children aged 6–59 months showed superior efficacy of the live attenuated vaccine, as compared with the inactivated vaccine.[15] However, this trial also showed a higher rate of hospitalization for any cause among children aged 6–11 months in the live-attenuated-vaccine group than in the inactivated-vaccine group.[15] Other RCT of the influenza vaccine showed similar efficacy of the inactivated vaccine to the live attenuated vaccine in children aged 1–16 years[16] and in school children aged 9–12 years.[17]

Several RCT[10,11] or cohort studies[12–14] have shown significant efficacy or effectiveness of TIV to reduce the incidence of influenza in children. However, efficacy or effectiveness of TIV in children less than 3 years old is scarce in evidence and even controversial.[12,14] Accordingly, a retrospective cohort study was conducted for evaluating the effectiveness of TIV among children aged 0–6 years in the 2011–2012 season in Sapporo City, Japan.

Methods

Every large day-care center was identified from 10 districts in Sapporo. Then, 1570 parents of children attending these 10 day-care centers were invited to participate in the survey, and eventually, 629 parents (40.1%) gave written, informed consent to participate in this survey. Age distribution of the study subjects at the end of April 2012, was as follows: 43 were 0 years old, 122 were 1 year old, 127 were 2 years old, 119 were 3 years old, 106 were 4 years old, and 112 were 5 or 6 years old. A self-administered and structured questionnaire was distributed to their parents at the end of April 2012, and they returned a filled-out questionnaire in May by mail. Each parent described whether a subject received TIV once or twice in the 2011–2012 season, and if so, we noted the exact dates of receiving TIV according to records in a maternal and child health handbook marked by a

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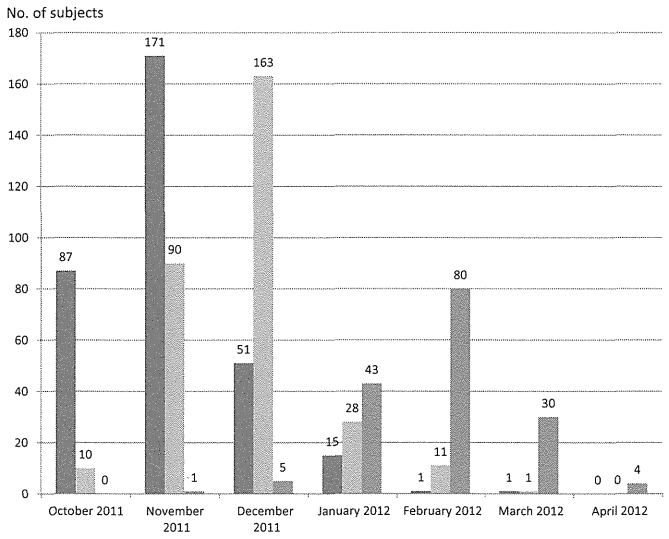

Fig 1. Distributions of the subjects in the first and second vaccinations of the trivalent inactivated vaccine and the incidence of influenza according to each month in the 2011–2012 season. ■, The first vaccination; ■, the second vaccination; ■, incidence of influenza.

pediatrician. TIV consisted of A/California/7/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Prisbane in the 2011–2012 season.[18] In addition, the questionnaire included inquiries about age, sex, size of household, number of siblings, number of smokers in the home, and so on.

The incidence of influenza was defined as being affected with influenza as diagnosed by a pediatrician. The exact date of the visit to a pediatrician and the name of the medical institute where the pediatrician worked were also obtained with the questionnaire. Cox's proportional model was used for calculating a hazard ratio (HR) and its 95% confidence interval (95%CI) of TIV on the influenza incidence. The start and end of observations were set at 1 October 2011, and 30 April 2012, respectively. SAS version 9.2 (SAS Institute, Cary, NC, USA) was utilized for every analysis. The significance level was set at 5%. This study was approved by the Ethical Committee of Sapporo Medical University (Approval date, 28 March 2012; Approval number, 23-2-76).

Results

From October 2011 to March 2012, 324 subjects among 629 participants (51.5%) received TIV at least once, and they were classified into the Vaccine group. In the Vaccine group, 302 subjects (93.2%) were fully vaccinated with two doses. As shown in Figure 1, the distribution of the subjects for the first vaccination according to months in the 2011–2012 season was as follows: 87 in October, 171 in November, 51 in December, 15 in January, one in February, and one in March. Furthermore, the distribution of the subjects on the second vaccination according to each month in the 2011–2012 season was as follows: 10 in October, 90 in November, 163 in December, 28 in January, 11 in February, and one in March.

Table 1 shows baseline characteristics of the subjects according to the status of receiving TIV, namely, the Vaccine and No vaccine groups. A subject who was vaccinated after being affected with influenza was classified into the No vaccine group. Various kinds of comorbidity were reported from 123 study children, including otitis media in 37 children and atopy or allergy in 19 children as the two most common comorbidities. The average age, proportion of boys, and presence of comorbidity were not different between the Vaccine and No vaccine groups. However, the distribution of day-care centers, size of the household, number of siblings, and number of smokers in the home were all significantly different between the two groups.

