

Immunogenicity of influenza H1N1 vaccination in mixed connective tissue disease: effect of disease and therapy

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OBJECTIVE: To assess the potential acute effects regarding the immunogenicity and safety of non-adjuvanted influenza A H1N1/2009 vaccine in patients with mixed connective tissue disease and healthy controls.

METHODS: Sixty-nine mixed connective tissue disease patients that were confirmed by Kasukawa's classification criteria and 69 age- and gender-matched controls participated in the study; the participants were vaccinated with the non-adjuvanted influenza A/California/7/2009 (H1N1) virus-like strain. The percentages of seroprotection, seroconversion, geometric mean titer and factor increase in the geometric mean titer were calculated. The patients were clinically evaluated, and blood samples were collected pre- and 21 days post-vaccination to evaluate C-reactive protein, muscle enzymes and autoantibodies. Anti-H1N1 titers were determined using an influenza hemagglutination inhibition assay. ClinicalTrials.gov: NCT01151644.

RESULTS: Before vaccination, no difference was observed regarding the seroprotection rates ($p=1.0$) and geometric mean titer ($p=0.83$) between the patients and controls. After vaccination, seroprotection (75.4% vs. 71%, $p=0.7$), seroconversion (68.1% vs. 65.2%, $p=1.00$) and factor increase in the geometric mean titer (10.0 vs. 8.0, $p=0.40$) were similar in the two groups. Further evaluation of seroconversion in patients with and without current or previous history of muscle disease ($p=0.20$), skin ulcers ($p=0.48$), lupus-like cutaneous disease ($p=0.74$), secondary Sjögren syndrome ($p=0.78$), scleroderma-pattern in the nailfold capillaroscopy ($p=1.0$), lymphopenia $\leq 1000/\text{mm}^3$ on two or more occasions ($p=1.0$), hypergammaglobulinemia $\geq 1.6 \text{ g/d}$ ($p=0.60$), pulmonary hypertension ($p=1.0$) and pulmonary fibrosis ($p=0.80$) revealed comparable rates. Seroconversion rates were also similar in patients with and without immunosuppressants. Disease parameters, such as C-reactive protein ($p=0.94$), aldolase ($p=0.73$), creatine phosphokinase ($p=0.40$) and ribonucleoprotein antibody levels ($p=0.98$), remained largely unchanged pre and post-vaccination. No severe side effects were reported.

CONCLUSIONS: The non-adjuvanted influenza A/H1N1 vaccination immune response in mixed connective tissue disease patients is adequate and does not depend on the disease manifestations and therapy.

KEYWORDS: Mixed Connective Tissue Disease; Influenza A Virus; H1N1 Subtype; Influenza Vaccine.

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INTRODUCTION

In 2009, there was a worldwide influenza A H1N1/2009 virus pandemic that caused many deaths and hospitalizations

and resulted in the WHO subsequently including this virus in the trivalent seasonal flu vaccine (1,2).

Patients with systemic autoimmune diseases are particularly susceptible to infections. This complication is an important cause of mortality and morbidity, reinforcing the importance of vaccination in this subgroup of patients. Immunosuppressed patients have been overrepresented among those who have experienced severe influenza A H1N1/2009 virus infections, demanding specific recommendations for the vaccination (3).

One dose of the non-adjuvant split-virion vaccine appears to be effective and safe for people without rheumatic

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diseases (4). Regarding mixed connective tissue disease (MCTD), there is only one study in the literature from our group focusing solely on the side effects of the vaccine and the overall immune response in a large cohort of patients with systemic autoimmune diseases (5). However, there are no data regarding the influence of disease and therapy on the immunogenicity of influenza H1N1 vaccination in MCTD. The possible effects of this vaccine on the clinical and laboratory parameters of this disease are also unknown.

The aims of this study were therefore to evaluate the possible influence of disease and therapy on the vaccination immune response and the potential effect of the vaccine on the clinical and laboratory MCTD parameters.

METHODS

Study design and participants

This prospective study enrolled patients with MCTD from the Outpatient Clinic of the Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, between March 22, 2010, and April 2, 2010, during the public health influenza A H1N1/2009 vaccine campaign for immunosuppressed patients. The protocol was approved by the Local Institutional Ethics Committee and registered in clinicaltrials.gov under #NCT01151644.

