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A/H1N1 influenza vaccination in patients with systemic lupus erythematosus: Safety and immunity

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ABSTRACT

Objectives: To determine the safety of and immunogenicity induced by A/H1N1 influenza vaccination in patients with systemic lupus erythematosus (SLE).
Research design and methods: The study population comprised 21 SLE patients and 15 healthy control subjects who underwent split-virion, inactivated monovalent A/H1N1 vaccination between December 2009 and January 2010. Sera were obtained before, three weeks after, and six months after vaccination. SLE disease activity index (SLEDAI) scores and autoantibodies were measured at every visit in SLE patients. Haemagglutination inhibition and the serum immunoglobulin G (IgG) level were calculated using the World Health Organization (WHO) procedure to evaluate the antibody responses. We also recorded current medications and past seasonal influenza vaccinations to analyse the interactions between vaccinations and the autoimmunity of SLE patients.
Results: The mean age of the enrolled population was 34.3 years for SLE patients and 39.4 years for control subjects. The average SLEDAI score for SLE patients was 4.1 at vaccination, 4.5 at three weeks, and 4.3 at six months. The seroprotection rate at three weeks was 76.2% in SLE patients and 80.0% in healthy control subjects; by six months, the seroprotection rate was 66.7% in SLE patients and 60% in healthy control subjects. The seroconversion rate was 76.2% in SLE patients and 80% in healthy controls at three weeks; by six months, the seroconversion rate was 52.4% in SLE patients and 53.3% in healthy controls. The response in SLE patients met the criteria of the European Committee for Proprietary Medicinal Products guidelines at three weeks, while the percentage of seroprotection did not at six months. The clinical disease activity and SLEDAI scores did not differ significantly from before to after vaccination in SLE patients, although the level of anticardiolipin IgG increased at three weeks after vaccination, but with no apparent clinical manifestations.
Conclusions: The A/H1N1 influenza vaccine is safe and effective in SLE patients and has no obvious adverse clinical effects. Treatment with a single immunosuppressive agent or combination therapy also leads to effective humoral immunity in these patients.

1. Introduction

The A/H1N1 and A/H3N2 influenza viruses have caused potent pandemics. A novel swine influenza A (A/H1N1) virus that clinically mimics seasonal influenza was identified in two children in the United States in March and April 2009 [1,2] and was responsible for an explosion of respiratory tract infections in Mexico [3]. When the transmission seemed to persist and increase in the Northern Hemisphere during the autumn and winter of 2009, the World Health Organization immediately proclaimed a worldwide pandemic, characterized by uncontained community-level transmission of the A/H1N1 virus in multiple areas of the world [4].

Some patients required admission to intensive care units for acute respiratory distress syndrome or septicaemia [5–7]. The elderly, the obese, and those with accompanying underlying medical conditions such as chronic obstructive pulmonary disease, immunodeficiency, and neurological diseases tended to have more severe disease [7–9]. Thus, in 2009 the Advisory Committee on Immunization Practices recommended the use of the monovalent A/H1N1 vaccine to prevent mortality and morbidity from A/H1N1 infection and to mitigate the pandemic [10]. Recent studies had

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confirmed the safety and immunogenicity of this A/H1N1 vaccine in Taiwanese [11–13].

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease. A defect in B lymphocytes causes abnormalities in apoptosis and persistent production of autoantibodies [14–16]. Dysregulation of B cells and immunosuppressive therapy in SLE patients might cause impaired humoral immune responses to the A/H1N1 influenza virus, which can occasionally lead to the complications of pulmonary infection and fatal organ dysfunction.

Previous reports recognized the safety and antibody response to influenza vaccination in SLE patients. Morbidity and mortality caused by A/H1N1 infection were diminished significantly after mass vaccination, although several reports claimed that vaccination might exacerbate autoimmunity [17]. Neither increased generation of autoantibodies nor clinical exacerbation of disease activity was observed in influenza-vaccinated SLE patients [18], but no information describing the safety and efficacy of this new A/H1N1 influenza vaccine in SLE patients is available.

In this report, we describe the clinical manifestations, autoimmunity, and humoral immune response including the seroprotection and seroconversion rates in A/H1N1 influenza-vaccinated SLE patients. The specific antibody response to A/H1N1 influenza virus in SLE patients met the European Committee for Proprietary Medicinal Products (CPMP) guidelines. We also demonstrated immunity in A/H1N1-vaccinated SLE patients under immunosuppressive therapy.

2. Materials and methods

2.1. Study design

Twenty-one patients with SLE defined according to the American College of Rheumatology criteria were selected during the 2009–2010 winter and, after giving informed consent, were vaccinated against A/H1N1 influenza virus. All patients were at low SLE disease activity index (SLEDAI scores < 8 and/or stable disease activity (defined as disease not demanding any increase in therapy for at least three weeks)) at the time of enrollment and without any contraindications (egg allergy or previous allergy to vaccine components). Patients infected by this unique swine A/H1N1 influenza virus in winter 2009–2010 were excluded. All SLE patients were taking one or more immunosuppressive agents including prednisolone, hydroxychloroquine (HCQ), disease-modifying antirheumatic drugs, or cytotoxic agents such as azathioprine (AZA) and cyclophosphamide. Fifteen sex- and age-matched normal healthy subjects were included as the control group from a group of voluntarily vaccinated people.

