INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Safety of a Pandemic Influenza Vaccine and the Immune Response in Patients with Duchenne Muscular Dystrophy

Tomoko Saito[1,2], Satoko Ohfuji[1], Tsuyoshi Matsumura[2], Toshio Saito[2], Kazuhiro Maeda[3], Akiko Maeda[1], Wakaba Fukushima[1], Harutoshi Fujimura[2], Saburo Sakoda[2] and Yoshio Hirota[1]

Abstract

Objective To examine the safety of and immune response to the influenza A(H1N1)pdm09 vaccine in patients with Duchenne muscular dystrophy (DMD).

Methods Forty-four non-ambulatory patients with DMD hospitalized in a muscle disease ward and 41 healthy healthcare workers each received one dose of the influenza A(H1N1)pdm09 vaccine. Serum samples were collected before and four weeks after vaccination to measure the hemagglutinin inhibition antibody titers.

Results No severe adverse events were noted in any of the subjects. The immune responses of the patients were comparable to those of the healthcare workers. Among the patients, tube feeding and a lower total protein level in the serum were identified to be significantly associated with a lower immune response.

Conclusion A single dose of the vaccine was found to be safe and induced an optimal level of immunity in the DMD patients. The nutritional status may be associated with the immune response in patients with DMD.

Key words: Duchenne muscular dystrophy, influenza vaccine, safety, immune response

(Intern Med 54: 1199-1205, 2015)
(DOI: 10.2169/internalmedicine.54.1186)

Introduction

Duchenne muscular dystrophy (DMD) is an inherited myogenic disorder characterized by progressive skeletal muscle involvement in which weakness of the respiratory muscles, distortion of the thorax and inability to perform postural changes result in the retention of secretions and chronic microatelectasis. In association with pulmonary dysfunction, respiratory infections, including influenza, can cause severe complications that further weaken the respiratory function, necessitating admission to an intensive care unit and potentially causing death (1, 2). Therefore, preventing respiratory infections is a matter of clinical importance. Although recent guidelines for the treatment of DMD recommend annual influenza vaccination (3, 4), there are currently no reports regarding the immune response in patients treated with these vaccines. Another concern is whether disease-related conditions, such as the patient's physical condition and nutritional status, are associated with the antibody response in cases of DMD.

In the present study, we investigated whether influenza vaccination is safe and immunogenic in patients with DMD as compared to that observed in healthy healthcare workers and identified the factors affecting the immune response in DMD patients.

Materials and Methods

Subjects

We invited 46 inpatients with DMD treated at National Hospital Organization Toneyama National Hospital from October 21 to 30, 2009, of whom 44 agreed to participate. All

[1]Department of Public Health, Osaka City University Graduate School of Medicine, Japan, [2]Department of Neurology, National Hospital Organization Toneyama National Hospital, Japan and [3]Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Japan
Received for publication June 17, 2013; Accepted for publication October 5, 2014
Correspondence to Dr. Tomoko Saito, tosaito@toneyama.go.jp

subjects were non-ambulatory, including 33 (75%) patients from the long-term care unit and 11 (25%) short-term inpatients. During the same period, 41 healthcare workers employed by the same hospital were also recruited to participate in the study as a control group. None of the subjects met the exclusion criteria, including prior episodes of influenza A(H1N1)pdm09 infection, acute febrile illnesses at the time of vaccination, history of anaphylaxis resulting from the vaccine components or other conditions making it inappropriate to undergo vaccination. Written informed consent was obtained from each participant or their guardian if younger than 20 years of age at the time of recruitment. The study protocol was reviewed and approved by the ethics committees of Osaka City University Graduate School of Medicine and Toneyama National Hospital.

Data collection

Prior to vaccination, all participants completed a self-administered questionnaire regarding sex, date of birth, height, weight and comorbid diseases. In addition, clinical information was extracted from the patients' medical records, including medications, ejection fraction of the left ventricle (EF) within the last six months, activities of daily living (ADLs), use of mechanical ventilation and levels of total protein, albumin, hemoglobin and hematocrit on routine laboratory tests.

Vaccine

A monovalent, unadjuvanted, inactivated, split influenza A (H1N1)pdm09 vaccine (Lot. HP01A; BIKEN) was used. All participants received a subcutaneous injection of the vaccine at a dose of 0.5 mL containing 15 μg of hemagglutinin antigens and 0.0008% thimerosal.