All of the patients with an MCTD diagnosis regularly followed at rheumatology outpatient clinics were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the Immunization Center of the same hospital. Healthy subjects who came to this center in response to the public health national campaign were invited to participate as control group.

After vaccination, there was a follow-up period of 21 days, during which the participants completed a personal diary card of side effects. The patients were clinically evaluated, and blood samples were collected pre- and 21 days post-vaccination. This period was chosen to evaluate the humoral response to influenza vaccine and is in accordance with previous studies (4).

We included the patients with MCTD who accepted the invitation and attended two visits for clinical and safety assessments and laboratory assays. All of the patients fulfilled Kasukawa's classification criteria (6). The healthy subjects were matched by gender and age and were included as the control group.

The participants were all ≥ 18 years of age and signed informed consent forms. The exclusion criteria were as follows: previous known infection with influenza A (H1N1) in 2009; anaphylactic response to vaccine components or egg proteins; acute infection with fever over 38°C at the time of vaccination; history of demyelinating syndromes or Guillain-Barré syndrome; previous vaccination with any live virus vaccine four weeks before inclusion or with any inactivated virus vaccine two weeks before the recruitment; seasonal flu vaccination in 2010; or blood transfusion within 6 months and hospitalization.

The doses of steroids and/or immunosuppressive agents remained the same throughout the evaluation.

Vaccine

The H1N1 vaccine (batch #1002027) was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil) using a novel monovalent, unadjuvanted, inactivated, split-virus

vaccine. The active substance was an inactivated split influenza virus containing antigen equivalent to the A/California/7/2009(H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. The vaccine was prepared in embryonated chicken eggs using the same standard techniques used to produce seasonal, trivalent, inactivated vaccine and was presented in 5 ml multi-dose vials with thimerosal added as a preservative ($45\text{ }\mu\text{g}$ per 0.5 ml dose).

All of the participants received a single intramuscular dose (0.5 ml) of $15\text{ }\mu\text{g}$ of hemagglutinin antigen specific for pandemic H1N1 A/California/7/2009-like virus (A/California/7/2009/Butantan Institute/Sanofi Pasteur) administered by a member of the nursing staff from the Immunization Center of Hospital das Clínicas, Medical School, University of São Paulo. The 69 patients with MCTD and 69 healthy subjects were vaccinated from March 22 to April 2, 2010.

Clinical assessments

The immune responses of the patients were analyzed according to the clinical features that they presented after their diagnosis with MCTD: muscle disease (muscle weakness associated with at least a two-fold elevation of creatine phosphokinase and/or aldolase in the absence of thyroid disease, infections or myopathy-inducing drugs); skin ulcers; SLE-like cutaneous disease (malar rash or photosensitivity); secondary Sjögren syndrome according to the American European Consensus Group (AECG) criteria (7); a scleroderma-pattern in nailfold capillaroscopy (presence of avascular areas or enlarged loops associated with at least one additional SD-parameter: microhemorrhages, reduced capillary density, enlarged loops and avascular areas); lymphopenia $\leq 1000\text{ cells/mm}^3$ on two or more occasions (not induced by cytotoxic drugs); hypergammaglobulinemia $\geq 1.6\text{ mg/dl}$; pulmonary arterial hypertension (estimated systolic pulmonary arterial pressure $\geq 40\text{ mmHg}$ or estimated mean pulmonary arterial pressure $>25\text{ mmHg}$ at echocardiogram); or pulmonary disease (presence of ground-glass opacity predominantly in the subcortical region at lower pulmonary lobes on chest high resolution computerized tomography).

Safety assessments

A 21-day diary card was given to each participant at the beginning of the study. This card listed 13 established side effects and requested yes or no responses. It also included the following items: local reactions (pain, redness, swelling and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea and nasal congestion). The diary cards were not pre-tested and were based on the adverse events reported in previous studies for the same vaccine in healthy subjects (4). The patients were instructed to return the cards 21 days after the vaccination for follow-up. All of the local reactions were considered to be related to the vaccine. The recorded systemic symptoms were checked by the investigators to determine the causality of solicited adverse events. Unsolicited adverse events were also assessed. Severe side effects were defined as those that required hospitalization or caused death.



