Vaccination against influenza in patients with systemic sclerosis

I. Litinsky[1], A. Balbir[2], D. Zisman[3], M. Mandelboim[4], E. Mendelson[4], J. Feld[3], Y. Braun[2], M. Anouk[1], I. Kaufman[1], D. Paran[1], D. Caspi[1], O. Elkayam[1]

[1]Department of Rheumatology, Tel Aviv Medical Center and the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [2]Department of Rheumatology, Rambam Health Care Campus Background, Haifa, Israel; [3]Rheumatology Unit, Carmel Medical Center, Haifa, Israel; [4]Central Virology Laboratory, Sheba Medical Center, Tel Aviv, Israel.

Ira Litinsky, MD
Alexandra Balbir, MD
Devy Zisman, MD
Michal Mandelboim
Ella Mendelson
Joy Feld, MD
Yolanda Braun, MD
Marina Anouk, MD
Ilana Kaufman, MD
Daphna Paran, MD
Dan Caspi, MD
Ori Elkayam, MD

Please address correspondence to:
Ira Litinsky, MD,
Department of Rheumatology,
Tel Aviv Medical Center,
6 Weizman Street,
Tel Aviv 64239, Israel.
E-mail: litinskyira@yahoo.com

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ABSTRACT

Objective. *To assess the efficacy and safety of the influenza virus vaccine in systemic sclerosis (SSc) patients compared to healthy controls.*

Methods. *Twenty-six SSc patients and 16 healthy controls were vaccinated with a trivalent influenza subunit vaccine (H1N1 A/Brisbane/59/2007(TGA 2008/81B) (H1N1), H3N2 A/Uruguay/716/2007 (A/Brisbane/10/2007-like, NIBSC8/124) (H3N2) and B B/Brisbane/60/2008 (TGA 2009/82/B) (B)). The subjects were evaluated on the day of vaccination and 6 weeks later. Disease activity was assessed by the Rodnan score, number of ulcers, number of tender and swollen joints, the presence of dyspnea, cough, dyspepsia and dysphagia, and patient (PDAI) and physician (PHDAI) disease activity evaluation by the visual activity score (VAS), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level. The humoral response was evaluated by haemagglutination inhibition (HI).*

Results. *At baseline, 62%, 15% and 88% of the SSc patients had protective levels against H1N1, H3N2 and B, respectively, versus 56%, 62% and 87% for controls. Six weeks later, the proportion of responders to H1N1 was significantly higher in the SSc patients (73%) compared to controls (37.5%) (p=0.0225). The proportion of responders to H3N2 and B was similar in both groups, and both had a significant increase in geometric mean titers for each antigen. A lower response to H1N1 was associated with interstitial lung disease, while patients on combination calcium channel blockers and iloprost therapy showed significantly better response to H1N1 and B antigens. Most underlying disease activity parameters remained unchanged.*

Conclusions. *The influenza virus vaccine was safe and generated a satisfactory humoral response in SSc patients.*

Introduction

Systemic sclerosis (SSc) is a relatively rare multisystem disease, characterised by excessive collagen deposition, vascular hyperactivity and obliterative microvascular phenomena. It is responsible for significantly decreased survival, especially in the diffuse form of the disease, with potential lung, digestive, renal and cardiac damage (1). Various therapeutic strategies have been used in SSc patients, including vasodilators, antifibrotic agents and immunosuppressants, corticosteroids, and haemapoietic stem cell transplantation.

Infections are the cause of death in 2–9% of SSc patients (2). It not clear whether the increased rate of infections is inherent to the disease itself, directly related to scleroderma organ involvement or due to the growing use of immunosuppressive drugs, such as cyclophosphamide, mofetil mycophenolate and corticosteroids. In a Swedish cohort of 249 SSc patients who had been followed for a mean duration of 5.8 years, 49 died and 6 of those deaths were due to infectious pneumopathy (3). Lung and haemapoietic stem cell transplantation as well as infected digital ulcers seem to further exacerbate the infectious complications (4, 5). While there is no doubt about the increased rate of infections, very few studies have specifically described the type of infections more likely to occur in SSc patients.

Pulmonary disease, including pulmonary parenchymatous disease and pulmonary arterial hypertension (PAH), is the leading cause of death in SSc (2). Respiratory infections may worsen the course of lung diseases and further increase the severity of PAH.

