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Concise report

Pandemic non-adjuvanted influenza A H1N1 vaccine in a cohort of patients with systemic sclerosis

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Abstract

Objective. To assess the possible effect of therapy, disease subtype and severity on H1N1 immunogenicity in patients with SSc.

Methods. Ninety-two patients and 92 age- and gender-matched healthy controls received adjuvant-free influenza A/California/7/2009 (pH1N1) vaccine. Blood samples were collected immediately before and 3 weeks after vaccination to evaluate antibody responses to the H1N1 virus. Efficacy was assessed by seroprotection (SP) and seroconversion (SC) rates and the factor increase in geometric mean antibody titre. Participants received a 21-day symptom diary card and were instructed to report local and systemic adverse events.

Results. SSc patients were predominantly females (91%) and 61% had limited SSc, 12% had severe skin involvement and 57.6% were on immunosuppressive (IS) therapy. SSc patients and controls presented comparable overall SP ($P = 0.20$) and SC ($P = 0.61$) rates. Further evaluation of the possible effect of disease and therapy revealed similar rates of SP and SC in patients with dcSSc *vs* lcSSc (SP $P = 0.62$ and SC $P = 0.66$), severe *vs* mild/moderate skin involvement (SP $P = 1$ and SC $P = 0.45$) and with *vs* without IS (SP $P = 0.26$ and SC $P = 0.10$). The frequency of mild local and minor systemic reactions was similar in patients with dcSSC *vs* lcSSc ($P = 0.70$ *vs* 0.32) and in those with and without severe skin involvement ($P = 0.59$ *vs* 0.28).

Conclusion. The non-adjuvanted influenza H1N1 virus vaccine proved to be safe and effective, independent of SSc clinical subtype, disease severity or therapy. These latter factors do not seem to contribute to mild adverse events observed in SSc. Our data support the annual influenza vaccination recommendation for these patients.

Trial registration: ClinicalTrials.gov (http://clinicaltrials.gov), NCT01151644

Key words: systemic sclerosis, scleroderma, influenza, vaccine, pandemic influenza A H1N1, non-adjuvanted influenza vaccine

CLINICAL SCIENCE

Rheumatology key messages

- The non-adjuvanted influenza H1N1 virus vaccine was safe and effective in SSc.
- The efficacy of influenza H1N1 vaccination was independent of SSc subtype, severity or therapy.
- The annual influenza vaccination can be recommended for SSc patients.

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Introduction

SSc is a chronic autoimmune disease characterized primarily by cutaneous involvement, but multiple internal organs may also be affected. SSc is characterized by the pathological triad of immune dysregulation, microvascular dysfunction and fibrosis affecting skin and internal organs. SSc aetiology is still unknown, but there is evidence that genetic and environmental factors may be important triggers for disease development [1].

Immunosuppressive (IS) drugs are often used to treat cutaneous and pulmonary involvement in SSc [2]. Consequently, the occurrence of infections, particularly in the respiratory tract, represents an important cause of morbidity and mortality in these patients [3, 4]. Vaccination must be considered in this context, as vaccines represent the most effective preventive measure to control virus dissemination and to reduce its associated complications [5].

Although the EULAR [5] and the 2010 recommendations of the Advisory Committee on Immunization Practices [6] have indicated immunocompromised patients should receive vaccine for seasonal and pandemic influenza, data regarding its immunogenicity and safety in SSc are scarce.

An Italian study observed satisfactory humoral immune response and clinical safety of a virosomal flu vaccine in 46 scleroderma patients without IS treatment compared with 20 controls [7]. The overall safety and adequate A(H1N1)pdm09 influenza vaccine response was further demonstrated in two cohorts of various autoimmune rheumatic diseases [8, 9], without a specific analysis of scleroderma subgroup. In addition, no data are provided regarding the possible influence of disease subtypes, severity or treatment on vaccine immune response in scleroderma patients.

Thus the aim of this study was to evaluate in SSc patients the impact of disease and therapy on humoral immune response to pandemic non-adjuvanted influenza A(H1N1)pdm09 vaccine.

Methods

All SSc patients and healthy controls were recruited during the Public Health Pandemic Influenza Vaccination Campaign between March and April 2010 in a large, prospective rheumatic disease cohort study conducted at a single centre (described in detail elsewhere [8]). The study was approved by the local institutional review board (Comissão de Ética em Pesquisa em Seres Humanos da Faculdade de Medicina da USP) and all participants signed the informed consent. The trial was registered at ClinicalTrials.gov (NCT01151644). This approval covered the current study so no additional approval was needed.