Assessment of adverse reactions

All vaccinated subjects recorded solicited local and systemic reactions occurring within 48 hours after vaccination using a self-administered questionnaire. For patients who were unable to independently fill in the questionnaire, nurses completed the form based on a direct interview and observation. Local reactions included redness, swelling, induration, itching and pain at the injection site. Systemic reactions included fever (axillary temperature ≥37.5℃), malaise, myalgia, headache and rashes.

Measurement of the antibody titer

Blood samples were collected 0-2 days before and 28-30 days after vaccination. The serum was stored at -20℃ until all samples were assayed at the same time in July 2010. The titer of serum antibodies to hemagglutinin was measured using the standard microtiter HAI method (5). All samples were tested at the laboratory of the Research Foundation for Microbial Diseases of Osaka University.

Statistical analysis

For comparisons of the baseline variables, adverse reactions and antibody responses between the subject groups (patients vs. healthcare workers), the Wilcoxon rank-sum test was used for continuous variables and the chi-square test, Fisher's exact test and Mantel-extension method were used for categorical variables.

Categorical variables included the pre-vaccination titer (<1:10, 1:10-1:20, and ≥1:40), ADLs (wheelchair use or bedridden status) and mechanical ventilation (none, noninvasive positive-pressure ventilation (NPPV) or tracheal positive-pressure ventilation (TPPV)). The immunogenicity endpoints were determined based on conventional international criteria, as follows: geometric mean titer (GMT), fold rise, seroprotection proportion (post-vaccination titer ≥1:40) and seroresponse proportion (fold rise ≥4) (6, 7). A titer of <1:10 was defined as 1:5 for the calculations. Reciprocal antibody titers were analyzed after logarithmic transformation. The results are presented in the original scale by calculating the antilogarithm.

We evaluated the independent effects of several factors on immunogenicity solely in the patient group, then calculated the odds ratio (OR) and 95% confidence interval (CI) using logistic regression models. Since only 44 patients were enrolled, care was taken to select explanatory variables for the multivariate models. In the first multivariate model (model 1), we controlled for age and pre-vaccination titer, which have been inconsistently reported to be related to immunogenicity from influenza vaccinations (8-10). In order to obtain meaningful calculation results, we combined the pre-vaccination subcategory titers 1:10-1:20 and ≥1:40 into one category of ≥1:10. In model 2, in order to identify additional potential confounders, we used a stepwise regression model (significance level for entry into the model =0.15), which resulted in the feeding method being included.

Two-sided p values less than 0.05 were considered to be statistically significant. All analyses were performed using the SAS software package, version 9.1 (SAS Institute, Inc., Cary, USA).

Results

Study subjects

Table 1 summarizes the baseline characteristics of the subjects. Most of the patients were in an advanced stage, with a high age, low cardiopulmonary function and low level of ADLs. None of the patients were currently receiving oral steroid therapy, although this therapy is now the standard treatment worldwide for young patients with DMD (11).

Vaccine safety

Solicited adverse reactions to the vaccine among the patients and healthcare workers are shown in Table 2. Both local and systemic reactions were less frequent in the patients. All symptoms were mild, and none of the affected subjects required medical treatment.

Table 1. Baseline Characteristics of the Study Subjects (n=85).

Characteristics	Total (n=85)	Patients (n=44)	Healthcare workers (n=41)	p value*
(Comparison between patients and healthcare workers)				
Sex: male, *n* (%)	62 (73)	44 (100)	18 (44)	0.001
Age (years)				
mean (SD)	35.8 (11.1)	30.9 (8.6)	41.2 (11.1)	<0.001
median (range)	33.0 (17-62)	31.3 (17-47)	43.0 (23-62)	
Body mass index (kg/m^2)				
mean (SD)	17.4 (4.5)	13.7 (2.4)	21.4 (2.4)	<0.001
median (range)	17.6 (10.2-30.5)	13.3 (10.2-19.5)	21.2 (17.1-21.2)	
Prevaccination titer				
<1:10, *n* (%)	57 (67)	31 (70)	26 (63)	0.857
1:10-1:20, *n* (%)	24 (28)	11 (25)	13 (32)	
≥1:40, *n* (%)	4 (5)	2 (5)	2 (5)	
Underling disease				
diabetes mellitus, *n* (%)	1 (1)	1 (2)	0 0	1.000
atopic dermatitis, *n* (%)	4 (5)	3 (7)	1 (2)	0.617
(Specific factors in patients)				
EF (%), *mean (SD)*		36.7 (15.3)		
Activity of daily living				
wheelchair user, *n* (%)		17 (39)		
bedridden, *n* (%)		27 (61)		
Respiratory status				
none, *n* (%)		2 (5)		
NPPV, *n* (%)		16 (36)		
TPPV, *n* (%)		26 (59)		
Feeding method				
tube feeding (-), *n* (%)		20 (45)		
tube feeding (+) **, *n* (%)		24 (55)		
Albumin (g/dl), *mean (SD)*		3.9 (0.5)		
Total Protein (g/dl), *mean (SD)*		7.1 (0.5)		
Hemoglobin (g/dl), *mean (SD)*		12.8 (1.5)		
Hematocrit (%), *mean (SD)*		38.1 (4.5)		