In the 2010–2011 season, 163 subjects (25.9%) were diagnosed as being affected with influenza by a pediatrician. As shown in Figure 1, the distribution of the subjects at the diagnosis of influenza according to months in the 2011–2012 season was as follows: one in November, five in December, 43 in January, 80 in February, 30 in March, and four in April.

Table 2 shows the sex-adjusted HR of TIV on the influenza incidence stratified by age. HR was significantly reduced in the subjects aged 1 year (relative risk = 0.24, 95%CI 0.10–0.56). Furthermore, sex- and age-adjusted HR were significantly

Table 1 Baseline characteristics of the subjects according to status of trivalent inactive vaccine in 2011–2012 season

Items	Vaccine group (n = 324)		No vaccine group (n = 305)		P-value
Age in years (mean, SD)	2.72	1.43	2.78	1.74	0.629
Boys (n, %)	155	47.8	149	48.9	0.799
Day-care center 1 (n, %)	44	13.6	19	6.2	<0.001
Day-care center 2 (n, %)	34	10.5	14	4.6	
Day-care center 3 (n, %)	37	11.4	40	13.1	
Day-care center 4 (n, %)	28	8.6	22	7.2	
Day-care center 5 (n, %)	19	5.9	32	10.5	
Day-care center 6 (n, %)	28	8.6	34	11.2	
Day-care center 7 (n, %)	42	13.0	49	16.1	
Day-care center 8 (n, %)	35	10.8	18	5.9	
Day-care center 9 (n, %)	32	9.9	32	10.5	
Day-care center 10 (n, %)	25	7.7	45	14.8	
Presence of comorbidity (n, %)	69	21.3	54	17.7	0.256
Size of household (mean, SD)	3.68	0.85	3.91	1.04	0.002
Number of siblings (mean, SD)	1.66	0.71	1.94	0.82	<0.001
Number of smokers in home (mean, SD)	0.49	0.66	0.63	0.71	0.011

Table 2 Sex-adjusted HR and its 95%CI of trivalent inactive vaccine on influenza incidence in 2011–2012 season

Age	Vaccine group				No vaccine group						
	n	Person-days	Incidence	Incidence rate‡	*n*	Person-days	Incidence	Incidence rate‡	HR	95%CI	*P*-value
0 years	3	506	1	19.8	40	6 921	4	5.8	2.23	0.20, 24.60	0.513
1 year	75	12 973	8	6.2	47	7 194	17	23.6	0.24	0.10, 0.56	0.001
2 years	85	13 971	19	13.6	42	6 675	14	21.0	0.66	0.33, 1.33	0.246
3 years	61	9 847	17	17.3	58	8 443	26	30.8	0.56	0.31, 1.04	0.067
4 years	53	8 455	15	17.7	53	8 205	16	19.5	0.90	0.44, 1.83	0.760
5 or 6 years	47	7 519	12	16.0	65	10 791	14	13.0	1.23	0.57, 2.67	0.602
Total	324	53 271	72	13.5	305	48 229	91	18.9	0.71†	0.52, 0.97	0.032

Incidence was defined as being affected with influenza diagnosed by pediatrician. †Age- and sex-adjusted HR in the total subjects. ‡Incidence rate per 10 000 person-days. HR, hazard ratio.

decreased in the total subjects (HR = 0.71, 95%CI 0.52–0.97). As shown in Table 3, the HR of TIV on the influenza incidence were not meaningfully changed even after adjusting potential confounding variables, such as the day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in the home in addition to age and sex of the patient. Namely, HR was significantly reduced in the subjects aged 1 year (HR = 0.22, 95%CI 0.09–0.54) as well as in the total subjects (HR = 0.72, 95%CI 0.52–0.99). Consequently, effectiveness of TIV was calculated as 78% for the subjects aged 1 year, and 28% for the total subjects.

Discussion

It was found that the HR of TIV on influenza incidence was significantly reduced in the subjects aged 1 year and in the total subjects, but not in the subjects aged 0 years, or 2–6 years. Fujieda *et al.*[14] reported, from the results of a follow-up study at 54 pediatric clinics in eight areas of Japan in the 2002–2003 season, that risk was significantly reduced in the group, aged 2.0–3.9 years, receiving an inactivated vaccine, but not those aged under 1.9 years or over 4.0 years. Similar to this study, they found an insignificantly increased risk of an inactivated vaccine among children less than 1 year of age, and they mentioned that there was a lower immune response to the influenza vaccine for those less than 1 year of age.[14]

Maeda *et al.*[12] showed, with a prospective cohort study in Japan, that the risk of an influenza-like illness was insignificantly reduced in the group receiving the inactivated vaccine of age strata from 1 year to 7 years of age. Similar to this study's results, they found a significantly decreased risk of the inactivated vaccine on influenza infection in the total number of children aged 1–7 years. As explained by Hirota *et al.*,[19] the variety in results comes from the fact that efficacy or effectiveness of the vaccine is influenced by the designs or conditions in the fields, such as a mixed epidemic with different strains, antigenic similarity between the vaccine strains and epidemic viruses, and inter-individual variation in the antibody response to the vaccine.

