RESEARCH PAPER

Human Vaccines & Immunotherapeutics 10:8, 2387–2394; August 2014; © 2014 Landes Bioscience

Immunogenicity of a monovalent influenza A(H1N1)pdm09 vaccine in patients with hematological malignancies

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Keywords: influenza vaccine, immunogenicity, reactogenicity, hematological malignancy, rituximab

Abbreviations: MFR, mean fold rise; GMT, geometric mean titer; sR, seroresponse proportion; sP, seroprotection proportion; HI, hemagglutination inhibition; sC, seroconversion proportion; EMA, European Medicines Agency; ORS, oculorespiratory syndrome; MDS, myelodysplatic syndrome; OR, odds ratios ; 95% CI, 95% confidence intervals

Patients with hematological malignancies have high risk for morbidity and mortality from influenza. This study was conducted to evaluate the immunogenicity and reactogenicity of an influenza A(H1N1)pdm09 vaccine among such subjects. Fifty subjects were vaccinated twice during the 2009–2010 season. The antibody response was expressed in terms of mean fold rise (MFR) of geometric mean titer, seroresponse proportion (sR), and seroprotection proportion (sP). The first vaccination induced only a small response, and additional antibody was acquired after the second dose (MFR 2.3 and 3.9, sR 32% and 54%, and sP 30% and 48% after the first and the second vaccination, respectively). Rituximab treatment showed an especially inhibitory effect (MFR 1.3, sR 9% and sP 0%). When analyzed using logistic regression models, only rituximab was found to have an independent effect; the adjusted odds ratio for sR was 0.09 ($P = 0.05$). Influenza vaccination of patients with hematological malignancies resulted in adepuate response, and the second vaccination induced additional antibody. It is therefore recommended to vaccinate this group twice.

Introduction

The United States Advisory Committee on Immunization Practices (US ACIP) recommends annual influenza vaccination for immunocompromised patients.[1-3] Patients with hematological malignancies have reduced immune response and therefore are at high risk for morbidity and mortality due to influenza.[4] It is said that their treatment with cytotoxic chemotherapy drugs induced a reduction of humoral response and led to increased susceptibility to infectious disease.[5-7] In fact, high mortality and morbidity of this population due to an influenza virus was reported.[8] On the other hand, it is unclear whether the underlying disease causes this lowered response. Thus more studies are required to evaluate the effectiveness of influenza vaccine in such patients and to protect this group from influenza. However there have been only a limited number of reports and they showed conflicting data.[9-16]

In March 2009, a novel influenza A(H1N1) virus was reported in North America.[17-19] This virus spread globally and brought about the 2009 influenza pandemic.[20-22] Because this virus was novel for human beings, we got an exceptional opportunity to study the immunogenicity of an influenza vaccine in a naive population. The objective of this study was to assess the immunogenicity and reactogenicity of a monovalent A(H1N1)pdm09 influenza vaccine in patients with hematological malignancies.

Results

Table 1 shows characteristics of the study subjects. The median age was 59 (range 21–83), and 48% of them were 60 or older. 40% of the subjects were males, 92% had pre-titer < 1:10, and 2 subjects had pre-titer ≥ 1:40 (1:40 and 1:80). Lymphoma was the most common underlying disease (42%). All lymphoma patients had non-Hodgkin's disease, and there was no patient with Hodgkin's disease. Steroid was the most frequently used chemotherapeutic agent (58%). Rituximab was being used on 11 (22%) patients only, but nearly half (48%) of the lymphoma patients were receiving rituximab.