EF: ejection fraction of the left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: Tracheal positive pressure ventilation

* Wilcoxon rank sum test, χ^2 test, Fisher's exact test or Mantel extension method for trend tests

** Tube feeding (+): gastrostomy feeding (n=5), nasal or oral tube feeding (n=19)

Table 2. Local and Systemic Reactions to the Vaccine.

	Patients (n=44)		Healthcare workers (n=41)		
Symptom	n	%	n	%	p value*
Local reactions					
Total	14	32	21	51	0.071
Redness	12	27	17	41	0.170
Swelling	4	9	8	20	0.171
Induration	0	0	7	17	0.005
Itching	0	0	8	20	0.002
Pain	2	5	13	32	0.001
Systemic reactions					
Total	3	7	12	29	0.007
Fever (>37.5˚C)	2	5	4	10	0.423
Malaise	0	0	8	20	0.002
Myalgia	0	0	2	5	0.230
Headache	0	0	2	5	0.230
Rash	1	2	0	0	1.000

* χ^2 test or Fisher's exact test

Immune response

The results for the antibody response in relation to the background factors are shown in Table 3. The only identified significant factor was the pre-vaccination titer, as a higher pre-titer value was associated with a greater post-vaccination GMT, lower seroresponse proportion and higher seroprotection proportion. There were no significant differences in any of the endpoints of immunogenicity between the subject groups. In the logistic regression analysis, the OR after adjustment for age and pre-vaccination titer in the patients as compared to the healthcare workers was 1.71 (95%CI: 0.50-5.87) for the seroresponse proportion and 0.88 (0.29-2.63) for the seroprotection proportion, neither of which were statistically significant.

Figure shows the pre- and post-vaccination GMTs in the patients based on several predictors. In a comparison of the fold rise between each factor, we found that the oral-fed patients exhibited better fold rise values than the tube-fed patients (16 vs. 7, p=0.047). We also examined the effects of disease-related factors on the seroresponse and seroprotection proportions, with the results shown in Tables 4 and 5. An older age was suggested to have a relationship with a greater seroresponse in model 2. Furthermore, the tube-fed patients demonstrated a decreased OR for the seroresponse proportion compared to the oral-fed patients, and a higher total protein level was found to be significantly associated with a higher seroprotection proportion. Variables of the functional status, such as EF, ADLs and the respiratory condition, were not related to the immune response.

Table 3. Immunogenicity to the Vaccine Based on the Background Factors (n=85).

		GMT		Fold rise	Seroresponse	Seroprotection
	N	S0	S1		n (%)	n (%)
Entire sample	85	7	72	9.7	68 (80)	61 (72)
Sex						
Male	62	8	79	10.5	51 (82)	45 (73)
Female	23	7	56	8.0	17 (74)	16 (70)
p value		0.488	0.274	0.428	0.381	0.785
Age (years)						
<34.5	42	8	74	9.6	34 (81)	31 (74)
≥34.5	43	7	70	9.9	34 (79)	30 (70)
p value		0.589	0.686	0.993	0.829	0.681
Body mass index (kg/m²)						
<18.5	45	7	70	9.6	37 (82)	31 (69)
≥18.5	40	8	75	9.8	31 (78)	30 (75)
p value		0.494	0.947	1.000	0.589	0.535
Pre-vaccination titer						
<1:10	57	5	51	10.2	46 (81)	35 (61)
1:10-1:20	24	12	127	10.4	21 (88)	22 (92)
≥1:40	4	95	320	3.4	1 (25)	4 (100)
p value		<0.001	0.002	0.222	0.035	0.010
Subject group						
Patients	44	7	75	10.5	37 (84)	31 (70)
Healthcare workers	41	8	69	9.0	31 (76)	30 (73)
p value		0.508	0.631	0.571	0.332	0.782

GMT: geometric mean titer, S0: pre-vaccination, S1: post-vaccination, Fold rise: S1/S0, Seroresponse: S1/S0 ≥4, Seroprotection:S1 ≥1:40

p value: The Wilcoxon rank-sum test or Kruskal-Wallis rank test were used to compare the GMT and fold rise, while the χ^2 test, Fisher's exact test or Mantel extension method were used to compare the seroprotection and seroresponse proportions.