Patients and healthy individuals

All SSc patients (according to the 1980 ARA criteria for the classification of SSc [10]) >18 years of age [mean age 52 years (S.D. 5.3)] regularly followed at the Systemic Sclerosis Outpatient Clinic were consecutively invited to participate. All participants signed the informed consent. Medical charts were extensively reviewed for additional clinical and treatment data. The following data were recorded: age, gender, disease duration, limited and diffuse variants of SSc, modified Rodnan skin score (mRSS) and use of immunosuppressive therapy (MTX, AZA, MMF and/or intravenous CYC). Exclusion criteria included a history of hypersensitivity to egg protein; personal or family history of Guillain–Barré syndrome or demyelinating disease; bleeding or any coagulation disorder; influenza illness; fever 72 h before vaccination; hospitalized patients; and previous vaccination with live virus vaccine <4 weeks, inactivated virus <2 weeks, anti-influenza virus <6 months and blood products transfusion in the last 6 months. Ninety-two age- and gender-matched healthy subjects were included as volunteers, after informed consent.

Vaccine

The A(H1N1)pdm09 vaccine, a novel, monovalent, non-adjuvanted, inactivated and split-virus vaccine, was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The active substance is a split, inactivated influenza virus containing antigens equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the World Health Organization. The vaccine was propagated in embryonated chicken eggs, with the same standard techniques that are used for the production of seasonal trivalent inactivated vaccines, and it was presented in 5 ml multidose vials, with thimerosal added as a preservative (15 μg/0.5 ml dose).

Study design

Patients and healthy individuals were assessed immediately before and 21 days after vaccination to determine seroprotection (SP) and seroconversion (SC) by haemagglutination inhibition assay (HIA) (Adolfo Lutz Institute, São Paulo, Brazil). Side effects (local pain, fever, arthralgia and flu symptoms) were also evaluated through a diary card.

Vaccination

All subjects were vaccinated with the pandemic influenza vaccine (A/California/7/2009/H1N1-like virus, Butantan Institute/Sanofi Pasteur). A single intramuscular dose (0.5 ml) of 15 μg haemagglutinin antigen, specific for the A/California/7/2009 (H1N1)-like virus, was administered.

Safety assessments

A 21 day diary card was given to each participant at study entry with a list of 13 possible adverse reactions, including local (pain, redness, swelling and itching) and systemic (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea and nasal congestion) reactions. Participants were required to return their diary cards at the end of the follow-up period (21 days after vaccination). All local reactions were considered to be related to the A(H1N1)pdm09 vaccine. Severe side effects were defined as those requiring hospitalization or leading to death.

Laboratory assays

Blood samples from patients and controls were collected at baseline and 3 weeks after vaccination for evaluation of the A(H1N1)pdm09 vaccination serological response. The immunogenicity of the A/California/7/2009 (H1N1)-like virus vaccine was evaluated by the use of an HIA. The two samples from each patient obtained immediately before and 21 days after vaccination were always tested in parallel in the same assay.

HIA

The influenza virus antigen used in this study was the A/California/7/2009 (H1N1) supplied by the Butantan Institute. Virus concentrations were determined by haemagglutinin antigen titration and the HIA test was performed after removing naturally occurring, non-specific inhibitors from the sera, as previously described [11]. Immunogenicity of A(H1N1)pdm09 vaccine was evaluated by determining levels of specific antibodies by HIA, and anti-H1N1 titres were determined by influenza HIA. The percentages of SP (titre $\geqslant$1:40) and SC (pre-vaccination titre $<$1:10, post-vaccination HIA titre $\geqslant$1:40, pre-vaccination titre $\geqslant$1:10 and a $\geqslant$4-fold rise post-vaccination), geometric mean titres (GMTs) and factor increases in GMTs (FI-GMTs) were calculated [8].

Statistical analysis

Age matching of the SSc group and healthy controls was carried out by random selection using SPSS software (version 15; IBM, Armonk, NY, USA). GMTs and FI-GMTs were calculated and analysed using log-transformed data. Comparisons between two groups were conducted using Student's *t* test or Mann–Whitney U test (continuous variables) and chi-squared test or Fisher's exact test (categorical variables). A predictor analysis including treatment (Fisher's exact test and U test) and age (Spearman correlation) was performed. *P*-values $<$0.05 were considered to be statistically significant.

Results

Demographics of SSc patients before vaccination

Among the 92 SSc patients, there was a predominance of lcSSc (61%) and female gender (91%), with a mean age of 46 years (S.D. 10.7), mean disease duration of 11.2 years (S.D. 7.3) and mean mRSS of 3.6 (S.D. 7.0). ANA, anti-Scl70 and anticentromere were positive in 95.7, 27.2 and 25% of patients, respectively.