Laboratory assays

The immunogenicity of the H1N1 A/California/7/2009-like virus vaccine was evaluated using a hemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute (São Paulo, Brazil). The influenza virus antigen used in this study was the H1N1 A/California/7/2009 provided by the Butantan Institute (São Paulo, Brazil). The viral concentration was previously determined using hemagglutinin antigen titration, and the HIA test was performed after removing the naturally occurring non-specific inhibitors from the sera as previously described. The immune response to the H1N1 vaccination was evaluated by determining the level of antibodies using HIA. The anti-H1N1 titer was determined by influenza HIA. The percentage of seroprotection (titer $\geq 1:40$), seroconversion (pre-vaccination titer $< 1:10$ and post-vaccination HIA titer $\geq 1:40$ or pre-vaccination titer $\geq 1:10$ and post-vaccination titer ≥ 4 -fold increase), geometric mean titers (GMTs) and factor increase in GMTs were calculated. The GMT is the geometric mean of the titers, the simple arithmetic mean of the logarithms of the last positive dilution of each serum. The factor increase in GMTs is the ratio of the GMT after vaccination to the GMT before vaccination.

The laboratory inflammatory activity of MCTD was evaluated pre- and post-vaccination by measuring the levels of aldolase, C-reactive protein (CRP) and creatine kinase (CK) using standard methods. The anti-ribonucleoprotein (anti-RNP) levels were also determined using an enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (INOVA Diagnostics).

Statistical analysis

Two-sided 95% CIs were calculated assuming binomial distributions for the dichotomous variables (Clopper-Pearson method) and log normal distributions for the hemagglutination inhibition titers. The categorical variables were compared using Fisher's exact test; the normally or non-normally distributed variables were compared using a t-test or Mann-Whitney rank sum test. All of the tests were two-sided, with a 0.05 significance level.

RESULTS

Seventy-five patients with MCTD accepted the invitation to participate in the study, but only 69 returned for the clinical and safety assessments and laboratory assays and were included. Sixty-nine healthy controls were also studied. As expected, the mean age (48.6 ± 12.6 vs. 48 ± 12 , $p=0.7$) and gender ratios (a female gender predominance; 95.6 vs. 95.6%, $p=1.00$) of the patients and controls were alike. The mean disease duration was 12.9 ± 8.9 years. The frequencies of MCTD manifestations were as follows: pulmonary fibrosis (52.2%), pulmonary arterial hypertension (20.3%), myositis (47.8%), lymphopenia (39.1%) and hypergammaglobulinemia (59.4%). At the beginning of the study, thirteen (18.84%) of the patients were not taking any drugs. Thirty-one (44.9%) of the patients were taking prednisone with a mean dose of 10.5 ± 7.2 mg/day. Current use of immunosuppressive agents was observed in 45 (65.2%) of the patients as follows: azathioprine (42.2%), methotrexate (28.9%), leflunomide (15.6%) and others (13.3%) (Table 1).

Table 1 - Laboratory data before and after the vaccination and treatment in 69 MCTD patients.

	MCTD patients (n = 69)		
	Before vaccine	After vaccine	p-value
Laboratory data			
ANTI-RNP (UI/ml)	2391.3 (1210.6)	2446.1 (1182.1)	0.98
CRP (mg/dl)	9.3 (13.4)	9.6 (13.9)	0.94
Aldolase	4.2 (2.8)	4.5 (3.0)	0.73
CK	199.3 (231.8)	153.1 (152.7)	0.40
Treatment			
Corticosteroids, n (%)	31 (44.9)		
mean dose, mg/day	10.5 (7.2)		
Chloroquine, n (%)	25 (36.2)		
Azathioprine, n (%)	19 (27.5)		
mean dose, mg/day	137.5 (42.2)		
Methotrexate, n (%)	13 (18.8)		
mean dose, mg/day	18.2 (5.9)		
Leflunomide, 20 mg/day, n (%)	7 (10.1)		

Data are expressed as numbers (%) or means (SD) unless otherwise specified. The medications that were prescribed to less than 10% of the patients were not included. CRP, C-reactive protein; CK, creatine kinase.