The vaccinated SLE patients underwent clinical evaluation and provided a detailed history that included experience of fever, malaise, headache, and myalgia. Laboratory evaluations of specific autoantibodies, peripheral blood lymphocyte subpopulations, and serum anti-A/H1N1 influenza virus antibody level were performed before, three weeks after, and six months after vaccination. We performed the same assessment in the healthy controls at the same times.

A single dose of 0.5 ml monovalent split swine A/H1N1 influenza vaccine without the adjuvant (A/California/7/2009 [A/H1N1]v like strain) (Adimmune, Taipei, Taiwan) was administered by the intramuscular route to SLE patients and normal controls. The vaccination included 15 μg of haemagglutinin per virus preparation.

2.2. Safety

The safety of the vaccine was monitored using the following clinical and laboratory parameters: general well-being of patients, the levels of complement C3 and C4, variation in autoantibody levels, clinical manifestation of flares, SLEDAI scores, and lymphocyte subpopulations before and after immunization. All examinations in both groups were approved by the Tri-Service General Hospital Institutional Review Board.

2.2.1. Autoantibodies

The levels of anti-double-stranded DNA (anti-dsDNA) and anti-extractable nuclear antigens (anti-ENA), immunoglobulin G (IgG) and M isotypes of anticardiolipin (aCL), and anti-β2 glycoprotein I antibodies (aGPI) were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Pharmacia Diagnostics, Milton Keynes, UK) according to the manufacturer's instructions and calibrated with the international standard. Lupus anticoagulant (LAC) was detected according to the guidelines of the International Society on Thrombosis and Haemostasis [19].

2.2.2. Lymphocyte subpopulations

Peripheral blood lymphocyte subpopulations were identified using a standard protocol for two-colour immunofluorescence flow cytometry [20–21]. Adequate amounts of fluorescein (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies against lymphocyte membrane markers were added to 0.1 ml of whole blood and incubated for 20 min at room temperature. The preparations were washed and fixed using FACS lysing solution (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

The following combinations of monoclonal antibodies were used: FITC–CD3 and PE–CD19 for recognizing T and B lymphocytes, respectively, and FITC–CD4 and PE–CD8 for identifying T helper and T cytotoxic lymphocytes, respectively. All monoclonal antibodies were purchased from Becton Dickinson.

Flow cytometry was performed using a FACScan (Becton Dickinson) flow cytometer. Forward and side light scatter were used to include only lymphocytes in the analysis. Red and green fluorescence (from PE and FITC conjugates, respectively) was measured using a single laser firing at 488 nm and appropriate filtering of the emitted light. Twenty thousand lymphocytes were analysed for each combination of monoclonal antibodies for each subject. Data were obtained by computerized calculations performed on contour plots generated by Consort 30 software.

2.2.3. Number and severity of flares, disease activity, and local or systemic adverse events

Flares for SLE patients were suspected on the basis of an increase in medication dose or the introduction of a new treatment in the presence of deterioration in an already active symptom or a manifestation of a new activity [22]. All SLE patients and normal controls were interviewed directly three weeks and six months after vaccination to identify possible local and systemic adverse reactions. One week after vaccination, all subjects were questioned about the occurrence of clinical systemic symptoms and/or local adverse effects around the injection site including fever (>37.5 °C), shaking chills, headache, malaise, arthralgia, myalgia, local pain, induration, or swelling. To detect possible infection with A/H1N1 virus, nasopharyngeal aspirates were collected from vaccinated patients and controls with symptoms of acute respiratory tract infection and fever >38 °C [23]. The nasopharyngeal aspirates were collected within 48 h of the onset of flu-like symptoms. SLEDAI scores were also calculated for SLE patients before and after the vaccination to evaluate disease activity.

2.3. Specific anti-A/H1N1 influenza antibodies

The sera were analysed by haemagglutination-inhibition (HAI) testing and an IgG ELISA according to standard procedures. Antigens used for testing were antigenically equivalent to the vaccine

formulation (A/California/7/2009). Each serum tested by the ELISA method was diluted 1:400, and the starting dilution of serum for HAI detection was 1:10.

The following variables were calculated: geometric mean titre (GMT) for HAI, the seroprotection rate (percentage of vaccine recipients with a serum HAI titre ≥ 1:40 after vaccination), and the seroconversion rate (the proportion of subjects with a prevaccination HAI antibody titre < 1:10 and a postvaccination titre HAI ≥ 1:40, or a prevaccination titre ≥ 1:10 and an increase in the titre by a factor of four or more). According to the guidelines of the European CPMP for the evaluation of influenza vaccines, a seroprotection rate >70% or a seroconversion rate >40% are considered the cut-off values for vaccine immunogenicity for adults 18–60 years of age [24].