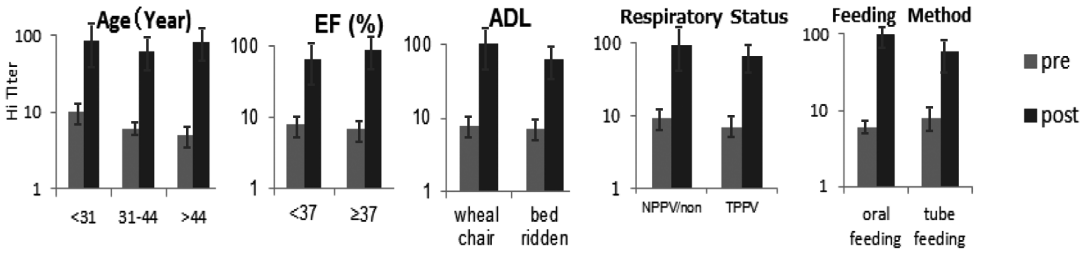

Figure. Pre- and post-vaccination HAI titers in the patients (n=44) based on age and disease-related factors. EF: ejection fraction, ADL: activities of daily living, NPPV: non-invasive intermittent positive pressure ventilation, TPPV: tracheal positive pressure ventilation. Age is presented according to tertile. The EF is presented according to the median. *In a comparison using the Wilcoxon rank-sum test or Kruskal-Wallis rank test, the p values for post-vaccination GMT were not significant, while the p value for the fold rise (post-/pre-vaccination GMT) in the feeding method was 0.047.

Discussion

No harmful adverse effects from vaccination were observed in any of the participants, while the patients with DMD experienced less frequent local and systemic reactions. Information bias derived from the self-administered questionnaire protocol may have been present, as the healthcare workers may have been more sensitive to subtle changes after vaccination. However, the patients showed lower risks for each objective reaction observed by the nurses, including redness, swelling and induration, and the lower frequency of induration was significant. There are likely several modifiers of inflammatory mediators, including sun exposure (12) and immobility, in patients with DMD that may decrease stimulation, although the pathophysiology of skin reactions remains unclear. Nevertheless, the present results are encouraging for both patients and clinicians concerned about risks associated with influenza vaccination.

The immune responses to the influenza vaccine were comparable between the patients with DMD and the health-

Table 4. Associations between Selected Characteristics and the Seroresponse Proportion in the Patients (n=44).

Category	Crude OR (95%CI)	Multivariate model 1* OR (95%CI)	Multivariate model 2* OR (95%CI)
Age			
1 year increased	1.10 (0.98-1.22)	1.14 (0.99-1.22)‡	1.23 (1.02-1.48)†
BMI			
1 kg/m^2 increased	1.38 (0.86-1.17)	1.38 (0.85-2.24)	1.29 (0.79-2.12)
Pre-vaccination titer			
≥1:10/<1:10	1.06 (0.18-6.30)	3.14 (0.36-27.12)	4.59 (0.37-56.70)
EF			
1% increased	1.00 (0.95-1.05)	1.02 (0.96-1.08)	1.02 (0.95-1.10)
Activities of daily living			
bedridden/wheelchair user	2.46 (0.48-12.72)	2.02 (029-13.82)	5.21 (0.53-51.58)
Respiratory status			
TPPV/NPPV or none	2.19 (0.43-11.2)	1.11 (0.14-9.07)	6.81 (0.51-92.28)
Feeding method			
tube feeding (+/-)	0.16 (0.02-1.44)	0.06 (0.01-0.83)†	(identical in model 1)
Albumin			
0.1 g/dL increased	1.00 (0.85-1.18)	1.03 (0.84-1.25)	0.92 (0.73-1.15)
Globulin**			
0.1 g/dL increased	1.10 (0.95-1.28)	1.08 (0.90-1.28)	1.24 (0.99-1.54)
Total protein			
0.1 g/dL increased	1.14 (0.96-1.37)	1.13 (0.93-1.38)	1.18 (0.95-1.49)
Hemoglobin			
0.1 g/dL increased	1.02 (0.97-1.08)	1.08 (0.90-1.28)	1.01 (0.95-1.07)
Hematocrit			
1% increased	1.08 (0.90-1.29)	1.09 (0.90-1.31)	1.01 (0.82-1.25)