Influenza A H1N1/2009 vaccine immunogenicity vs. healthy controls

At study onset, the seroprotection rates ($p=1.0$) and GMT ($p=0.83$) were similar between the patients and controls. After vaccination, the seroprotection rate (75.4% vs. 71%, $p=0.70$), seroconversion rate (68.1% vs. 65.2%, $p=1.0$) and factor increase in GMT (10.0 vs. 8.0, $p=0.40$) remained similar in both groups (Table 2).

Effect of therapy in the influenza A H1N1/2009 vaccine immune response

The comparison of MCTD patients post-vaccination with and without therapy revealed comparable seroprotection ($p=1.0$), seroconversion ($p=1.0$) and FI GMT ($p=0.61$). Similarly, the seroconversion rates were alike in patients with and without the following therapies: glucocorticoids ($p=0.80$), chloroquine ($p=0.79$), azathioprine ($p=0.26$), methotrexate ($p=1.0$) and leflunomide ($p=0.68$). Patients with and without immunosuppressive agents also had a similar post-vaccination seroprotection rate (75.6%; 95% CI, 62.3-88.9% vs. 75%; 95% CI, 59-91%; $p=1.0$), FI GMT (13.5; 95% CI, 8.2-22.1 vs. 6.4; 95% CI, 4.3-9.5; $p=0.06$) and seroconversion rate (73.2%; 95% CI, 59.4-86.9 vs. 57.1; 95% CI, 38.5-76%; $p=0.2$).

Effect of disease in the influenza A H1N1/2009 vaccine immune response

Analysis of clinical parameters revealed comparable rates of seroconversion in MCTD patients with and without current or previous history of the following factors: muscle disease (75.7%; 95% CI, 60.9-90.6% vs. 58%; 95% CI, 42-74.7%; $p=0.2$), skin ulcers (80%; 95% CI, 53.9 - 106.1% vs. 64%; 95% CI, 52.1-76.7%; $p=0.48$), SLE-like cutaneous disease (72.7%; 95% CI, 45.1-100.3% vs. 66%; 95% CI, 53.2-77.8%; $p=0.74$), secondary Sjögren syndrome (63.2%; 95% CI 40.9-85.4% vs. 67%; 95% CI, 54.1-80.6%; $p=0.78$), nailfold capillaroscopy scleroderma-pattern (65.8%; 95% CI, 50.5-81.0 vs. 67%; 95% CI, 46-87.3%; $p=1.0$), lymphopenia ≤ 1000 cells/mm³ on two or more occasions (66.7%; 95%



Table 2 - Seroprotection, seroconversion, geometric mean titers and factor increase in geometric mean titers before and after vaccination.

Subset	Pre-vaccination		Post-vaccination			
	GMT	Seroprotection	GMT	Seroprotection	FI in GMT	Seroconversion
MCTD	8.3 (6.8-10.3)	10.1 (3.0-17.2)	83.3 (59.0-117.6)	75.4 (65.2-85.6)	10.0 (7.0-14.2)	68.1 (57.1-79.1)
Controls	8.3 (6.9-9.9)	10.1 (2.9-17.3)	66.1 (49.6-88.1)	71 (60.2-81.8)	8.0 (6.0-10.6)	65.2 (53.9-76.5)
Glucocorticoid						
Yes	8.9 (6.2-12.8)	13.0 (1.0-25.0)	78.2 (42.9-142.4)	74.0 (59.0-90.0)	8.7 (4.8-15.9)	65.0 (47.0-82.0)
No	7.9 (6.2-9.8)	7.9 (-0.7-16.6)	87.6 (59.9-128.2)	76.3 (62.6-90.0)	11.1 (7.5-16.4)	68.4 (53.4-83.4)
Chloroquine						
Yes	7.8 (5.7-10.6)	8.0 (-3.0-19.0)	67.7 (41.4-110.7)	76.0 (59.0-93.0)	8.7 (5.0-14.8)	64.0 (45.0-83.0)
No	8.6 (6.6-11.3)	11.3 (1.8-20.8)	93.6 (59.5-147.2)	75.0 (62.0-87.9)	10.8 (6.9-16.8)	68.1 (54.2-82.1)
Azathioprine						
Yes	7.7 (5.4-10.9)	1.1 (-0.05-1.16)	119.5 (55.9-255.1)	79 (60.0-98.0)	15.4 (2.0-31.3)	79.0 (60.0-98.0)
No	8.6 (6.7-11.0)	1.1 (1.0-1.23)	72.6 (50.3-104.7)	74 (61.7-86.3)	8.4 (5.7-12.4)	62.0 (48.4-75.6)
Methotrexate						
Yes	6.5 (4.2-9.9)	8.0 (-7.0-23.0)	49.5 (27.0-90.5)	77 (53-101.0)	7.6 (3.8-14.9)	69.0 (43.0-95.0)
No	8.8 (7.0-11.1)	10.7 (2.5-18.8)	93.9 (63.7-138.6)	75 (63.5-86.4)	10.6 (7.1-15.7)	66.0 (53.5-78.6)
Leflunomide						
Yes	8.2 (3.8-17.7)	14.3 (-13.7-42.3)	119.5 (55.9-255.1)	79.0 (60.0-98.0)	15.4 (2.0-31.3)	79.0 (60.0-98.0)
No	8.3 (6.7-10.3)	9.6 (2.2-17.0)	73.9 (53.4-102.5)	75.8 (65.0-86.5)	8.8 (6.4-12.1)	67.7 (56.0-79.4)