The immunosuppressant therapy was used by 53 patients (57.6%): MTX [mean dosage 14.5 mg/week (S.D. 3.8)] in 21.7%, AZA [mean dosage 118.4 mg/day (S.D. 25.2)] in 19.6%, CYC in 8.7% and MMF in 6.5% of the patients.

Overall immunogenicity of A(H1N1)pdm09 vaccine

SSc patients and controls presented similar pre-vaccination SP rates (20.7 *vs* 12%; $P = 0.11$) and GMTs (11.3 *vs* 8.8; $P = 0.42$). After vaccination, the SP rate (83.7 *vs* 76.1%; $P = 0.20$), SC rate (76.1 *vs* 72.8%; $P = 0.61$) and FI-GMT (14.7 *vs* 11.8; $P = 0.34$) were comparable in patients and controls. Of note, the GMT was higher in patients than controls (166.1 *vs* 104.1; $P = 0.03$).

Influence of SSc clinical presentation and IS therapy on vaccine humoral immune response

Patients with the diffuse *vs* limited subtype had similar SP rates (86.1 *vs* 82.1%; $P = 0.62$), SC rates (75 *vs* 76.8%; $P = 0.66$), GMTs (209.5 *vs* 143.1; $P = 0.26$) and FI-GMTs (13.5 *vs* 15.5; $P = 0.68$) after vaccination. Likewise, patients with severe skin involvement (mRSS $\geqslant$14) *vs* mild/moderate skin involvement had comparable SP rates (81.8 *vs* 84%; $P = 1$), SC rates (63.6 *vs* 77.8%; $P = 0.45$), GMTs (132.4 *vs* 171.3; $P = 0.55$) and FI-GMTs (8.5 *vs* 15.8; $P = 0.23$) after vaccination (Table 1).

Patients on IS vs without IS therapy were alike regarding SP rate (79.2 *vs* 89.7%; $P = 0.26$), SC rate (69.8 *vs* 84.6%; $P = 0.10$), GMT (166.4 *vs* 165.8; $P = 0.82$) and FI-GMT (13.7 *vs* 16.1; $P = 0.74$) after vaccination (Table 1). When analysed separately, patients on MTX *vs* without IS [SP rate 75.0 *vs* 89.7% ($P = 0.25$), SC rate 65.0 *vs* 84.6% ($P = 0.11$), GMT 117.1 *vs* 165.8 ($P = 0.36$) and FI-GMT 10.2 *vs* 16.1 ($P = 0.25$)] and on AZA *vs* without IS [SP rate 84.2 *vs* 89.7% ($P = 0.67$), SC rate 78.9 *vs* 84.6% ($P = 0.72$), GMT 206.6 *vs* 165.8 ($P = 0.69$) and FI-GMT 16.6 *vs* 16.1 ($P = 0.98$)] confirmed these results. There was no association between vaccine response parameters and age, steroid use and IS drugs use (data not shown).

Overall vaccine side effects

SSc patients and controls presented similar rates of local side effects (7.6 *vs* 10.9%; $P = 0.45$) and minor systemic reactions (25 *vs* 31.5%; $P = 0.33$). No severe events occurred in these patients post-vaccination.

Influence of SSc clinical presentation and treatment on vaccine side effects

Patients with the diffuse *vs* limited subtype had comparable frequencies of local side effects (5.6 *vs* 8.9%; $P = 0.70$) and minor systemic reactions (19.4 *vs* 28.6%; $P = 0.32$). Similarly, patients with severe (mRSS $\geqslant$14) *vs* mild/moderate skin involvement had similar frequencies of local side effects (0 *vs* 8.6%; $P = 0.59$) and minor systemic reactions (9.1 *vs* 27.2%; $P = 0.28$). Patients on IS *vs* without IS were alike regarding local side effects (7.5 *vs* 7.7%; $P = 1$) and minor systemic reactions (26.4 *vs* 23.1%; $P = 0.71$).

Discussion

The present study showed that SSc disease subtypes, skin severity and treatment do not seem to compromise the immunogenicity, safety and efficacy of non-adjuvanted H1N1 influenza vaccine. The major advantage of our study is the inclusion of a sizeable sample of scleroderma patients, a rare disease, allowing the investigation of disease and therapy factors that could interfere with humoral immune response to the H1N1 virus vaccine.