2.4. Statistical analysis

All statistical analyses were performed using SPSS 14 (SPSS, Chicago, IL, USA). The difference in change in the GMT of the SLE and control groups was tested using the Mann–Whitney *U*-test and Kruskal–Wallis *H*-test. The seroconversion and seroprotection rates were compared between groups using the χ^2 test with Yates' correction for continuity. For all other comparisons, the χ^2 test or Fisher's exact test was used, depending on the size of the expected counts. The significance of the differences between data with an approximately normal distribution was determined using analysis of variance (ANOVA) with the Bonferroni–Dunn post-test or Student's *t*-test for paired data. The differences between other data were analysed by Friedman and Wilcoxon tests for paired data. A p value < 0.05 was considered significant.

3. Results

3.1. Demographics

Twenty-one SLE patients and 15 normal controls were enrolled into this study. All SLEDAI scores of the SLE patients were <8 before vaccination. One SLE patient (4.8%) and six (40%) of the normal controls had received the seasonal trivalent influenza vaccination in winter 2009 before this H1N1 vaccination ($p = 0.013$). Eighteen (85.7%) of the SLE patients and eight controls (53.3%) had never before received an influenza vaccination ($p = 0.058$). The demographic features of the SLE patients and normal controls are shown in Table 1. Four SLE patients were not taking prednisolone, three were not taking AZA, and six were not taking HCQ. Median doses for the drugs were 4.9 mg/day for prednisolone, 45 mg/day for AZA, and 162 mg/day for HCQ. The baseline characteristics such as sex, age, and past influenza vaccination did not differ between the SLE patients and normal controls.

Table 1
Characteristics in SLE patients and normal controls.

	SLE patients ($n = 21$)	Normal controls ($n = 15$)
Sex (male)	1 (4.8)	5 (33.3)
Age (mean ± SD (years))	34.3 ± 11.8	39.4 ± 13.9
Influenza vaccination in 2009/2010	1 (4.8)	6 (40)† (0.013)
No influenza vaccination	18 (85.7)	8 (53.3) (0.058)
SLEDAI scores	4.1 (0–7)	NA
No immunosuppressive agents	0 (0)	NA
Prednisolone	17 (81.0)	NA
Median (range), mg/day	4.9 (2.5–17.5)	NA
Azathioprine	18 (85.7)	NA
Median (range), mg/day	45 (25–100)	NA
Hydroxychloroquine	15 (74.4)	NA
Median (range), mg/day	162 (100–400)	NA
NSAID	5 (23.8)	NA

Note: Except where indicated otherwise, values are the number (%); SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; NA: not applicable; NSAID: non-steroidal anti-inflammatory drugs.
† p value < 0.05 versus normal controls.

3.2. Safety

No significant difference in complement C3 level occurred in the SLE patient group three and six months after vaccination (from 76.1 mg/dl to 81.1 mg/dl and 79.0 mg/dl, $p = 0.057$ and 0.326, respectively). The titres of anti-dsDNA and LAC did not differ at any of the three times (before, three weeks after, and six months after vaccination). The aCL IgG antibody level increased significantly from 6.01 GPL U/ml to 7.95 GPL U/ml (normal < 10 GPL U/ml) ($p = 0.003$) at three weeks in SLE patients, but did not differ significantly at six months (six months versus before vaccination, from 6.01 GPL U/ml to 5.44 GPL U/ml, $p = 0.468$). The aCL IgG antibody level increased from 1.71 GPL U/ml to 2.8 GPL U/ml ($p = 0.022$) at six months in normal controls without signs of clinical thromboembolism. By contrast, aGPI IgG level decreased from 1.94 to 1.48 mg/ml ($p = 0.002$) at six months in SLE patients and from 1.53 to 0.64 mg/ml ($p = 0.002$) at three weeks in the normal control group. The anti-ribonucleoprotein antibody level increased from 1.1 U/ml to 1.5 U/ml at three weeks and six weeks in normal controls ($p = 0.008$ and 0.018, compared with before vaccination, respectively). The anti-Smith antibody level also increased from 0.13 to 0.28 U/ml ($p = 0.002$) six months after vaccination without clinical manifestations of lupus (Table 2).

$CD19^+$ lymphocyte percentage decreased from 14.0% to 10.9% ($p < 0.001$) three weeks after vaccination in the normal control group but did not change in SLE patients. There was no difference at six months in both groups (six months versus before vaccination) (Table 3).

One SLE patient who had exhibited bilateral optic neuritis without neurological sequelae four years before this vaccination experienced general malaise, sore throat, fever, and blurred vision two weeks after the vaccination. Her prevaccination immunosuppressive agents included prednisolone 2.5 mg/day and HCQ 200 mg/day. The nasopharyngeal aspirate for rapid test and RT-PCR examinations showed no detectable A/H1N1 influenza virus infection. Ophthalmic physical examinations and magnetic resonance imaging of the brain showed bilateral optic neuritis and demyelization. An increase from 4 to 12 in SLEDAI score occurred at the same time. Consequently, this patient was given pulse methylprednisolone therapy of 3000 mg under a diagnosis of SLE flare and had a satisfactory response. Neither blurred vision nor flares of lupus in other organs developed in the following six months. Apart from this, no SLE patient demonstrated neurological or psychiatric manifestations of SLE before and after this vaccination. None of the other vaccinated SLE patients experienced significant flares or increase in SLEDAI score (Table 2). One of the normal control subjects developed symptoms suggestive of influenza infection. The nasopharyngeal aspirate and RT-PCR tests showed no evidence of A/H1N1 influenza virus infection. No other systemic adverse reactions emerged in vaccinated healthy controls, and they did not undergo physical examination or provide a blood sample during the follow-up. Neither local pain nor induration developed at the site of the injection in either group.