Table 3 HR and its 95%CI of trivalent inactive vaccine on influenza incidence in 2011–2012 season, after adjusting potential confounding variables†

Age	HR	95%CI	*P*-value
0 years	2.47	0.08, 73.63	0.602
1 year	0.22	0.09, 0.54	0.001
2 years	0.60	0.28, 1.28	0.185
3 years	0.66	0.35, 1.27	0.215
4 years	0.75	0.36, 1.54	0.427
5 or 6 years	1.37	0.62, 3.04	0.438
Total	0.72	0.52, 0.99	0.042

†Distribution of day-care center, presence of comorbidity, size of household, number of siblings, and number of smokers in home, were adjusted in addition to sex and age. HR, hazard ratio.

The efficacy of the influenza vaccine has been reported to be higher in fully vaccinated children with two doses than in partially vaccinated children with one dose.[20,21] However, Gruber *et al.*[10] showed that a single dose of TIV produced a sufficient serologic rise to influenza viral antigen, and might protect against viral infection. It should be mentioned that the research by Gruber *et al.*[10] was performed among school-age children, and immunological backgrounds may be different from pre-school children. Because a majority of the vaccinated subjects (93.2%) were fully vaccinated with two doses of TIV, it was not possible to compare the effectiveness between one and two doses in this study.

The influenza incidence was defined as that diagnosed by a pediatrician, although information was not obtained about either cultural confirmation or the subtype of influenza. A report about the sampling study on the cultural confirmation of suspected specimen from clinics in Sapporo City showed that 91.4% of them were the influenza virus.[18] Furthermore, according to surveillance by Sapporo City Hygiene Research Center,[22] endemic of the influenza virus A/H3N2 was observed from the 51st week of 2011 to the 14th week of 2012, and its peak was at the 4th week of 2012. In addition, the spread of the influenza virus B was observed from the 3rd week of 2012 to the 20th week of 2012. The proportion of patients with influenza was reported to be about 71% in influenza A/H3N2 and about 28% in influenza B in the entire 2011–2012 season. We considered that the endemic of influenza in the study population was consistent with endemic of influenza in the entire Sapporo City. In addition, it was reported that the antigenicity of 2011–2012 endemic influenza A (H3N2) and B strains were concordant with those of 2011–2012 vaccine strains in around 60% and 70%, respectively (IASR 33: 288–294, 2012).

Although the amount of influenza vaccine given to children increased in the 2011–2012 season from 0.1 mL to 0.25 mL for those aged 0 years, from 0.2 mL to 0.25 mL for those aged 1–2 years, and from 0.2 mL to 0.5 mL for those aged 3–5 years, it was not possible for us to evaluate the effect of these increments, because the appropriate comparative population could not be obtained. Although we set the initial date of observation at 1 October 2011, the initial date of observation for each subject with or without vaccination is controversial for analysis with the Cox model. Therefore, we applied analysis by the logistic regression model in addition to analysis by the Cox model. As a result, we could obtain the similar risk estimates in association of influenza vaccination with influenza infection between these two analyses (the odds ratios obtained with the logistic regression analysis are not shown in this article).

As a limitation of this study, only 40% of study candidates responded to the request to participate in this study. Accordingly, a selection bias might exist in this study. Ideally the incidence of influenza should be confirmed by observing protocols at every medical institution, or observing records of high fever in every day-care center. However, it was not practical for us to access medical records at all medical institutions or records of high fever at the day-care centers. It was thought that distribution of the day-care centers, size of household, number of siblings, and the number of smokers in home were all potential confounding factors in the association between vaccination and influenza incidence. Especially, different status of influenza endemic was observed in 10 day-care centers as shown in Table 1, and one day-care center showed a significantly increased risk of influenza infection (HR = 2.53, 95%CI 1.48–4.34). However, it was not the case in this study, because HR of TIV on the influenza incidence were not altered even after adjusting all of them, as shown in Table 3.

In conclusion, HR of TIV on the influenza incidence was significantly reduced in the subjects aged 1 year and in the total subjects, but not in the subjects aged 0 years, or 2–6 years. Further studies are necessary in different seasons, places, and populations to clarify the effectiveness of the influenza vaccine in children.