Logistic regression model CI: confidence interval, OR: odds ratio, EF: ejection fraction of left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: tracheal positive pressure ventilation, †p<0.05, ‡ p<0.10

*model 1: adjusted for age and pre-vaccination titer

*model 2: adjusted for all variables in model 1 plus feeding method

** globulin=total protein - albumin

care workers in the present study. The primary factor significantly associated with immunogenicity was the pre-vaccination titer, as a higher pre-vaccination titer was found to be significantly associated with a higher post-vaccination titer, lower seroresponse and higher seroprotection proportion. The inverse association with the seroresponse reflects an effect of "the law of initial values" or "negative feedback" (8, 9). It is important to take the pre-vaccination titer into account when evaluating the immune response to pandemic vaccines. As such studies are often performed during pandemic waves and asymptomatic infections in the study population are inevitable, it is difficult to predict how the immune status prior to vaccination has been modified. We believe that our multivariate analysis including the pre-vaccination titer was adequate to appropriately examine the immune response in this study.

We also found that an increased age was associated with an increased seroresponse in the patient population. Previous studies have reported decreased immune responses in elderly individuals 65 years of age or older (10, 13). However, the mean age of our patients was 30.9 years, with the oldest patient being 47; thus, we cannot simply compare our results to those of other studies. The oldest group in the present study had the lowest pre-GMT and highest post-GMT values (Figure). On the other hand, the EF, ADLs and respiratory status were not significantly associated with the immune response, which indicates that the disease stage or severity is not associated with immunity. Although several specific factors are assumed to be related to long-term survival in DMD patients (14), a superior antibody response in older patients has not been previously reported. Further cell biological and epidemiological investigations of the immune status of long-term survivors with DMD will provide new insight.

The significant OR values for tube feeding and total protein in the present study indicate that the nutritional status is an independent predictor of the antibody response in patients with DMD. Our results are consistent with those of previous studies showing that the nutritional status is associated with immunogenicity in elderly persons (15-17). These findings may help to increase awareness regarding the higher burden of infection in tube-fed patients with DMD.

This study is associated with several limitations. The investigation was conducted in a single hospital and the number of subjects was small; therefore, the study power was limited. In addition, most of the patients were in an advanced stage of disease, which may limit the generalizability

Table 5. Associations between Selected Characteristics and the Seroprotection Proportion in the Patients (n=44).

Category	Crude OR (95%CI)	Multivariate model 1* OR (95%CI)	Multivariate model 2* OR (95%CI)
Age			
1 year increased	0.98 (0.91-1.06)	1.05 (0.95-1.15)	1.05 (0.94-1.16)
BMI			
1 kg/m^2 increased	1.03 (0.78-1.36)	0.84 (0.59-1.21)	0.87 (0.60-1.26)
Pre-vaccination titer			
≥1:10/<1:10	NA	NA	NA
EF			
1% increased	1.01 (0.97-1.06)	1.03 (0.97-1.10)	1.03 (0.97-1.10)
Activities of daily living			
bedridden/wheelchair user	0.62 (0.16-2.44)	1.12 (0.23-5.53)	1.33 (0.25-7.10)
Respiratory status			
TPPV/NPPV or none	0.87 (0.23-3.26)	2.73 (0.39-18.92)	5.45 (0.51-58.35)
Feeding method			
tube feeding (+/-)	0.42 (0.11-1.64)	0.35 (0.08-1.62)	(identical in model 1)
Albumin			
0.1 g/dL increased	1.17 (1.00-1.36)†	1.14 (0.95-1.37)‡	1.11 (0.92-1.34)
Globulin**			
0.1 g/dL increased	1.03 (0.93-1.15)	1.07 (0.93-1.24)	1.13 (0.95-1.33)
Total protein			
0.1 g/dL increased	1.24 (1.04-1.47)†	1.28 (1.03-1.60)†	1.45 (1.04-2.01)†
Hemoglobin			
0.1 g/dL increased	1.05 (1.00-1.10)‡	1.05 (0.99-1.10)	1.04 (0.98-1.10)
Hematocrit			
1% increased	1.17 (1.00-1.37)‡	1.16 (0.97-1.40)	1.14 (0.94-1.38)