Table 1 Serological data before and after influenza H1N1/2009 vaccine in controls and systemic sclerosis patients

Subset	Pre-vaccination		Post-vaccination			
	GMT	SP (%)	GMT	SP (%)	FI-GMT	SC (%)
SSc patients (n = 92)	11.3 (8.8, 14.6)	20.7 (12.3, 29.0)	166.1 (119.6, 230.8)	83.7 (76.1, 91.3)	14.7 (10.6, 20.3)	76.1 (67.3, 84.9)
Controls (n = 92)	8.8 (7.4, 10.4)	12 (5.3, 18.6)	104.1 (77.8, 139.4)	76.1 (67.3, 84.9)	11.8 (9.1, 15.5)	72.8 (63.7, 82)
P-value	0.42	0.11	0.03	0.20	0.34	0.61
Diffuse SSc (n = 36)	15.6 (9.8, 24.8)	30.6 (15.3, 45.8)	209.5 (123.2, 356.3)	86.1 (74.7, 97.6)	13.5 (8, 22.5)	75 (60.7, 89.3)
Limited SSc (n = 56)	9.2 (7.0, 12.2)	14.3 (5.0, 23.5)	143.1 (94.2, 217.4)	82.1 (72, 92.3)	15.5 (10.2, 23.6)	76.8 (65.6, 87.9)
P-value	0.06	0.06	0.26	0.62	0.68	0.66
mRSS $\geqslant$14 (n = 11)	15.5 (5.2, 46.1)	27.3 (0, 54.9)	132.4 (54.9, 319.4)	81.8 (57.9, 100)	8.5 (3.4, 21.5)	63.6 (33.8, 93.5)
mRSS <14 (n = 81)	10.9 (8.5, 13.9)	19.8 (11, 28.5)	171.3 (120.1, 244.4)	84 (75.9, 92)	15.8 (11.2, 22.3)	77.8 (68.7, 92)
P-value	0.92	0.69	0.55	1	0.23	0.45
On IS (n = 53)	12.2 (8.6, 17.2)	22.6 (11.3, 34)	166.4 (105.3, 263)	79.2 (68.2, 90.3)	13.7 (8.6, 21.7)	69.8 (57.3, 82.3)
No IS (n = 39)	10.3 (7.2, 14.8)	17.9 (5.7, 30.2)	165.8 (103.5, 265.4)	89.7 (80.1, 99.4)	16.1 (10.3, 25.1)	84.6 (73.1, 96.1)
P-value	0.45	0.58	0.82	0.26	0.74	0.10

Data are expressed as n (95% CI for GMT, FI-GMT) and % (95% CI for SP, SC). P-values relate to comparison with the preceding group.

In this regard, SSc is a complex autoimmune disease driven by an interplay between inflammation, cytokine disturbances and fibroblast activation [12]. Although diffuse SSc has shown a significantly poorer survival compared with limited SSc [3, 4], our findings showed that this clinical difference in prognosis of the disease did not affect the immunogenicity of influenza A H1N1 virus vaccine. Reinforcing this finding, patients with severe skin involvement (mRSS $\geqslant$14) did not have impaired immune response to the H1N1 virus vaccine. Previous studies [7, 9] estimating virosomal influenza vaccine effectiveness in a small group of SSc patients failed to address these potentially interfering clinical variables on the immunogenicity of that vaccine.

In contrast with our findings, ageing has been reported as a potential negative bias on the effectiveness of influenza vaccine in a previous study [13]. The inclusion of an age-matched control group minimized the effect of this factor in our group and the predictor analysis confirmed that age was not associated with the vaccine response.

Another important finding of our study was the observation that IS treatment (MTX, AZA, CYC and MMF) did not affect H1N1 vaccine efficacy in SSc patients in spite of the fact that more than half of these patients were on these therapies. Previous studies in patients with axial SpA and RA have also shown that, with the exception of rituximab, other immunosuppressants did not affect the immunogenicity of the seasonal flu vaccine [14–16]. In contrast, DMARDS (except for HCQ and SSZ) were identified as the main determinants of impaired vaccine response in another study that evaluated 173 patients with inflammatory rheumatic diseases [13]. Likewise, pandemic influenza A H1N1/2009 vaccine response was diminished in SLE patients on IS therapy [17].

Although pulmonary disease is an important cause of morbidity in patients with SSc, the rate of influenza vaccination of these patients is still low, mainly due to a lack of information and fear of adverse events [18]. In a previous study evaluating 199 French patients with a variety of autoimmune rheumatic diseases (including SSc) after A/H1N1 flu vaccination, local reactions were found in 18% and a flulike syndrome was seen in 7.5%, with only two flu episodes considered associated with the vaccination [9]. Patients analysed herein presented similar rates of local side effects and minor systemic reactions after non-adjuvanted influenza A H1N1 vaccine, despite SSc clinical variants, severity and IS therapy.

Importantly, SSc patients regardless of disease subtype, disease severity or the use of IS therapy achieved all three current immunologic standards established for seasonal vaccines/pandemic influenza vaccines to be licensed in healthy adults 18–60 years of age: SP >70%, SC >40% and FI-GMT >2.5 [19, 20].

We therefore strongly recommend seasonal influenza vaccination for SSc patients to minimize viral infections in this high-risk group.

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