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ORIGINAL ARTICLE

Influenza A(H1N1)pdm09 vaccine effectiveness and other characteristics associated with hospitalization in chronic liver disease patients

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effectiveness – hospitalization – influenza A (H1N1)pdm09 vaccine – liver function – risk factors

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Abstract

Background & Aims: To date, few studies have investigated the clinical effectiveness of influenza vaccine in chronic liver disease patients. The aim of this study was to examine the effectiveness of monovalent inactivated influenza A (H1N1)pdm09 vaccine and other characteristics associated with hospitalization in patients with chronic hepatitis C. *Methods:* We conducted a hospital-based cohort study during influenza A(H1N1)pdm09 pandemic. A total of 408 patients (132 vaccinated, 276 unvaccinated) with detectable HCV-RNA were followed up with respect to any hospitalization using a weekly postal questionnaire. Reported hospitalizations were verified by medical records. *Results:* During the epidemic period, 28 hospitalizations (6 vaccinated, 22 unvaccinated) were observed. After adjustment for potential confounders, vaccination decreased the odds ratio (OR) for hospitalization with marginal significance (OR = 0.43, 95%CI = 0.16–1.17). Besides, positive association with hospitalization was observed in patients with albumin levels <3.5 g/dl (OR = 8.40, 3.66–19.3) and steroid users (OR = 5.58, 0.98–31.7). *Conclusions:* Among patients with chronic hepatitis C, A(H1N1) pdm09 vaccine appeared to have a protective effect against hospitalization. Those patients with a higher risk for hospitalization should be carefully followed during the influenza season, even when vaccinated.

Patients with chronic liver disease are classified as a high-risk group for influenza-related complications (1, 2). Influenza infection can cause hepatic decompensation and hospitalization in patients with advanced liver disease (3, 4). Thus, preventing severe influenza that requires hospitalization has been an important issue in patients with chronic liver disease.

As influenza vaccination is the most effective method for preventing influenza and its complications, the Advisory Committee on Immunization Practices in the USA has recommended annual influenza vaccination for patients with chronic liver disease since 2007 (5). 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 clinical effectiveness of influenza vaccine among patients with chronic liver disease. To the best of our knowledge, there has been only one study on this topic until now. The Korean study indicated that seasonal influenza vaccine decreased the incidence of laboratory-confirmed influenza and associated symptoms in cirrhotic patients (6). However, no studies so far have demonstrated the effectiveness of influenza vaccine to prevent hospitalization in these patients.

Thus, the primary objective of this study was to examine the effectiveness of influenza A(H1N1)pdm09 vaccine in preventing hospitalization among patients with chronic liver disease. Using these data, the other characteristics associated with hospitalization were also assessed as a secondary objective.

Material and methods

Study subjects

In Japan, monovalent inactivated unadjuvanted split-virus influenza A(H1N1)pdm09 vaccine became available for tiered use in October 16, 2009. Vaccination was scheduled first for healthcare workers, pregnant women and then provided to patients with underlying illnesses (including the present study subjects) from November 2009, according to the order of priority of the groups. The present hospital-based cohort study was performed under the constraint of this national vaccination strategy.

Between November 2009 and January 2010 (i.e. recruitment), patients with chronic hepatitis C who had been under clinical follow-up at three medical institutions in Osaka, Japan, were invited to participate in this study. Eligible patients were those with detectable HCV-RNA levels at the time of recruitment, whereas those with a prior episode of influenza A(H1N1)pdm09 virus infection were excluded. A total of 416 subjects who agreed to participate were enrolled. All study subjects provided their written, informed consent after the nature and possible consequences of this study had been explained.

The study protocol was approved by the Ethics Committees at the Osaka City University Faculty of Medicine, Osaka City Juso Hospital and Osaka City General Hospital, and was performed in accordance with the Declaration of Helsinki.

Information collection

Three kinds of data were collected for each subject. Two kinds of data, physical and environmental characteristics, as well as clinical characteristics, were collected for use as baseline data, whereas data regarding subsequent hospitalization were collected weekly in the follow-up survey. Information on the following physical and environmental characteristics was collected using a self-administered questionnaire: status of influenza A (H1N1)pdm09 vaccination and date of vaccination (if vaccinated); sex, age (years), height (cm) and weight (kg); steroid treatment for two or more consecutive weeks within the last 6 months; underlying illnesses other than liver disease (hereinafter referred to as 'other chronic diseases') including diabetes mellitus, chronic heart disease, chronic renal disease, neuromuscular disease, asthma and chronic respiratory disease; smoking and alcohol habits; number of family members; and total room space in the patient's house (m^2).

In addition, information about clinical characteristics was collected using a structured questionnaire that was completed by the physician-in-charge at the time of recruitment. The questionnaire gathered information about: current treatment with interferon; hepatocellular carcinoma; ascites; hepatic encephalopathy; and laboratory data such as platelet count ($\times 10^4/mm^3$), albumin (g/dl) and prothrombin activity (%). Using these data, Child–Pugh Scores were calculated according to the conventional method (7). Child–Pugh Scores of 5 or more were considered to indicate cirrhosis.

With respect to the follow-up survey, the subjects were requested to fill out a weekly postal questionnaire about the following episodes during the preceding week: physician-diagnosed influenza, results of rapid antigen testing, if applicable, and hospitalization. The postal questionnaire was to be returned to the Department of Public Health, Osaka City University Faculty of Medicine each week during the follow-up period, which was between recruitment and the 15th week of 2010 (April 12–18). For subjects who had been vaccinated within 2 weeks before recruitment, to consider the time length required for a sufficient immune response, the follow-up started 2 weeks after vaccination (8). Reported hospitalizations were verified by medical records at three participating hospitals.