Immunisation is the most efficient way to prevent infections. Although vaccination against influenza is currently recommended for patients who suffer from chronic diseases, including autoimmune diseases such as SSc (6), the

safety and efficacy of vaccination in this patient population has not been established. We, and others, have shown that vaccination against influenza is safe, and that it induces a satisfactory humoral response in rheumatoid arthritis (RA) and in ankylosing spondylitis (AS), although to a lesser extent than that of healthy controls (7-9). There are few published reports on the efficacy and risks associated with vaccination of patients with SSc (10). The purpose of our current study was to assess the efficacy and clinical safety of the influenza virus vaccine in patients with SSc in comparison with healthy controls.

Methods

Subjects

Twenty-six consecutive SSc outpatients routinely treated at three rheumatology centers (Tel Aviv, Rambam and Carmel Medical Centers), classified according to the extent of skin involvement into limited cutaneous SSc (11) and diffuse cutaneous SSc (11), and 16 healthy hospital personnel, matched for age and gender, participated in this study. The vaccination consisted of a trivalent influenza subunit vaccine AGRIPPAL S1, 2009/2010 season influenza vaccine, surface antigen, inactivated (Novartis, Italy) including H1N1 A/Brisbane/59/2007 (TGA 2008/81B) (H1N1), H3N2 A/Uruguay/716/2007 (A/Brisbane/10/2007-like, NIBSC 8/124) (H3N2) and B B/Brisbane/60/2008 (TGA 2009/82/B) administered intramuscularly in the deltoid. Exclusion criteria were pregnancy, a history of past vaccination allergy and known allergy to egg products. Patients were evaluated clinically and blood was drawn for serological testing, both on the day of vaccination and 6 weeks later. The study was approved by the institutional medical ethics committee, and written informed consent was obtained from all participants.

Clinical assessment

Each subject gave a complete history including the current use of medications and underwent a physical examination before receiving the vaccination. Baseline and 6-week clinical assessment included the Rodnan score, the number of ulcers, the number of tender and swollen joints, changes in clinical signs (dyspnea, cough, dyspepsia and dysphagia), the presence or absence of interstitial lung disease (ILD) defined as combination of restriction pattern on pulmonary function test (PFT) and evidence of ILD on HRCT of lung and a patient (PDAI) and physician (PHDAI) disease activity evaluation for the past week by a visual analogue score (VAS), in which 10 represent an extremely severe disease activity and 0 no disease activity (anchored at 0 = ”no disease activity” and at 10 = “very severe disease activity”), asking patient “how severe was your overall health in the week” and asking physician “ how would you rate the patient condition in the last week” Laboratory assessment of disease activity included erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level, on the day of vaccination and 6 weeks later.

Haemagglutination inhibition test

The immunogenicity of the vaccine was tested by the haemagglutination inhibition (HI) test. Influenza virus has two important surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Antigen classification and subtyping of influenza viruses are based on these two glycoproteins. HA has a key role in virus cell entry by binding to cell surface receptors, which are also found on red blood cells of certain species. Binding to red cells results in haemagglutination and this can be observed as a carpet of agglutinated red cells at the bottom of a tube or microtiter well. In the HI test, antibodies directed against the viral HAs block the virus from binding to the blood cells and thus inhibit the haemagglutination reaction.

The pre- and post-immunisation HI antibodies were tested at the Central Virology Laboratory of the Israeli Ministry of Health using the HI test according to a standard WHO procedure (12). Serum samples were separated, code labelled, and stored at -20°C until tested. They were treated with receptor-destroying enzyme cholera filtrate to remove non-specific inhibitors, and with Turkey red blood cells to remove non-specific agglutinins. The treated sera were evaluated by an HI test against the three above-specified antigens included in the vaccine. The working dilution (test dose) of each antigen contained four units in 25 μL antigen. Test doses were diluted in phosphate-buffered saline and added to serial dilutions of antiserum. The HA inhibition titer was determined as the highest dilution of serum that completely inhibited the haemagglutination of the red blood cells. A titer of antiserum that did not show any inhibition was recorded as <1/10. Humoral response was defined as either a ≥4-fold rise in titer, or a rise from a nonprotective baseline level of <1/40 to a protective level ≥1/40 in HI antibodies 6 weeks after vaccination (13, 14). Geometric mean titers (GMTs) of antibodies were calculated to assess the immunity of the whole group.

Data analysis

Comparison of response to vaccination between the patient and control groups was by the Chi-squared tests. A pairwise comparison was done using the false discovery rate method for significance level adjustment whenever there was a significant difference between them. One-way analysis of variance or the Kruskal-Wallis non-parametric test was used, as applicable, to compare the patient and the control group for all continuous variables. Pair-wise comparisons were performed using the Ryan-Einot-Gabriel-Welsch Multiple Range Test for multiple comparisons whenever this analysis yielded a significant result. Various combinations of the patient and control groups were compared using the *t*-test or the Mann-Whitney non-parametric test, as applicable. Association between continuous variables was evaluated using Pearson correlation coefficients. All statistical analyses were performed using the SAS for Windows version 9.1.3.