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Submitted: 02/21/2014; Revised: 04/23/2014; Accepted: 05/02/2014; Published Online: 05/08/2014;
http://dx.doi.org/10.4161/hv.29094

Table 1. Characteristics of patients with hematological malignancy

Characteristics	Patients (n = 50)	
Age Median (range)	59	(21–83)
Gender Male	20	(40)
Prevaccination titer < 1:10 1:10–1:20 ≥ 1:40	46 2 2	(92) (4) (4)
Underlying disease Lymphoma Acute Leukemia Myeloma MDS[a] Aplastic anemia Other [b]	21 14 8 3 2 2	(42) (28) (16) (6) (4) (4)
Chemotherapy [c] Steroid Immunosuppressive agent Anticancer agent [d] Rituximab	29 6 16 11	(58) (12) (32) (22)
Chemotherapy by Underlying disease Lymphoma Steroid Immunosuppressive agent Anticancer agent [d] Rituximab Acute leukemia Steroid Immunosuppressive agent Anticancer agent [d] Rituximab Myeloma Steroid	21 15 2 13 10 14 8 3 2 1 8 4	(100) (71) (10) (62) (48) (100) (57) (21) (14) (7) (100) (50)

Note: Number in parentheses is expressed as percentage if not otherwise specified; [a]MDS (myelodysplastic syndrome);[b]Other includes chronic myelogenous leukemia and myelofibrosis; [c]Each treatment is not mutually exclusive; [d]Anticancer agents does not include rituximab;

Immunogenicity of the vaccine is summarized in **Table 2**. In the entire sample, GMT did not reach the protective level (≥ 1:40) even after the second vaccination (S2 = 1:22). MFR reached 2.3 after the first and 3.9 after the second vaccination. sR became 32% and 54% and sP became 27% and 46% after the first and the second vaccination, respectively Females had 1/3 or less the GMT of males both after the first and the second vaccinations with clearly lower MFR, sR and sP. In the two categories with pre-titer ≥ 1:10 there was almost no increase in MFR, and both sR and sP were 0%. Patients with lymphoma, acute leukemia, or myeloma as the underlying disease showed significant increase in MFR after the second vaccination, but they differed in the extent of this increase (MFR of 2.0 for lymphoma, 4.6 for acute leukemia and 9.5 for myeloma). Similarly, the sR (S1/S0 = 10% and S2/S0 = 33%) and sP (S1 = 10% and S2 = 19%) were significantly low in lymphoma patients compared with patients with other underlying diseases. The values of various parameters of categories of patients who were under different types of chemotherapy were generally lower than those of the entire sample, irrespective of the drug used. The values were particularly low with rituximab, MFR, sR and sP being 1.3, 9% and 0% respectively after the second vaccination.

MFR after the second vaccination was 3.9 for the entire sample, which satisfied the EMA criterion (2.5). The two categories with pre-titer ≥ 1:10 both had sR 0% after the first and the second vaccinations, and sC also became 0%. Because of this, sC for the entire sample was 26% (13/50) after the first vaccination and 44% (22/50) after the second vaccination. Thus, as with MFR, the vaccine satisfied the EMA criterion (40%) for sC also for the entire sample.

Table 3 shows the results of logistic regression analysis of sR (MFR S2/S0 ≥ 4) after the second vaccination. In the univariate analysis, significantly reduced ORs were seen for lymphoma (P = 0.01), steroid (P = 0.02), anticancer agents (P = 0.02) and rituximab (P = 0.01), and there was marginal significance (P = 0.09) for gender. In the multivariate analysis of model 1, where age, gender and underlying disease were included, the OR for lymphoma showed marginal significance (P = 0.06), but there was no significant reduction in the OR for gender (P = 0.50). In model 2, where gender was not taken into account, the OR for lymphoma showed statistical significance (OR = 0.08, 95% CI = 0.01–0.95). In model 3, where age and chemotherapy were included, statistical significance was seen only for rituximab (OR = 0.08, 95% CI = 0.01–0.86). Finally, in model 4, which included age, lymphoma and rituximab, only rituximab showed significance, that too only marginal (OR = 0.09, 95% CI = 0.01–1.04). Lymphoma did not have significant effect (OR = 0.43, 95% CI = 0.08–2.18).