3.3. Immunity

The results of the assessment of immunity to the vaccine are shown in Table 4. HAI titre ≥ 40 developed in two SLE patients before the vaccination. The serum levels of both specific HAI and IgG antibodies toward the antigens (A/California/7/2009) increased significantly in SLE patients and normal controls, and met the CPMP

Table 2
Autoimmunity and disease activity in SLE patients and normal controls before and three weeks after the A/H1N1 influenza vaccination.

Autoantibodies	SLE patients ($n=21$)			Normal controls ($n=15$)			p value
	0 day	3 weeks	6 months	0 day	3 weeks	6 months	
C3	76.1	81.1	79.0	110.9	106.7	116.9	
C4	13.2	13.8	24.7	21.4	24.6	21.1	
LA	1.1	1.1	1.1	1.0	1.0	1.0	
aGPI IgG	1.94	1.98	1.48‡ (0.002)	1.53	0.64‡ (0.002)	0.8	§(0.002)
aGPI IgM	2.27	2.37	2.45	3.68	1.4‡ (0.002)	1.61	
aCL IgG	6.01	7.95‡ (0.003)	5.44	1.71	2.1	2.8† (0.022)	§(0.030)
aCL IgM	2.05	1.74	3.21	0.64	0.52	1.5	
Anti-dsDNA	70.1	50.1	38.6	1.6	3.1	1.9	
Anti-Sm	2.26	3.59	3.66	0.13	0.14	0.28‡ (0.002)	
Anti-RNP	24.5	25.5	21.9	1.1	1.5† (0.008)	1.5† (0.018)	
Anti-RO	125.0	121.2	106.1	0.37	0.42	1.3	
Anti-LA	9.2	11.0	6.9	0.23	0.26	0.29	
SLEDAI scores	4.1	4.5	4.3	NA	NA	NA	

Note: The bracket represent the p value compared pre-vaccination with pot-vaccination in SLE patients and normal controls, respectively; SLE: systemic lupus erythematosus; LA: lupus anticoagulant; aGPI IgG: anti-beta-2 glycoprotein I IgG; aGPI IgM: anti-beta-2 glycoprotein I IgM; aCL IgG: anti-cardiolipin IgG antibody; aCL IgM: anti-cardiolipin IgM antibody; anti-ENA include anti-sm, anti-RNP, anti-RO, and anti-LA.
† p value < 0.05 = versus baseline (0 days).
‡ p value < 0.01 = versus baseline (0 days).
§ p value versus normal controls at 3 weeks.

Table 3
Percentage distribution of peripheral blood lymphocyte subpopulations in SLE patients and normal controls before and three weeks after the A/H1N1 influenza vaccination.

Lymphocyte subpopulations	SLE patients ($n=21$)			Normal controls ($n=15$)			p value
	0 day	3 weeks	6 months	0 day	3 weeks	6 months	
$CD19^+$	10.5 ± 9.7	11.1 ± 8.9	9.2 ± 7.2	14.0 ± 3.9	10.9 ± 3.2‡ (<0.001)	14.3 ± 6.7	§(0.015)
$CD3^+$	80.4 ± 13.7	81.3 ± 11.5	80.3 ± 14.5	70.6 ± 9.5	73.9 ± 10.1	69.6 ± 12.2	
$CD4^+$	36.0 ± 12.5	37.0 ± 13.5	39.2 ± 13.4	38.2 ± 9.6	37.7 ± 11.7	39.5 ± 12.5	
$CD8^+$	47.1 ± 12.7	46.9 ± 16.6	44.4 ± 13.9	37.7 ± 9.0	37.2 ± 10.2	36.6 ± 10.3	

Note: Values are the number (%); SLE: systemic lupus erythematosus.
‡ p value < 0.01 versus baseline.
§ p value versus normal controls after 3 weeks.

guidelines three weeks and six months after vaccination. The antibody levels did not differ significantly between groups ($p=0.532$ and 0.286 for GMT at three weeks and six months, respectively; $p=1.000$ and 1.000 for seroprotection rate at three weeks and six months, respectively; $p=1.000$ and 1.000 for seroconversion rate at three weeks and six months, respectively). The GMT, seroprotection rate, and seroconversion rates were lower in SLE patients and normal controls at six months than at three weeks. The seroprotection rates of both SLE patients and controls did not meet the CPMP criteria at six months (66.7% in SLE patients and 60% in normal controls).

The HAI titre varied widely in the SLE patients. To identify any influence of immunosuppressive agents on vaccination efficacy, we analysed the seroprotection rate and seroconversion rates at three weeks and six months after vaccination in SLE patients using immunosuppressive agents. We compared the effect of each of these drugs (prednisolone, AZA, or HCQ). At three weeks, the GMT of serum HAI and seroprotection rate increased significantly, compared with those before vaccination ($p=0.03$, 0.04, and 0.04 for GMT, respectively; $p<0.001$ for seroprotection rate in each group). The seroconversion rate in each group met the CPMP guidelines. No difference was found in the GMT, the percentages of seropro-

Table 4
A/H1N1 influenza vaccination specific seroprotection rate and seroconversion rate in SLE patients and normal controls before and three weeks after the vaccination.