Outcome definitions and epidemic

The study outcome was defined as hospitalization that occurred during the epidemic period of influenza A (H1N1)pdm09. The epidemic period was determined using the surveillance data in Osaka Prefecture and was defined as the period in which the weekly number of influenza patients remained at ≥1 per sentinel (9). Based on the epidemic curve (Fig. 1), the epidemic peaked in November (when this study started) and continued to the 7th week of 2010 (February 15–21). All influenza viruses isolated in Osaka Prefecture during this period were influenza A(H1N1)pdm09 virus strains.

Statistical analysis

Baseline characteristics were compared between vaccinated and unvaccinated subjects using the χ^2 test and the Wilcoxon rank sum test. To evaluate the association between baseline characteristics and outcome, univariate and multivariate logistic regression models were employed to obtain crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI).

In constructing a multivariate model, nine variables were selected for inclusion in the initial model, as three variables were distributed differently between vaccinated and unvaccinated subjects ($P < 0.1$) and the remaining variables were considered medically significant in relation to outcomes. Then, the reduced model was constructed, as the initial model included too many variables for the number of outcome events. In this process, variables that had no association with hospitalization in the results of initial models were excluded. Eventually, the final model included the following four variables: vaccination; other chronic diseases; steroid treatment within the last 6 months; and albumin level.

The results were also verified in the subgroup who was not receiving interferon therapy, as subjects

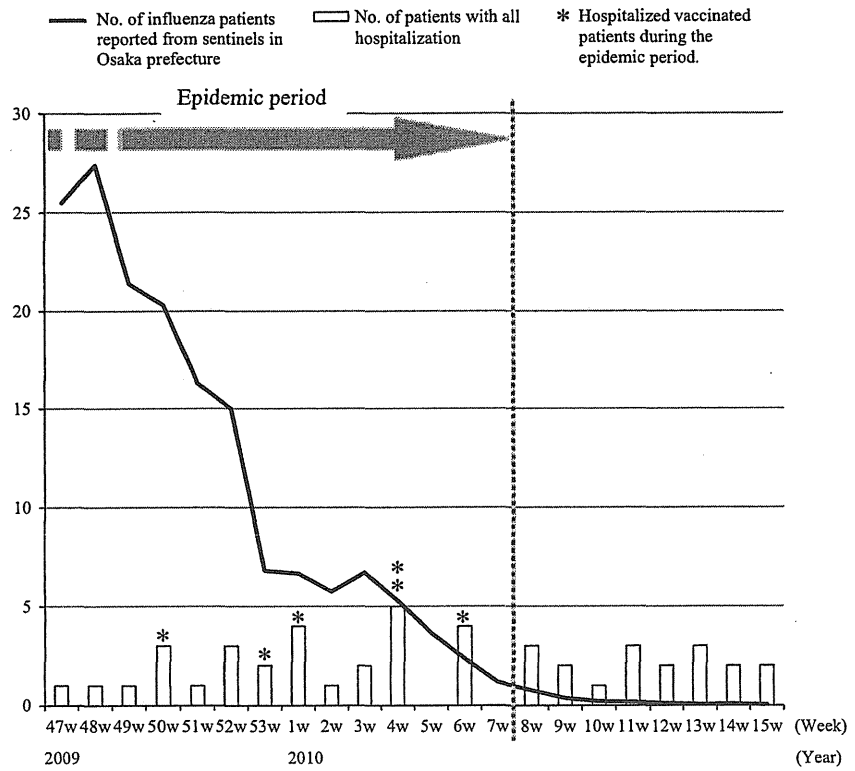

Fig. 1. Data from regional surveillance (line) and from follow-up surveys of study subjects (bars).

receiving interferon therapy were likely to develop influenza-like symptoms because of the side effects of interferon, which might affect the results.

Furthermore, to consider the vaccine effectiveness according to liver function, stratified analysis by platelet counts or albumin levels was also conducted. All tests were two-sided. All analyses were performed using SAS version 9.1.3 software (SAS Institute, Cary, NC, USA).

Results

Baseline characteristics of study subjects

Of the 416 patients with chronic hepatitis C, eight unvaccinated patients (2%) were excluded because of incomplete data in the follow-up surveys. Eventually, data from a total of 408 patients (132 vaccinated, 276 unvaccinated) were analysed.

Table 1 compares baseline characteristics between vaccinated and unvaccinated patients. The vaccinees included a smaller proportion of males (23% vs. 41%, $P < 0.001$), and had less habit of smoking (never smokers: 78% vs. 64%, $P = 0.015$) and alcohol drinking (never-drinkers: 77% vs. 66%, $P = 0.038$). Variables that were thought to be potentially associated with influenza, such as age, body mass index, steroid treatment, other chronic diseases, room space per person, interferon treatment, hepatocellular carcinoma and laboratory data suggesting cirrhosis, were distributed similarly between the vaccinated and unvaccinated patients.