Table 4 shows the results for sP after the second vaccination (S2 ≥ 1:40). Significantly reduced ORs were seen in the univariate analysis for gender (P = 0.03), lymphoma (P = 0.01) and anticancer agents (P = 0.05), and there was marginal significance (P = 0.09) for myeloma. The antibody titer did not reach the seroprotective level in any of the patients under rituximab treatment. Therefore we could not include rituximab in the model. In the multivariate model 1, which included age, gender and underlying disease, the OR for lymphoma maintained a significance (P = 0.04) and the OR for gender was not significant (P = 0.32). In model 2, where gender was not taken into account, OR for lymphoma showed even greater decrease (OR = 0.07, 95% CI = 0.01–0.76). In model 3, where age and chemotherapy were included, no variable showed statistically significant OR. Finally in model 4, which included age, lymphoma and chemotherapy, only lymphoma showed a significant decrease in OR (OR = 0.10, 95% CI = 0.02–0.58).

We examined the associations among these explanatory variables by calculating Cramer's V. Gender and lymphoma had Cramer's V of 0.42 (P < 0.01). In fact, a higher proportion of females than males had lymphoma (3/20 males and 18/30 females). In the univariate analysis, females showed a lower OR because of this skew. Cramer's V was 0.53 (P < 0.001) between lymphoma and rituximab. When the frequency of rituximab treatment was compared between lymphoma and non-lymphoma patients, it was seen that mostly lymphoma patients had received the treatment (10/21 lymphoma patients and 1/29 non-lymphoma

Table 2. Immunogenicity of monovalent 2009 influenza A (H1N1) vaccine on hematological malignancy patients

		GMT					MFR						sR						sP					
													S1/S0 ≥ 4			S2/S0 ≥ 4			S1 ≥ 1:40			S2 ≥ 1:40		
Category	N	S0		S1		S2 n	S1/S0 (%) n			S2/S0 (%) n			(%)		n	(%)								
Entire sample	50	6		13		22	2.3			3.9			16	(32)		27	(54)		13	(27)		22	(46)	
Age 21–59 60–83	27 23	6 5		16 11		27 18	2.3 2.4	* *		4.3 3.2	* *		9 7	(33) (30)		15 12	(56) (52)		8 5	(32) (22)		12 10	(48) (44)	
Gender Male Female	20 30	7 5		26 8	†	43 14	†	4.0 1.6	* *	†	6.5 2.8	* *	†	10 6	(50) (20)	**	13 14	(65) (47)		8 5	(44) (17)	**	12 10	(67) (33)	**
Prevaccination titer < 1:10 1:10–1:20 ≥ 1:40	46 2 2	5 10 57	†	13 7 57		22 10 80	2.5 0.7 1.0	*	†	4.4 1.0 1.4	*		16 0 0	(35)		27 0 0	(59)		13 0 -	(28)		22 0 -	(48)	
Underlying disease Lymphoma Acute leukemia Myeloma MDS [a] Aplastic anemia Other [b]	21 14 8 3 2 2	5 6 5 5 20 5	†	7 13 20 40 20 320	†	10 30 48 40 40 160	†	1.4 2.0 4.0 8.0 1.0 64.0	* *	†	2.0 4.6 9.5 8.0 2.0 32.0	* * *	†	2 5 4 3 0 2	(10) (36) (50) (100) (100)	**	7 9 6 3 0 2	(33) (64) (75) (100) (100)	**	2 3 4 2 0 2	(10) (23) (50) (67) (100)	**	4 8 6 2 0 2	(19) (62) (75) (67) (100)	**
Chemotherapy [c] Steroid Immunosuppressive agent Anticancer agent [d] Rituximab	29 6 16 11	6 8 5 6		10 13 8 6	† †	18 25 11 8	† †	1.7 1.6 1.5 1.0	*	† † †	2.9 3.2 2.1 1.3	* *	† † †	6 1 2 0	(21) (17) (13)	‡ ‡	11 2 5 1	(38) (33) (31) (9)	‡ ‡ ‡	5 1 2 0	(19) (20) (13)		10 2 4 0	(37) (40) (25)	

Note: GMT (geometric mean titer); MFR (mean fold rise); sR (seroresponse proportion); sP (seroprotection proportion), those with S0 ≥ 1:40 were excluded; [a]MDS (myelodysplastic syndrome); [b]Other includes chronic myelogenous leukemia and myelofibrosis; [c]Each treatment is not mutually exclusive and comparisons were conducted with absent group; [d]Anticancer agents does not include rituximub;* $P < 0.05$ in Wilcoxon signed rank test for intra-category comparisons; † $P < 0.05$ in Wilcoxon rank sum test or Kruskal-Wallis test for inter-category comparisons; ** $P < 0.05$ in $\chi 2$ test; ‡ $P < 0.05$ in $\chi 2$ test (compared with patients who were not administrated corresponding chemotherapeutics).