Results

Characteristics of the patients and controls

Forty-two participants were included in this study: 26 were SSc patients, of whom 12 (46.1%) suffered from the diffuse type, 14 (53.9%) had limited pSS, and 16 were healthy controls. The

patient and control groups were similar in gender (mostly women) (Table I), and age (mean age 51.7 years for SSc and 44.5 years for controls). At the time of vaccination, 7 (26.9%) patients were on immunosuppressive therapy (5 prednisone, 2 mofetil mycophenolate, 2 methotrexate, and 1 Cuprimine,while 17 (65.4%) patients were on combination therapy with calcium channel blockers and iloprost.

Effect of vaccination against influenza on disease activity

Vaccination against influenza was not associated with a significant worsening of any clinical or laboratory index of disease activity (Table II). The only adverse events following vaccination were mild upper respiratory tract infection in two SSc patients. There was no local reaction to the vaccination in any group.

Immunogenicity of influenza vaccine

Pre-vaccination HI antibody protective levels were found for H1N1 in16 (62%) of SSc patients compared to 9 (56%) controls, for H3N2 antibodies in 4 (15%) SSc patients compared to 10 (62%) controls, and for B antigen in 23 (88%) of SSc patients compared to 14 (87%) controls. Six weeks after vaccination, all study participants displayed significant increases in the GMTs of HI antibody against each of the three tested antigens, suggesting a good humoral response for the whole cohort (Table III).

Individual responses of SSc patients and controls to vaccination against influenza

Although as a group, both the SSc patients and controls responded to vaccination, the vaccine did not appear to be uniformly immunogenic in all participants. Six weeks after vaccination, the proportion of responders (either a ≥4-fold rise in titer or a rise from a non-protective baseline level of <1/40 to ≥1/40 in HI antibodies 6 weeks after vaccination) in the SSc group was 73% for the H1N1 antigen compared with 37.8% in the control group (p=0.0225). The proportion of responders against the H3N2 antigen was similar in both groups (42.3% for the SSc patients *vs.* 37.8% for the controls), while the proportion of responders against the B antigen was 50% in the SSC group and 37.5% in the control group (Fig. 1).

Table I. Clinical and demographic characteristic of SSc patients and control subjects.

Characteristics	SSc (n=26)	Healthy controls (n=16)
Age, years (mean±SD)	51.7 ± 12.9	44.5 ± 15.3
Male: Female ratio	1:5.5	1:7
Disease duration, years (mean)	8.29 ± 6.28	
Diffuse type, n	12 (46.1%)	
CREST type, n	14 (53.9%)	
Digital ulcers, n	9 (34.6%)	
PAH, n	7 (26.9%)	
GIT involvement, n	15 (57.7%)	
Musculoskeletal involvement, n	11 (42.3%)	
Raynaud's phenomenon, n	26 (100%)	
Immunosuppressive treatment, n	7 (26.9%)	

F: female; M: male; SSc: Scleroderma; PAH: pulmonary arterial hypertension; GIT: gastrointestinal tract.

Table II. Effects of vaccination on disease activity in scleroderma patients.

Disease activity measures	Before vaccination	After vaccination	p-value
Tender joints (n)	0.5 (1.44)	0.52 (1.23)	0.25
Swollen joints (n)	0.43 (1.07)	0.25 (0.78)	1.0
Digital ulcers (n)	0.46 (1.02)	1.34 (3.04)	0.0625
Rodnan score	10.42 (9.99)	10.3 (9.95)	0.625
PDAI (VAS)	5.12 (2.53)	4.36 (2.48)	0.00156
PHDAI (VAS)	3.75 (2.49)	3.95 (1.92)	0.234
ESR	27.85 (16.84)	27.5 (18.34)	0.826
CRP	1.83 (1.92)	2.59 (5.77)	0.94

Values are mean (SD). PDAI: Patient Disease Activity Index; PHDAI: Physician Disease Activity; VAS: visual activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table III. Geometric mean titers of haemagglutination inhibition (HI) antibodies (μg/ml) against influenza antigens in scleroderma (SSc) patients and controls before and six weeks after vaccination.

Antigen	SSc (n=26)			Controls (n=16)		
	Week 0	Week 6	p-value	Week 0	Week 6	p-value
H1N1	29.35	356.0*	<0.0001	33.63	76.6*	0.02
H3N2	3.28	51.3*	<0.001	41.77	113.13*	<0.01
B	62.9	198*	<0.0001	80	153.21*	0.04

*p<0.05.