	SLE patients ($n=21$)	Normal controls ($n=15$)	§p value
Geometric mean titer			
$t=0$ day	28.28	28.28	NS
$t=21$ days	148.74	116.19	NS
$t=6$ months	60.14	44.50	NS
Seroprotection rate			
$t=0$ day	9.5% (2/21)	6.7% (1/15)	NS
$t=21$ days	76.2% (16/21)‡ (<0.001)	80.0% (12/15)‡ (<0.001)	NS
$t=6$ months	66.7% (14/21)‡ (<0.001)	60.0% (9/15)‡ (<0.001)	NS
Seroconversion rate			
21 days	76.2% (16/21)	80.0% (12/15)	NS
6 months	52.4% (11/21)	53.3% (8/15)	NS

Note: The intra-bracket values are true subject numbers; the haemagglutination inhibition (HAI) titers met the seroprotection criteria before the vaccinations is excluded in calculating the seroprotection rate, so was seroconversion rate; seroprotection = titers ≥ 40; seroconversion rate = the proportion of subjects with a prevaccination HAI antibody titer <1:10 and a post-vaccination titer ≥ 1:40, or a pre-vaccination titer ≥ 1:10 and an increase in the titer by a factor of four or more; SLE: systemic lupus erythematosus; NS: non-significant.
§ p value versus normal controls.
‡ p value < 0.01 versus baseline.

Table 5
A/H1N1 influenza vaccination specific seroprotection rate and seroconversion rate in SLE patients of different immunosuppressive agents.

	Prednisolone (n = 17)	AZA (n = 18)	HCQ (n = 15)
Geometric mean titer			
t = 0 day	30.31	30.31	25.20
t = 21 days	127.0† (0.03)	113.1† (0.04)	152.27† (0.04)
t = 6 months	55.08	53.84	58.10
Seroprotection rate			
t = 0 day	5.9% (1)	5.6% (1)	0
t = 21 days	70.6% (12)‡ (<.0001)	72.2% (13)‡ (<.0001)	80.0% (12)‡ (<.0001)
t = 6 months	64.7% (11)‡ (<.0001)	61.1% (11)‡ (<.0001)	73.3% (11)‡ (<.0001)
Seroconversion rate			
t = 21 days	70.6% (12)	72.2% (13)	80.0% (12)
t = 6 months	47.1% (8)	55.6% (10)	66.7% (10)

Note: The intra-bracket values are true subject numbers; the haemagglutination inhibition (HAI) titers met the seroprotection criteria before the vaccinations is excluded in calculating the seroprotection rate, so was seroconversion rate; seroprotection = titers ≥ 40; seroconversion rate = the percentage of vaccine recipients with an increase in serum anti-A/H1N1 IgG by at least four times after vaccination compared with titers before vaccination; SLE: systemic lupus erythematosus.

† p value < 0.05 versus baseline.

‡ p value < 0.01 versus baseline.

Table 6
A/H1N1 specific immunity in SLE patients with multiple immunosuppressive agents.

	Prednisolone & AZA (n = 15)	AZA & HCQ (n = 12)	HCQ & prednisolone (n = 13)
Geometric mean titer			
t = 0	33.6	28.3	28.3
t = 21 days	99.0	109.6	134.5
t = 6 months	48.3	49.2	51.5 I
Seroprotection rate			
t = 0 day	5.9% (1)	5.6% (1)	0
t = 21 days	70.6% (12)‡ (<.0001)	75.0% (9)‡ (<.0001)	76.9% (10)‡ (<.0001)
t = 6 months	60% (9)‡ (<.0001)	66.6% (8)‡ (<.0001)	69.2% (9)‡ (<.0001)
Seroconversion rate			
t = 21 days	66.7% (10)	75.0% (9)	76.9% (10)
t = 6 months	40.0% (6)	58.3% (7)	61.5% (8)

Note: The intra-bracket values are true subject numbers; the haemagglutination inhibition (HAI) titres met the seroprotection criteria before the vaccinations is excluded in calculating the seroprotection rate, so was seroconversion rate; seroprotection = titres ≥ 40; seroconversion rate = the percentage of vaccine recipients with an increase in serum anti-A/H1N1 IgG by at least four times after vaccination compared with titres before vaccination; SLE: systemic lupus erythematosus.

‡ p value < 0.01 versus baseline.

tection and seroconversion rate among these three groups. At six months, GMT, percentage of seroprotection, and seropconversion rate decreased. Only SLE patients taking HCQ met the CPMP criteria for seroprotection. The seroconversion rate was more than 40% in each group using single immunosuppressive agent. Nevertheless, No difference was found in the GMT, the percentages of seroprotection and seroconversion rate among these three groups (Table 5).