Association of influenza A(H1N1)pdm09 vaccine with hospitalization

Figure 1 shows the distribution of outcome events from the follow-up surveys of study subjects (bars). During the epidemic period (from the 47th week of 2009 to the 7th week of 2010), there were 28 hospitalizations (7%), including 6 vaccinated patients.

Table 2 shows the crude and adjusted ORs of influenza vaccine for hospitalization during the epidemic period. Compared with unvaccinated patients, vaccinated lowered the OR for hospitalization to about half in the crude analysis (OR = 0.55, 95%CI = 0.22–1.39). After adjustment for potential confounders, the decreased OR of vaccination reached the marginally significant level (OR = 0.43, 95%CI = 0.16–1.17). Even in the subjects who were not receiving interferon therapy, both the proportion of outcome events and the ORs of vaccination were almost the same as for the entire study subjects. However, ORs of vaccination somewhat fluctuated according to their liver function, and subjects with better liver disease status (i.e. platelet count $\geq 10.0 \times 10^4/mm^3$ or albumin level ≥ 3.5 g/dl) seemed to be more likely to manifest vaccine effectiveness. Especially in subjects with platelet count $\geq 10.0 \times 10^4/mm^3$, vaccination was associated with a decreased OR for

Table 1. Comparison of baseline characteristics between patients with influenza A(H1N1)pdm09 vaccination and those without vaccination

Characteristics	Category	Vaccinated (n = 132)	Unvaccinated (n = 276)	P*
Sex	Male	31 (23)	112 (41)	<0.01
Age (years)	65.0+	85 (64)	176 (64)	0.90
Body mass index (kg/m^2)	25.0+	20 (15)	49 (18)	0.49
	Data missing		2	
Steroid treatment within the last 6 months	Received	5 (4)	5 (2)	0.22
	Data missing	1		
Underlying illness other than liver disease		57 (43)	106 (39)	0.37
Diabetes mellitus		14 (11)	33 (12)	0.68
Chronic heart disease		11 (8)	15 (5)	0.27
Chronic renal disease		7 (5)	13 (5)	0.80
Neuromuscular disease		7 (5)	10 (4)	0.43
Malignant neoplasm		3 (2)	14 (5)	0.29
Asthma		6 (5)	8 (3)	0.40
Blood dyscrasia		3 (2)	11 (4)	0.37
Others†		8 (6)	20 (7)	0.65
	Data missing		1	
Smoking habit	Never	103 (78)	176 (64)	0.02
	Ever	16 (12)	53 (19)	
	Current	13 (10)	47 (17)	
Alcohol drinking habit	Never	102 (77)	181 (66)	0.04
	Ever	21 (16)	57 (21)	
	Current	9 (7)	38 (14)	
Room space per person (m^2)	Mean (SD)	43.2 (25.9)	40.8 (26.8)	0.37
	Unknown	3	6	
Clinical characteristics at the time of recruitment				
Interferon treatment	Receiving	40 (30)	105 (38)	0.12
	Data missing		1	
Hepatocellular carcinoma	Present	26 (20)	56 (21)	0.82
	Data missing		5	
Laboratory data				
Platelet count ($\times 10^4$/mm^3)	<10.0	42 (32)	89 (32)	0.89
	Data missing		2	
Albumin level (g/dl)	<3.5	22 (17)	41 (15)	0.61
	Data missing	1		
Prothrombin activity (%)	<80	19 (18)	23 (13)	0.35
	Data missing	24	105	
Child–Pugh score	5+	43 (40)	72 (42)	0.68
	A (5–6)	37 (34)	62 (36)	0.80
	B (7–9)	5 (5)	10 (6)	
	C (10+)	1 (1)	0 (0)	
	Data missing	24	106	

SD, standard deviation. Data expressed as n (%) unless otherwise indicated.
*The χ^2 test or Wilcoxon rank sum test was employed where appropriate.
†Others included 11 atopic disease, 7 pregnancy, 5 collagen disease, 4 cerebrovascular disease, 3 chronic respiratory disease and 1 immunosuppressive disease.

hospitalization with marginal significance (OR = 0.19, 95%CI = 0.03–1.22).

Association of other clinical variables with hospitalization

Table 3 shows the association of other baseline characteristics with hospitalization during the epidemic period. Patients with other chronic diseases had about a two-fold increased OR for hospitalization with marginal significance in the crude analysis (crude OR = 2.10, 95%CI = 0.97–4.57). After adjustment for potential confounders, however, the increased OR was not significant. Instead, OR of steroid use showed a marginal association with hospitalization (adjusted OR = 5.58, 95% CI = 0.98–31.7). In addition, patients with a lower albumin level had significantly increased ORs for hospitalization both in the crude and adjusted analyses (adjusted OR = 8.40, 95%CI = 3.66–19.3).