Table 3. Association between selected characteristics and SeroResponse proportion (S0 to S2) (n = 46)

			Crude		Multivariate model 1[a]		Multivariate model 2[b]		Multivariate model 3[c]		Multivariate model 4[d]	
Category		sR (%)	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age	60–83/36–59	52/60	0.73 (0.23–2.28)	0.59	1.58 (0.37–6.74)	0.53	1.52 (0.37–6.37)	0.56	0.71 (0.18–2.80)	0.63	0.96 (0.22–4.15)	0.95
Gender	Female/ Male	47/72	0.34 (0.10–1.18)	0.09	0.60 (0.14–2.63)	0.50						
Underlying disease Lymphoma Acute leukemia Myeloma	 +/- +/- +/–	 33/74 69/51 75/53	 0.18(0.05–0.61) 2.13 (0.55–8.21) 2.71 (0.49–15.1)	 0.01 0.27 0.25	 0.09 (0.01–1.12) 0.40 (0.03–4.83) 0.39 (0.02–6.96)	 0.06 0.47 0.52	 0.08 (0.01–0.95) 0.40 (0.03–4.76) 0.47 (0.03–7.71)	 0.05 0.47 0.59			 0.43 (0.08–2.18)	 0.31
Chemotherapy Steroid Immunosuppressive Agents Anticancer agents* Rituximab	 +/- +/- +/–	 41/76 40/58 30/69 10/68	 0.22 (0.06–0.76) 0.48 (0.07–3.17) 0.21 (0.06–0.75) 0.05 (0.01–0.45)	 0.02 0.45 0.02 0.01					 0.75 (0.11–4.93) 0.42 (0.04–4.49) 0.56 (0.08–3.84) 0.08 (0.01–0.86)	 0.76 0.47 0.55 0.04	 0.09 (0.01–1.04)	 0.05

Logistic regression model. CI: confidence interval; OR: odds ratio; [a]Model include age, sex, lymphoma, acute leukemia, and myeloma; [b]Model include age, lymphoma, acute leukemia and myeloma. [c]Model include age, steroid, immunosuppressive agents, anticancer agents and rituximab; [d]Model include age, lymphoma and rituximab; *Anticancer agents does not include rituximab.

patients). This strongly suggested that lymphoma maintained significant association in model 4 of **Table** 4 because this model did not include rituximab.

Table 5 shows the proportion of subjects who had adverse events. No mortality or serious adverse event was reported. Only 2 patients reported adverse events after the first vaccination. One patient (2%) had a systemic reaction while another (2%) had a localized reaction. No patient reported symptoms of ORS. On the other hand, this syndrome was reported after the second vaccination by 4 patients (8%). After the second vaccination, systemic reaction was reported by 12 (24%) patients and localized reaction by 10 (20%). However, all the adverse events were of grade 1.

Discussion

In the present study, we gave two vaccinations of an influenza A(H1N1)pdm vaccine to patients with hematological malignancies. The immunological indices of the subjects were considerably lower (MFR 3.9 times, sR 54% and sP 46%) than in healthy adults.[23-29] There have been quite a few reports about the low immunogenicity of influenza vaccines in patients with hematological malignancies.[30-38] The results obtained by use here agree with their findings. In healthy adults usually the induction of antibody reaches a plateau after one vaccination.[23-29] However in patients with hematological malignancies antibodies were found to be induced further even after the second vaccination. We recommend two vaccinations for such patients as an additional effect can be expected from the second vaccination. Very recent research on patients of lymphoid tumors has also revealed an additional effect of the second vaccination, and the authors of those studies have also recommended vaccinating such patients twice.[39,40]