In patients taking dual immunosuppressive treatments (prednisolone and AZA, AZA and HCQ, or HCQ and prednisolone), the seroprotection rate at three weeks met the CPMP criteria but did not at six months. The seroconversion rate of every group at three weeks and six months also met the CPMP guideline. The evaluation of GMT, the percentages of seroprotection and seroconversion rate among these three groups revealed no specific differences (Table 6).

4. Discussion

Published studies of trivalent influenza vaccination (A/H1N1, A/H3N2, and type B influenza) in SLE patients investigated whether disease activity and autoantibodies change after vaccination, especially in patients with pre-existing nephritis or neurological manifestations [25–31]. Recent reports proved the safety of trivalent influenza vaccination in patients with quiescent SLE, but the vaccine may have lower efficacy [32] and patients may have reduced humoral and cell-mediated immunity, such as a more rapid decline in anti-influenza antibody titres [33–35].

The immune system should respond to A/H1N1 influenza in the first week following the vaccination. Thus, we should theoretically observe any flare or deteriorations in SLEDAI scores in SLE patients caused by side-effects from specific anti-A/H1N1 antibodies [26–39]. Only one SLE patient experienced a flare presenting as recurrent optic neuritis. Our study showed no significant increase in SLEDAI scores and autoantibodies six months after vaccination, suggesting the reliability and safety of the A/H1N1 vaccine in SLE patients. Flares of SLE depend on the complex activation of the immune system including cytokine releases and T and B lymphocyte activation. More evidence is needed to evaluate accurately the safety of vaccine administration.

Trivalent influenza vaccination might not predispose to the formation of antiphospholipid antibodies in SLE patients [37,40]. aCL IgG antibody level increased three weeks after vaccination in SLE patients but was not elevated six months after vaccination. aCL IgG level tended to increase in the normal controls in the follow-up period. Nonetheless, the aGPI IgG level both had decreased in SLE patients and normal controls. Antiphospholipid antibodies did not exceed normal range and thromboembolic events did not occur in either group after six months of observation, which might indicate the safety of vaccination.

The subpopulations of lymphocytes did not differ significantly in SLE patients. Previous reports also showed no significant variation in lymphocyte subpopulations in SLE patients after the trivalent influenza vaccine [39]. Other reports showed that some people develop adverse neurological symptoms such as optic neuritis [41],

myalgia, and fatigue following this new A/H1N1 vaccination. The influenza vaccination is not associated with an increased risk of optic neuritis [42], and recent studies show no adverse neurological effects of this A/H1N1 vaccine [11–13]. The SLE patient in our study who reported previous lupus-associated optic neuritis experienced recurrence after the vaccination. The blurred vision disappeared within one week of onset after the pulse methylprednisolone therapy and did not relapse in the six-month follow-up. Our study has the longest follow-up period compared with previous studies of similar variables such as autoantibody formation and clinical effects. More research is needed to differentiate vaccine-dependent neurological manifestations from those associated with flares of SLE.

The seroprotection rate and the seroconversion rate increased significantly at three weeks and six months in both groups and met the CPMP criteria. The seroprotection rate was lower in SLE patients than in controls, but the GMT of HAI was higher in SLE patients, although the serum GMT and rates of seroprotection and seroconversion did not differ between groups. Some studies have shown that the efficacy of A/H1N1 vaccination within a trivalent influenza vaccine is the same for SLE patients and normal controls [29–31,38], whereas others have disagreed [32,43,44]. However, the vaccine is reliable and effective in protecting SLE patients against the new A/H1N1 influenza virus.

A trend toward a lower humoral response to influenza vaccination in SLE patients taking prednisolone at a dose of >10 mg/day or taking AZA has been reported [45]. Though our reports showed relative higher GMT level, seroprotection rate, and seroconversion rate in patients prescribed with HCQ, the response did not differ between patients treated with a single immunosuppressive agent or combination therapy. Multiple immunosuppressive agents are often prescribed for SLE patients. Our study demonstrated no significant variations among dual therapy.

Antibody responses to A/H1N1 included within the trivalent influenza vaccination in the general population demonstrated either decreased antibody responses, the same, or better responses [46–52]. Our data showed no difference in the humoral response to the new A/H1N1 influenza virus between SLE patients and normal controls who had not had previous influenza vaccinations. There might be cross-reactivity between these two different A/H1N1 subtype influenza viruses because of antigen similarity. That may explain why we simultaneously detected serum IgG levels in excess of HAI titres; further investigation is needed to confirm this possibility.

Our study has some limitations. The enrolled population was relatively small, whereas the comparisons were multidimensional. Not all SLE patients had similar lupus activity, prevaccination medications, or dysregulation of autoimmunity. Studies of larger populations are needed to obtain full data on the response to the A/H1N1 vaccine in SLE patients. The response of cell-mediated immunity to the A/H1N1 vaccinations also needs to be studied. Information about cell-mediated immunity is scarce even for the prevailing trivalent influenza vaccination. Finally, more studies are needed to clarify the cross-reactivity between the influenza vaccines and to determine whether repeated vaccination is necessary to increase the seroprotection rate and the seroconversion rate for SLE patients.