Data are expressed in percentages or values (95% CI). GMT, geometric mean titer; FI in GMT, factor increase in GMT after vaccination; MCTD, mixed connective tissue disease.

CI 48.6-84.8% *vs.* 68%; 95% CI, 52.8-82.2%; $p=1.0$), hypergammaglobulinemia ≥ 1.6 g/d (63.4%; 95% CI, 48.5-78.3 *vs.* 71%; 95% CI, 52.3-89.4%; $p=0.60$), pulmonary arterial hypertension (64.3; 95% CI, 38.2 - 90.3% *vs.* 67%; 95% CI 54.8 - 79.8%; $p=1.0$) and pulmonary fibrosis (63.9%; 95% CI, 47.9 - 79.8 *vs.* 69.7%; 95% CI, 53.8 - 85.6%; $p=0.80$).

Vaccine safety

No severe side effects were reported. The frequencies of minor local reactions were similar between the patients and controls (13% *vs.* 29%, $p=0.11$). The systemic reaction that was most frequently reported by patients was myalgia (11.5%), but the reported level was not different relative to that of the controls ($p=1.0$).

DISCUSSION

This is the first study to determine that the immune response to influenza H1N1 vaccine in MCTD patients is adequate and independent of the clinical aspects of the disease and therapy.

Regarding other rheumatic diseases, we and others have previously demonstrated appropriate pandemic 2009 influenza A (H1N1) response and vaccine safety in patients with rheumatoid arthritis, ankylosing spondylitis, systemic sclerosis, psoriatic arthritis, Behçet's disease, primary antiphospholipid syndrome, dermatomyositis, primary Sjögren syndrome, Takayasu's arteritis, polymyositis, granulomatous polyangiitis and juvenile autoimmune rheumatic disease (juvenile systemic lupus erythematosus (SLE), juvenile idiopathic arthritis, juvenile dermatomyositis, juvenile scleroderma, and vasculitis) compared with healthy controls (5,8,9). In contrast, similar studies with SLE patients have demonstrated an impairment in the immune response to influenza vaccination evaluated by the assessment of autoantibodies (10-12). It is possible that these discrepancies could be related to variations in the diseases, variations in the vaccines and the usage of several medications.

No harmful effect of the disease was observed, which may be related to the use of unadjuvanted vaccine in the present

study. Unadjuvanted vaccines offer the theoretical advantage of minimizing the risk of potentiating the humoral response and avoiding the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) (13). However, a large meta-analysis revealed no difference in the incidence of adverse events of autoimmune origin between subjects who received influenza vaccinations with and without adjuvant (14).

In contrast with SLE, in which several activity indexes are available and widely used (15) and there are well-defined serological markers, such as anti-dsDNA and complement levels (16), there are no such tools for MCTD. In SLE, controversy remains regarding whether disease flare occurs after immunization with H1N1 vaccination (10,17) and whether there are changes in the levels of SLE-related auto antibodies (10,18). In this group of MCTD patients, the stability of the clinical, laboratory and treatment parameters throughout the study supports the notion that pandemic H1N1 is not harmful to this disease.