Comparison of various immunological indices in patients stratified for various characteristics showed that the indices were low in females, lymphoma patients, and those treated with steroids, anticancer agents or rituximab (**Table 2**). The values were particularly low with rituximab, with only one patient showing a 4-fold increase in antibody titer even after two vaccinations, and none reaching titer ≥ 1:40. Recent studies have also shown the low immunogenicity of influenza vaccines in lymphoma patients and patients on rituximab treatment.[30-43] The results obtained by us here are in agreement with those findings. Subjects having pre-titer ≥ 1:10 did not show any increase in antibody response after the second vaccination. This may have happened by chance, because such subjects were very few. Larger studies are needed to confirm this.

In the present study we performed multivariate logistic analysis only with subjects having pre-titer < 1:10 in order to eliminate the effect of pre-titer level on antibody induction. Pre-titer is an important factor in immune response and this calls for special

Table 4. Association between selected characteristics and SeroProtection proportion (after S2) (n = 46)

			Crude		Multivariate model 1[a]		Multivariate model 2[b]		Multivariate model 3[c]		Multivariate model 4[d]	
Category		sR (%)	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age	60–83/36–59	44/48	0.83 (0.27–2.60)	0.75	2.14 (0.42–10.8)	0.36	1.98 (0.41–9.60)	0.40	0.93 (0.28–3.11)	0.91	2.02 (0.44–9.26)	0.37
Gender	Female/ Male	33/67	0.25 (0.07–0.86)	0.03	0.48 (0.11–2.06)	0.32						
Underlying disease Lymphoma Acute leukemia Myeloma	 +/- +/- +/–	 19/68 62/40 75/40	0.12 (0.03–0.46) 2.40 (0.65–8.86) 4.50 (0.81–25.2)	 0.01 0.19 0.09	0.09 (0.01–0.94) 0.66 (0.08–5.46) 0.78 (0.06–10.4)	 0.04 0.70 0.85	0.07 (0.01–0.76) 0.66 (0.08–5.27) 1.01 (0.08–12.2)	 0.03 0.69 1.00			0.10 (0.02–0.58)	0.01
Chemotherapy Steroid Immunosuppressive Agents Anticancer agents* Rituximab	 +/- +/- +/–	 37/57 40/47 25/56 0/58	0.44 (0.14–1.41) 0.77 (0.12–5.06) 0.26 (0.07–0.98) NA	 0.17 0.78 0.05					1.11 (0.20–6.08) 0.57 (0.06–5.51) 0.23 (0.04–1.43)	 0.91 0.63 0.12	0.69 (0.10–4.71) 0.88 (0.08–9.99) 0.96 (0.10–9.12)	 0.70 0.92 0.97

Logistic regression model. CI: confidence interval; OR: odds ratio; [a]Model include age, sex, lymphoma, acute leukemia, and myeloma; [b]Model include age, lymphoma, acute leukemia and myeloma; [c]Model include age, steroid, immunosuppressive agents and anticancer agents; [d]Model include age, lymphoma and anticancer agents;*Anticancer agents does not include rituximab.

care in the analysis of the data.[44,45] Here we used a vaccine against the A(H1N1)pdm09 influenza strain, which is a novel strain to which most people had not been exposed. Therefore, very few subjects (n = 4) had the antibody (titer ≥ 1:10) before the vaccination. Exclusion of these subjects from the analysis did not have a major effect.

Gender-stratified ORs, which showed significance in univariate analysis, lost their significance in the multivariate analysis of sR and sP when lymphoma was simultaneously included in the analysis, the ORs nearly reaching the value of 1 (**Tables 3 and 4**). On the other hand, the ORs for sR and sP adjusted for lymphoma which simultaneously took the gender also into account showed strong reduction. This suggested that the significance of the gender-stratified ORs seen here was because of association with lymphoma. Among the chemotherapies, only the adjusted OR for rituximab showed significance (**Table 3**), and there was an association between rituximab treatment and lymphoma. As with gender there was the possibility of the effect being due to the association with lymphoma. We therefore simultaneously adjusted for rituximab and lymphoma in the final model. As a result, lymphoma's OR for sR became close to 1 whereas rituximab's OR remained about the same. This suggests that not lymphoma but rituximab was blocking the immune response. The inhibitory effect of rituximab on antibody induction has been reported by many, but we believe that ours is the first report that demonstrates this effect by eliminating the effect of the underlying disease (lymphoma), through multivariate logistic regression analysis.[19,39-42] There is a possibility that the observed effect was related to the fact that the multivariate logistic regression analysis did not include Hodgkin's lymphoma. The Japanese population has fewer patients of Hodgkin's lymphoma than other populations, and our patients also did not have this disease. Future studies with other racial population are required to resolve this.