Despite such restrictions, ours is the first prospective cohort study on the safety of and immunogenicity induced by the 2009 A/H1N1 vaccine in SLE patients. SLE patients can obtain significant immunity from the vaccination without experiencing clinical changes in autoimmunity. It is safe for SLE patients to receive the vaccination to prevent the high mortality and morbidity caused by this new A/H1N1 swine influenza virus infection. The seroprotection rate decreased and did not meet the CPMP criteria six months after vaccination. The effect of immunosuppressive therapy need more advanced laboratory examinations and clinical observation.

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References

[1] Centers for Disease Control and Prevention (CDC). Swine influenza A (A/H1N1) infection in two children—Southern California, March–April 2009. MMWR Morb Mortal Wkly Rep 2009;58:400–2.
[2] Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, et al. Antigenic and genetic characteristics of swine-origin 2009 A(A/H1N1) influenza viruses circulating in humans. Science 2009;325:197–201.
[3] Centers for Disease Control and Prevention (CDC). Outbreak of swine-origin influenza A (A/H1N1) virus infection—Mexico, March–April 2009. MMWR Morb Mortal Wkly Rep 2009;58:467–70.
[4] World Health Organization. New influenza A (A/H1N1) virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009;84:249–57.
[5] Kumar A, Zarychanski R, Pinto R, et al. Critically ill patients with 2009 influenza A(A/H1N1) infection in Canada. JAMA 2009;302:1872–9.
[6] ANZIC Influenza Investigators, Webb SA, Pettila V, et al. Critical care services and 2009 A/H1N1 influenza in Australia and New Zealand. N Engl J Med 2009;361:1925–34.
[7] Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 A/H1N1 influenza in the United States, April–June 2009. N Engl J Med 2009;361:1935–44.
[8] Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (A/H1N1) virus in humans. N Engl J Med 2009;360:2605–15.
[9] Centers for Disease Control and Prevention (CDC). Intensive-care patients with severe novel influenza A(A/H1N1) virus infection—Michigan, June 2009. MMWR Morb Mortal Wkly Rep 2009;58:749–52.
[10] Centers for Disease Control and Prevention (CDC). Use of influenza A (A/H1N1) 2009 monovalent vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2009;58:1–8.
[11] Lu CY, Shao PL, Chang LY, et al. Immunogenicity and safety of a monovalent vaccine for the 2009 pandemic influenza virus A (H1N1) in children and adolescents. Vaccine 2010;16(28):5864–70.
[12] Kung HC, Huang KC, Kao TM, et al. A clinical study to assess the immunogenicity and safety of a monovalent 2009 influenza A (H1N1) vaccine in an area with low-level epidemics of pandemic influenza. Vaccine 2010;21(28):7337–43.
[13] Kao TM, Hsieha SM, Kung HC, et al. Immune response of single dose vaccination against 2009 pandemic influenza A(H1N1) in the Taiwanese elderly. Vaccine 2010;28:6159–63.
[14] Yurasov S, Wardemann H, Hammersen J, et al. Defective B cell tolerance checkpoints in systemic lupus erythematosus. J Exp Med 2005;201:703–11.
[15] Mohan C, Datta SK. Lupus: key pathogenic mechanisms and contributing factors. Clin Immunol Immunopathol 1995;77:209–20.
[16] Pugh-Bernard AE, Cambier JC. B cell receptor signaling in human systemic lupus erythematosus. Curr Opin Rheumatol 2006;18:451–5.
[17] Wraith DC, Goldman M, Lambert PH. Vaccination and autoimmune disease: what is the evidence? Lancet 2003;362:1659–66.
[18] Mercado U, Acosta H, Avendaño L. Influenza vaccination of patients with systemic lupus erythematosus. Rev Invest Clin 2004;56:14–8.
[19] Brandt JT, Triplett DA, Alving B, et al. Criteria for the diagnosis of lupus anticoagulant: an update. Thromb Haemost 1995;74:1185–90.
[20] Fattorossi A, Matricardi PM, Pizzolo JG, et al. Lack of specificity in the mechanisms involved in the enhancement of the concanavalin A driver human T lymphocyte stimulation by B-endorphin: studies on activation marker expression, cell cycle and interleukin release. J Biol Regul Homeost Agents 1991;5:91–7.
[21] Biselli R, Farrace S, D'Amelio R, et al. Influence of stress on lymphocyte subset distribution. A flow cytometric study in young student pilots. Aviat Space Environ Med 1993;64:116–20.
[22] Gladman DD, Ibanez D, Urowitz B. Systemic lupus erythematosus disease activity index. J Rheumatol 2002;29:288–91.
[23] Fuenzalida L, Blanco S, Prat C, et al. Utility of the rapid antigen detection BinaxNOW A&B test for detection of novel influenza A (A/H1N1) virus. Clin Microbiol Infect 2009 Dec 29 [Epub ahead of print].
[24] Belshe RB, Newman FK, Cannon J, et al. Serum antibody responses after intradermal vaccination against influenza. N Engl J Med 2004;351:2286–94.
[25] Choen AD, Shoenfeld Y. Vaccine-induced autoimmunity. J Autoimmun 1996;9:699–703.
[26] Hess EV, Hann HB. Influenza immunization in lupus erythematosus: safe, effective? Ann Intern Med 1978;88:833–4.
[27] Williams GW, Steinberg AD, Reinsen L. Influenza immunization in SLE. A double blind trial. Ann Intern Med 1978;88:729–34.