Other liver function markers were also investigated by incorporating them into the model instead of the albumin level, as the positive association between a lower albumin level and hospitalization seemed to represent an

Table 2. Association of influenza A(H1N1)pdm09 vaccine with hospitalization during the epidemic period, according to the selected clinical condition subgroup: crude and adjusted analyses

Stratified category	Vaccination status	*N*	*n* (%)	Crude OR (95%CI)	Adjusted* OR (95%CI)
Entire study subjects	Unvaccinated	276	22 (8)	1.00	1.00
	Vaccinated	132	6 (5)	0.55 (0.22–1.39)	0.43 (0.16–1.17)
Interferon therapy					
Not receiving	Unvaccinated	170	15 (9)	1.00	1.00
	Vaccinated	92	5 (5)	0.59 (0.21–1.69)	0.43 (0.14–1.35)
Receiving	Unvaccinated	105	7 (7)	1.00	1.00
	Vaccinated	40	1 (3)	0.36 (0.04–3.01)	0.40 (0.04–3.87)
Platelet count ($\times 10^4/mm^3$)					
≥ 10.0	Unvaccinated	185	12 (6)	1.00	1.00
	Vaccinated	90	2 (2)	0.33 (0.07–1.50)	0.19 (0.03–1.22)
<10.0	Unvaccinated	89	10 (11)	1.00	1.00
	Vaccinated	42	4 (10)	0.83 (0.25–2.82)	0.75 (0.21–2.66)
Albumin level (g/dl)					
≥3.5	Unvaccinated	235	13 (6)	NA	NA
	Vaccinated	109	0 (0)		
<3.5	Unvaccinated	41	9 (22)	1.00	1.00
	Vaccinated	22	6 (27)	1.33 (0.40–4.40)	1.13 (0.32–4.00)

OR, odds ratio; CI, confidence interval; NA, not applicable.
*Model includes underlying illnesses other than liver disease, steroid treatment within the last 6 months and albumin level, other than the stratified variable.

Table 3. Association of influenza A(H1N1)pdm09 vaccine and other baseline characteristics with hospitalization during the epidemic period: crude and adjusted analyses

Baseline characteristics	*n* (%)	Crude OR (95%CI)	Adjusted* OR (95%CI)
Vaccination status			
Unvaccinated	22 (8)	1.00	1.00
Vaccinated	6 (5)	0.55 (0.22–1.39)	0.43 (0.16–1.17)
Underlying illness other than liver disease			
Absent	12 (5)	1.00	1.00
Present	16 (10)	2.10 (0.97–4.57)	1.82 (0.80–4.14)
Steroid treatment within the last 6 months			
Not received	26 (7)	1.00	1.00
Received	2 (20)	3.57 (0.72–17.7)	5.58 (0.98–31.7)
Albumin level (g/dl)			
<3.5	15 (24)	7.96 (3.57–17.7)	8.40 (3.66–19.3)
3.5+	13 (4)	1.00	1.00

OR, odds ratio; CI, confidence interval; NA, not applicable.
*Model includes all variables in this table.

association with advanced liver disease. The adjusted ORs for hospitalization of any liver function markers were also increased: platelet count <10.0 × $10^4/mm^3$ (OR = 2.10, 95%CI = 0.96–4.60), prothrombin activity <80% (OR = 4.32, 95%CI = 1.69–11.1), Child–Pugh Score of 5 or more (OR = 3.51, 95%CI = 1.38–8.92) and hepatocellular carcinoma (OR = 3.09, 95%CI = 1.38–6.91).

Discussion

In this study among patients with chronic hepatitis C, there was an indication of vaccine effectiveness for preventing severe outcomes requiring hospitalization during an epidemic. Although the limited number of outcome events made it difficult to detect significant vaccine effectiveness, the present results support the usefulness of influenza vaccine for patients with chronic hepatitis C.

To date, no study has reported the effectiveness of influenza A(H1N1)pdm09 vaccine against hospitalization in patients with specific underlying medical conditions including chronic liver disease. However, based on the reports about vaccine effectiveness among subjects with any high-risk condition, a cohort study in Denmark showed that vaccine conferred protection against influenza-related hospitalization to 44% (−19–73%) among subjects <65 years with underlying illnesses (10). A matched case–control study in the Netherlands indicated that the vaccine effectiveness for influenza-related hospitalization was 19% (−28–49%) among subjects with

high-risk conditions (11). Although these studies did not refer to vaccine effectiveness in patients with individual underlying illnesses, the present results among patients with chronic hepatitis C would correspond to those in subjects with any high-risk conditions.