Logistic regression model CI: confidence interval, OR: odds ratio, EF: ejection fraction of left ventricle, NPPV: non-invasive positive pressure ventilation, TPPV: tracheal positive pressure ventilation, NA: not applicable,
†p<0.05, ‡ p<0.10
*model 1: adjusted for age and pre-vaccination titer
*model 2: adjusted for all variables in model 1 plus feeding method
** globulin=total protein - albumin

of our findings. Furthermore, since none of the study patients were given oral corticosteroids, we were unable to evaluate the influence of immunosuppressive therapy on the efficacy of vaccination. Additional studies with larger cohorts including young patients and long-term survivors are thus needed to thoroughly investigate immunogenicity to influenza vaccination in cases of DMD.

In conclusion, we found that the influenza A(H1N1)pdm 09 vaccine safely induced a good immune response in patients with DMD. Influenza infection is sometimes lethal in DMD patients. The present results provide useful information for preventing influenza infection in patients with DMD.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

We are grateful to the patients and staff at Toneyama National Hospital for their understanding and cooperation while participating in this study.

This study was supported by a grant for Research on Emerging and Re-emerging Infectious Diseases as a part of Health and Labor Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan.

References

1. Bach JR, Ishikawa Y, Kim H. Prevention of pulmonary morbidity for patients with Duchenne muscular dystrophy. Chest **112**: 1024-1028, 1997.
2. Finder JD, Birnkrant D, Carl J, et al. Respiratory care of the patient with Duchenne muscular dystrophy: ATS consensus statement. Am J Respir Crit Care Med **170**: 456-465, 2004.
3. Bushby K, Finkel R, Birnkrant DJ, et al; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol **9**: 177-189, 2010.
4. Practical guideline for Duchenne muscular dystrophy Working Group. Practical guideline for Duchenne muscular dystrophy 2014. Nankodo, Tokyo, 2014: 81-82 (in Japanese).
5. World Health Organization. WHO manual on animal influenza diagnosis and surveillance [Internet]. [cited 2013 June 17]. Available from: http://whqlibdoc.who.int/hq/2002/who_cds_csr_ncs_2002.5.pdf
6. Committee for Proprietary Medical Products. Note for guidance on harmonization of requirements for influenza vaccines. [Internet]. [cited 2013 June 17]. Available from: http://www.ema.europa.

eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf

7. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices- United States, 2013-2014: MMWR 62(RR-07): 1-43, 2013.

8. Hobson D, Baker FA, Curry RL. Effect of influenza vaccines in stimulating antibody in volunteers with prior immunity. Lancet **2**: 155-156, 1973.

9. Hirota Y, Kaji M, Ide S, Goto S, Oka T. The hemagglutination inhibition antibody responses to an inactivated influenza vaccine among healthy adults: with special reference to the prevaccination antibody and its interaction with age. Vaccine **14**: 1597-1602, 1996.

10. Beyer WE, Palache AM, Baljet M, Masurel N. Antibody induction by influenza vaccines in the elderly: a review of the literature. Vaccine **7**: 385-394, 1989.

11. Bushby K, Finkel R, Birnkrant DJ, et al; DMD Care Considerations Working Group. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol **9**: 77-93, 2010.

12. Henri P, Lambert A, Philippe E. Laurent Intradermal vaccine delivery: Will new delivery systems transform vaccine administration? Vaccine **26**: 3197-3208, 2008.

13. Zhu FC, Wang H, Fang HH, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med **361**: 2414-2423, 2009.

14. Ishizaki M, Ueyama H, Masida T, Nishida Y, Imamura S, Ando Y. Two cases of Duchenne muscular dystrophy over 40 years after onset. Rinshou Shinkeigaku (Clin Neurol) **53**: 293-298, 2013 (in Japanese, Abstract in English).

15. Hara M, Tanaka K, Hirota Y. Immune response to influenza vaccine in healthy adults and the elderly: association with nutritional status. Vaccine **23**: 1457-1463, 2005.

16. Hui SL, Chu LW, Peiris JS, Chan KH, Chu D, Tsui W. Immune response to influenza vaccination in community-dwelling Chinese elderly persons. Vaccine **24**: 5371-5380, 2006.

17. Fülöp T Jr, Wagner JR, Khalil A, Weber J, Trottier L, Payette H. Relationship between the response to influenza vaccination and the nutritional status in institutionalized elderly subjects. J Gerontol A Biol Sci Med Sci **54A**: M59-M64, 1999.