Predictors of immunogenicity

We attempted to identify clinical or laboratory indices which might predict a poor response to the vaccine. SSc patients suffering from interstitial lung disease (ILD) demonstrated a significant lower response (p=0.02 for the H1N1 antigen and p=0.03 for the H3N2 antigen), but not for the B antigen (Table IV). The use of immunosuppressive drugs did not affect the humoral response (Table V), when studied in terms of proportion of responders. However, the increase in GMT against H3N2 was not significant in patients treated with immunosuppressive drugs. The combination therapy of iloprost and calcium channel blockers significantly increased the humoral response to the H1N1 and B antigens (p<0.0001 and p=0.0007, respectively).

Discussion

Vaccination against influenza is the primary strategy to reduce the mortality and morbidity associated with influenza. Published data on the safety

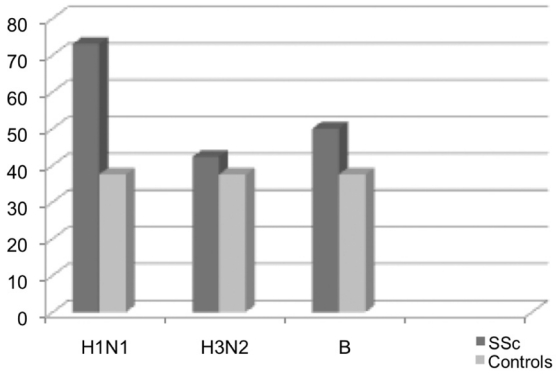

Fig. 1. Percentages of responders among groups of scleroderma patients and controls. The proportion of responders was significantly higher in the patient group for H1N1 antigen and similar to that of the controls for the H3N2 and B antigens.

Table IV. Proportion of responders to each antigen with regard to the presence or absence of ILD. The results are presented in percentages.

Antigen	Scleroderma without ILD	Scleroderma with ILD	*p*-value
H1N1	88.2	44.4	0.02*
H3N2	58.8	11.1	0.03*
B	52.9	44.4	1.0

Table V. Geometric mean titers of haemagglutination inhibition (HI) antibodies (μg/ml) against influenza antigens in scleroderma patients (SSc) subgroups with regard to the use of immunosuppressive drugs, before and six weeks after vaccination.

Antigen	SSc with IS (7)			SSc without IS treatment (19)		
	Week 0	Week 6	*p*-value	Week 0	Week 6	*p*-value
H1N1	4.18	5.66	0.036*	3.08	5.95	<0.0001*
H3N2	1.58	2.63	0.097	1.04	4.41	<0.0001*
B	4.18	4.87	0.017*	4.12	5.43	0.0001*

*$p<0.05$. IS: immunosuppressive treatment.

and immunogenicity of vaccination in SSc patients are scarce. The findings of our current study demonstrated that vaccination against influenza was safe and that it generated a good humoral response in SSc patients, with no untoward side effects. Our results confirmed those of a recent report on the efficacy, clinical safety and immune effects of the flu vaccine in 46 SSs patients (12): those authors showed a satisfactory humoral immune response, with protective titers of antibodies having been achieved in about 80% of their patients. However, the vaccine was found to be less effective in SSc patients in comparison to normal individuals, both in terms of antibody titer and cellular immunity (12).

Mercado *et al*. investigated vaccination against *Streptococcus pneumonia* in SSc patients and showed that patients with both limited and diffuse SSc are able to generate antibodies against the antigens that were included in the multivalent pneumococcal vaccine, and that this response was independent of treatment with immunosuppressive drugs (15). This lack of influence of immunosuppressive drugs on the humoral response is concordant with our current results on vaccination against influenza in SSc patients and with our previous observations in RA patients, in whom the immune response was not significantly affected by the use of prednisone, disease-modifying antirheumatic drugs (DMARD) or TNF alpha antagonists (7).

Concerns have been raised with regard to the role of vaccination in triggering the onset or exacerbation of autoimmune diseases (16). Sporadic cases of localised and generalised morphea have been described after vaccination against tetanus and hepatitis B (17-19). However, in our study, similarly to previous ones (8, 15, 20), there was no observable sign of exacerbation of the underlying disease. These results support the recently published recommendations to vaccinate patients with SSc for immunisation against influenza and pneumococcus, especially those with pulmonary involvement and those receiving immunosuppressive therapy (21).

We are aware of the limitations of this study, which included a relatively small number of patients and controls and an unexplained low proportion of responders in the control group. Large-scale studies are needed in order to confirm our conclusions. However, our results serve to further support the compelling evidence on the safety and efficacy of inactivated vaccines in patients with autoimmune diseases and the recommendation to annually vaccinate SSs patients against seasonal influenza.

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