Influenza infection occasionally causes hepatic decompensation without typical influenza symptoms in patients with chronic liver disease (4, 6), which might bring about delayed antiviral therapy and increase influenza-related mortality. Thus, it was an important finding that influenza vaccine had some effect for reducing hospitalization during the epidemic period, although the present results were not significant. According to the previous studies, vaccination for cirrhotic patients lowered the incidence of laboratory-confirmed influenza and atypical influenza symptoms such as myalgia, hepatic decompensation, oliguria and uncontrolled ascites during influenza season (6). Furthermore, some reports have indicated that influenza vaccine was sufficiently immunogenic in patients with cirrhosis (12–15). Taken together, it would be reasonable to advise vaccination for patients with chronic liver disease. In fact, the Advisory Committee on Immunization Practices in the USA has recommended annual influenza vaccination for patients with chronic liver disease since 2007 (5), and the WHO position paper has indicated that patients with specific chronic medical conditions continue to be an appropriate target group for annual influenza vaccination (16).

In this study, however, subjects with advanced liver disease (represented by lower albumin level) had a higher risk for hospitalization during the epidemic period, irrespective of their vaccination status (Table 3). These results corresponded to a previous case report in which influenza infection caused hepatic decompensation and hospitalization in patients with advanced liver disease (4). Influenza virus itself could cause hepatitis (17), and influenza infection could induce toxic metabolites and proinflammatory cytokines such as TNF-α, IL-1 and IL-6, which contribute to hepatic damage (18, 19). These seemed to result in disease deterioration, especially in patients with advanced liver disease. Thus, it would be better for subjects with advanced liver disease to be followed with special attention during the season, even when vaccinated.

In addition, steroid treatment and the presence of other chronic diseases were related to hospitalization during the epidemic period, independent of vaccination status or liver function. Steroid treatment and the presence of chronic diseases have been the known high-risk factors for influenza and its complications (5). In the 2009 influenza pandemic, immunosuppressive therapy and chronic diseases (especially asthma) were among the highest comorbid conditions in critically ill patients with influenza A(H1N1)pdm09 infection in the USA (20), Canada (21), Australia (22) and Mexico (23). The present results agreed with these findings. Patients on immunosuppressive therapy have impaired vaccine responses (24), and patients with asthma are expected to have similar vaccine responses, as they often receive steroid treatment. These backgrounds of poor immunological responses might bring about the high sensitivity for influenza infection and severe outcomes owing to influenza.

When interpreting the present results, however, the following limitations should be considered. Firstly, the insufficient statistical power owing to the small sample size and the limited number of outcome events is obviously important. This limitation made it difficult to detect significant vaccine effectiveness. If more subjects could be recruited, more meaningful results would be obtained. However, studies on pandemic influenza vaccine must be conducted under strict time constraints, as pandemic influenza virus had circulated and pandemic influenza vaccines became available during the epidemic. In addition, the epidemic subsided after sufficient distribution of the vaccines. This tight time schedule represented a major obstacle to recruiting a sufficient number of vaccinated and unvaccinated subjects for any observational prospective cohort study.

Secondly, voluntary enrolment in the observational study might lead to selection bias in the vaccination status. In fact, female patients, non-smokers and non-drinkers tended to receive vaccination in this study, which might lead to a healthy vaccinee effect. However, even when additional analyses that adjusted for these variables were conducted, similar results were obtained (ORs of vaccination were 0.45 (95%CI = 0.16–1.26). On the other hand, the determination of vaccination status relied on patients' self-reports and could not be confirmed by their medical records, as patients usually received any vaccination in their neighbouring clinic. Thus, some non-differential misclassification in the vaccination status might have occurred.

Thirdly, there might be some concern about outcome misclassification, as hospitalization is a less specific outcome for influenza. In this study, however, the methods in which outcomes were confined into the epidemic period would have helped to minimize outcome misclassification and obtain a higher specificity of influenza for hospitalization. Furthermore, hospitalization is essentially considered an objective outcome that can be verified by the medical records, and therefore misclassification owing to non-influenza illness, if any, would be non-differential between vaccinated and unvaccinated patients (25). Such misclassification leads to an underestimation of vaccine effectiveness and does not materially affect the validity of the results.

Finally, previous immunity in unvaccinated patients might affect the underestimation of vaccine effectiveness to some extent. Based on a serological study, about one-third of subjects aged ≥65 years was reported to have pre-existing antibody before the epidemic, as many had been exposed to antigens similar to influenza A(H1N1) pdm09 virus during childhood (26). In this study, however, although about two-thirds of subjects were ≥65 years old, the proportion of subjects with pre-existing

antibody was expected to be lower than in previous studies, because the immunogenicity study of influenza A(H1N1)pdm09 vaccine, in which part of this study subjects participated, indicated that only about 5% of subjects had the pre-existing antibody at the beginning of the pandemic (12). Thus, the effect of previous immunity, if any, would be very minimal.

In conclusion, among patients with chronic hepatitis C, influenza A(H1N1)pdm09 vaccine was suggested to have some protective effect against hospitalization during the epidemic period. As patients with advanced liver disease, steroid treatment and other chronic diseases (especially asthma) are considered to be at higher risk for hospitalization during the epidemic period, they should be followed up with special attention during the season, even when vaccinated.

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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); prevaccination 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 ($P < 0·01$). 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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