Rituximab is a monoclonal antibody that specifically recognizes the CD20 antigen and induces phagocytosis of B cells.[46,47] The CD20 antigen is expressed on malignant B cells and also on mature B cells.[48,49] Therefore, administration of rituximab causes destruction of malignant B cells as well as mature B cells, and persons under rituximab treatment show depletion of B cells. This type of B cell depletion can persist for long periods of time. It has been reported that even patients who had been under complete remission for long (≥ 6 mo) had low ability to induce antibodies against influenza vaccines.[41,50] After receiving rituximab treatment such patients do not attain the optimum antibody titer through influenza vaccination for a long time. Therefore, we need to inform people who come into close contact with such patients to get vaccinated for influenza and to adopt other measures to prevent the spread of the infection to them.

There was no report of mortality or any serious adverse events after the vaccination in the present study. All the adverse events

Table 5. Reactgenicity of patients with hematological malignancy

	after first vaccination		after second vaccination	
Oculorespiratory syndrome [a]	0		4	(8)
Any	0		2	(4)
Red eyes	0		1	(2)
Facial edema	0		3	(6)
Respiratory symptoms				
Systematic reactions [b]	1		12	(24)
Any	1		3	(6)
Feaver (> 37 °C)	0	(2)	8	(16)
Malaise	0	(2)	5	(10)
Myalgia	0		8	(16)
Headache	0		1	(2)
Rash				
Local reactions [b]	1		10	(20)
Any	0		7	(14)
Redness	0	(2)	3	(6)
Swelling	1	(2)	3	(6)
Induration	1	(2)	5	(10)
Itching	0		1	(2)
Pain				

Note: Number in parentheses is expressed as percentage; [a]symptoms within 24 h after vaccination; [b]symptoms within 48 h after vaccination.

reported were common ones and of grade 1, suggesting that the vaccine was tolerated well.

In this study, we have shown adequate immunogenicity and good tolerance of the A(H1N1)pdm09 vaccine and demonstrated the effect of two dose vaccination of immunocompromised patients by using epidemiological methods. Unfortunately, our multivariate model suggested that rituximab treatment had an inhibitory effect on the immune response.

This study was conducted at the time of the influenza pandemic season. The data of immunogenicity and reactogenicity of the vaccine was required urgently. So study subjects were limited. Thus we could not include pre-vaccination immune function and pre-existing medical conditions other than malignancies in the analysis. Our present limited study yielded the aforesaid results. Future multicenter studies may provide more compelling evidence.

To sum up, various antibody indices measured after a second vaccination with an influenza A(H1N1)pdm09 vaccine were adequate in patients of hematological malignancies, and all the EMA criteria were satisfied. Furthermore because the second vaccination showed an additional effect, we recommend that such patients be vaccinated twice. Multivariate logistic analysis showed that rituximab interfered with immunogenicity of the influenza vaccine. Thus it is necessary to pay attention to the fact that vaccination of patients under rituximab treatment could possibly result in failure to achieve the required antibody levels.

Materials and Methods

Subjects

We recruited 50 patients with hematological malignancies from St. Mary's hospital in Fukuoka, Japan during October 2009. In Japan, a pdm09 strain was first reported in May, and the epidemic reached its peak in November. A similar trend was observed at the study location. Exclusion criteria were post-partial remission malignancy, fever of over 38 °C, history of past vaccination allergy, known allergy to egg products, and bleeding tendency due to DIC. This study was approved by the ethics review committees of the Osaka City University, St. Mary's College and St. Mary's hospital. Written informed consents were obtained from the patients or their guardians.