[28] Ristow SC, Douglas RG, Condemi JJ. Influenza vaccination of patients with SLE. Ann Intern Med 1978;88:786–9.
[29] Broadman R, Gilfillan R, Glass D. Influenza vaccine response in SLE. Ann Intern Med 1978;88:735–40.
[30] Louie JS, Nies KM, Shoji KT, et al. Clinical and antibody responses after influenza immunization in SLE. Ann Intern Med 1978;88:790–2.
[31] Herron A, Dettleff G, Hixon B, et al. Influenza vaccination in patients with rheumatic diseases. Safety and efficacy. JAMA 1979;242:53–6.
[32] Holvast A, Huckriede A, Wilschut J, et al. Safety and efficacy of influenza vaccination in systemic lupus erythematosus patients with quiescent disease. Ann Rheum Dis 2006;65:913–8.
[33] Rimmelzwaan GF, Nieuwkoop N, Brandenburg A, et al. A randomized, double blind study in young healthy adults comparing cell mediated and humoral immune responses induced by influenza ISCOM vaccines and conventional vaccines. Vaccine 2000;19:1180–7.
[34] Rimmelzwaan GF, Nieuwkoop N, Brandenburg A, et al. Vaccination of the immunosuppressed adult patient with rheumatologic disease. Rheum Dis Clin North Am 1999;25:567–84.
[35] Holvast A, van Assen S, de Haan A, et al. Studies of cell-mediated immune responses to influenza vaccination in systemic lupus erythematosus. Arthritis Rheum 2009;60:2438–47.
[36] Abu-Shakra M, Zalmanson S, Neumann L, et al. Influenza virus vaccination of patients with systemic lupus erythematosus: effects on disease activity. J Rheumatol 2000;27:1681–5.
[37] Abu-Shakra M, Press J, Sukenik S, et al. Influenza virus vaccination of patients with SLE: effects on generation of autoantibodies. Clin Rheumatol 2002;21:369–72.
[38] Kanakoudi-Tsakalidou F, Trachana M, Pratsidou-Gertsi P, et al. Influenza vaccination in children with chronic rheumatic diseases and long-term immunosuppressive therapy. Clin Exp Rheumatol 2001;19:589–94.
[39] Del Porto F, Laganà B, Biselli R, et al. Influenza vaccine administration in patients with systemic lupus erythematosus and rheumatoid arthritis. Safety and immunogenicity. Vaccine 2006;24:3217–23.
[40] Tarján P, Sipka S, Lakos G, et al. Influenza vaccination and the production of anti-phospholipid antibodies in patients with systemic lupus erythematosus. Scand J Rheumatol 2006;35:241–3.
[41] Lapphra K, Huh L, Scheifele DW. Adverse neurologic reactions after both doses of pandemic H1N1 influenza vaccine with optic neuritis and demyelination. Pediatr Infect Dis J 2010 [Epub ahead of print].
[42] DeStefano F, Verstraeten T, Jackson LA, et al. Vaccinations and risk of central nervous system demyelinating diseases in adults. Arch Neurol 2003;60:504–9.
[43] Holvast B, Huckriede A, Kallenberg CG, et al. Influenza vaccination in systemic lupus erythematosus. Autoimmun Rev 2007;6:300–5.
[44] Więsik-Szewczyk E, Romanowska M, Mielnik P, et al. Anti-influenza vaccination in systemic lupus erythematosus patients: an analysis of specific humoral response and vaccination safety. Clin Rheumatol 2010;29:605–13.
[45] Abu-Shakra M, Press J, Varsano N, et al. Specific antibody response after influenza immunization in systemic lupus erythematosus. J Rheumatol 2002;29:2555–7.
[46] Pyhala R, Kumpulainen V, Alanko S, et al. HI antibody kinetics in adult volunteers immunized repeatedly with inactivated trivalent influenza vaccine in 1990–1992. Vaccine 1994;12:947–52.
[47] Gross PA, Sperber SJ, Donabedian A, et al. Paradoxical response to a novel influenza virus vaccine strain: the effect of prior immunization. Vaccine 1999;17:2284–9.
[48] Govaert TM, Sprenger MJ, Dinant GJ, et al. Immune response to influenza vaccination of elderly people: a randomized double-blind placebo-controlled trial. Vaccine 1994;12:1185–9.
[49] De Bruijn I, Remarque EJ, Beyer WE, et al. Annually repeated influenza vaccination improves humoral responses to several influenza virus strains in healthy elderly. Vaccine 1997;15:1323–9.
[50] De Bruijn I, Remarque EJ, Jol-van der Zijde CM, et al. Quality and quantity of the humoral immune response in healthy elderly and young subjects after annually repeated influenza vaccination. J Infect Dis 1999;179:31–6.
[51] Keitel WA, Cate TR, Couch RB. Efficacy of sequential annual vaccination with inactivated influenza virus vaccine. Am J Epidemiol 1988;127:353–64.
[52] Keitel WA, Cate TR, Couch RB, et al. Efficacy of repeated annual immunization with inactivated influenza virus vaccines over a five-year period. Vaccine 1997;15:1114–22.