Vaccination and HI assay

The monovalent unadjuvanted inactive A(H1N1) pdm09 split-virus vaccine (Lot.HP01A: BIKEN, Osaka, Japan) contains 30μg/mL of hemagglutinin [A/California/7/2009 (H1N1)]. 0.5 ml of the vaccine was administered subcutaneously twice, 4 wk apart. Blood samples were drawn at baseline (S0), 4 wk after each of the first vaccination (S1) and the second vaccination (S2). All serum samples were stored at -80 °C until used. Hemagglutination inhibition (HI) assay was conducted as described previously.[51]

Information collection

Information about underlying disease (disease name) and chemotherapy (whether administered and duration) was obtained from medical charts. Frequency and severity of adverse events were examined using self-administrated questionnaires on oculorespiratory syndrome (ORS) (within 24h) and systemic reactions and local reactions (within 48 h).[3] All adverse events were graded as follows: grade 1 (present but not interfere with dairy activities), grade 2 (moderate) and grade 3 (prevents daily activities).[19] We also collected information about ORS, because it has been reported occasionally within 24 h after seasonal trivalent influenza vaccination.[52,53] Serious adverse events were defined as reports of death, life-threatening illness, hospitalization or prolongation of hospitalization, or permanent disability, according to the Vaccine Adverse Events Reporting System (VAERS).[54]

Statistical analysis

The antibody response was assessed by calculating the following indices: mean fold rise (MFR) of geometric mean titer (GMT), seroresponse proportion (sR, the proportion of subjects showing ≥ 4-fold rise) and seroprotection proportion (sP, the proportion with postvaccination titer ≥ 1:40).[55] We also calculated seroconversion proportion (sC, proportion with baseline titer < 1:10 and postvaccination titer ≥ 1:40 or baseline titer ≥ 1:10 and ≥ 4-fold rise), and compared our results with the European Medicines Agency (EMA) criteria (sC > 40%, MFR > 2.5 and sP > 70%).[56]

For data processing, HI titer < 1:10 was regarded as 1:5. Reciprocal titers were used for analyses after logarithmic transformation. The results were presented on the original scale by calculating the antilogarithms. Wilcoxon signed-rank test was applied for intracategory comparisons of MFR, and either Wilcoxon rank-sum test or Kruskal-Wallis test was used for intercategory comparisons of GMT. Chi-square test or Mantel-extension test was performed, as appropriate, for comparisons of sR and sP.

Antibody response was assessed for populations stratified for age, gender, underlying disease and type of chemotherapy. Cyclosporine and tacrolimus were grouped with immunosuppressive agents. The categories of chemotherapy were not exclusive.

We conducted multivariate logistic regression analysis to examine the effect of each factor on objective variables (sR and sP after second dose). This multivariate analysis was limited to patients with prevaccination antibody titer (pre-titer) < 1:10 (n = 46). Myelodysplatic syndrome (MDS), aplastic anemia and other disease were not included in the models as there were only a few such patients (n = 3, 2 and 2, respectively). Multivariate models were analyzed in two separate steps because of the limited number of subjects. Models of the first step analysis included underlying disease, and those of the second step analysis included chemotherapeutic agents, as explanatory variables, along with age and gender. The final model included factors selected in each previous analysis step to enable identification of the factors that were more prominently associated with the lowered immune response, from among the underlying medical conditions and treatments adopted for those conditions. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. We also calculated Cramer's V to detect relationships among the variables. A two-sided *P* value of less than 0.05 was considered statistically significant. A *P* value less than 0.10 and larger than or equal to 0.05 was regarded as marginally significant.

All statistical analyses were performed using SAS ver. 9.3 (SAS institute, NC, USA).

Disclosures

No conflict of interest statement declared.

Acknowledgment

The present study was performed as a part of the "Analytical epidemiological study for influenza and currently-concerned respiratory infectious diseases (Principal investigator Yoshio Hirota)" funded by a 2009 Health and Labor Sciences Research Grant.

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