Conversely, the possible influence of disease manifestation in the post-vaccination immune response is a matter of concern, but none of the clinical or laboratory MCTD parameters evaluated were associated with a diminished humoral response. In contrast, the efficacy of the H1N1 pandemic vaccine was impaired in lupus patients with lymphopenia (19), and in HIV-infected individuals, a lower mean nadir CD4 cell count and longer duration of infection were associated with reduced seroconversion (20).

Despite the use of immunosuppressive drugs, MCTD patients did not present any impairment in their immune response. The influenza vaccine response appears to differ in individual rheumatic diseases (5). In this regard, our large cohort analysis of 555 lupus patients revealed that immunomodulators appear to be a more relevant factor for a reduced pandemic vaccine immune response than the disease itself (21). Similarly, we observed a methotrexate-impaired H1N1 vaccine-induced humoral response in RA patients (22). In children with systemic autoimmune diseases, glucocorticoid was identified as the only drug that decreased seroconversion in multivariate analysis (9). Immunosuppressive drugs, such as methotrexate, azathioprine, leflunomide, mycophenolate



mofetil, cyclosporine and glucocorticoid, were associated with lower antibody titers in patients with inflammatory rheumatic diseases (9,11,23).

The non-adjuvanted influenza A/H1N1 vaccination immune response in MCTD patients is appropriate and independent of their disease manifestations and therapies. In addition, the overall vaccine safety supports its recommendation.

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■ AUTHOR CONTRIBUTIONS

Miossi R contributed to the design, planning, interpretation of the data and statistical analysis, and writing. Fuller R contributed to the design, planning, interpretation of the data, and writing. Moraes JC, Ribeiro AC, Saad CG, Aikawa NE contributed to the execution and planning. Miraglia J and Ishida MA contributed to the execution (laboratory). Bonfá E contributed to the design and planning. Caleiro MT contributed to the design, planning, execution, and writing.

■ REFERENCES

- Centers for Disease Control and Prevention. Questions and Answers: Vaccine Selection for the 2010-2011 Influenza Season. http://www.cdc.gov/flu/about/qa/1011_vac_selection.htm (accessed 10 August 2011).
- Centers for Disease Control and Prevention. Questions and Answers: Vaccine Selection for the 2011-2012 Influenza Season. <http://www.cdc.gov/flu/about/qa/vaccine-selection.htm> (accessed 10 August 2011).
- van Assen S, Agmon-Levin N, Elkayam O, Cervera R, Doran MF, Dougados M, et al. EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis*. 2011;70(3):414-22. <http://dx.doi.org/10.1136/ard.2010.137216>.
- Liang XF, Wang HQ, Wang JZ, Fang HH, Wu J, Zhu FC, et al. Safety and immunogenicity of 2009 pandemic influenza A H1N1 vaccines in China: a multicenter, double-blind, randomized, placebo-controlled trial. *Lancet*. 2010;375(9708):56-66. [http://dx.doi.org/10.1016/S0140-6736\(09\)62003-1](http://dx.doi.org/10.1016/S0140-6736(09)62003-1).
- Saad CGS, Borba EF, Aikawa NE, Silva CA, Pereira RM, Calich AL, et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic disease. *Ann Rheum Dis*. 2011;70(6):1068-73. <http://dx.doi.org/10.1136/ard.2011.150250>.
- Kasukawa R. Mixed connective tissue disease. *Intern Med*. 1999;38(5):386-93. <http://dx.doi.org/10.2169/internalmedicine.38.386>.
- Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61(6):544-58.
- Elkayam O, Amir S, Mendelson E, Schwaber M, Grotto I, Wollman J, et al. Efficacy and safety of vaccination against pandemic 2009 influenza A (H1N1) virus among patients with rheumatic diseases. *Arthritis Care Res*. 2011;63(7):1062-67. <http://dx.doi.org/10.1002/acr.20465>.
- Aikawa NE, Campos LM, Silva CA, Carvalho JF, Saad CG, Trudes G, et al. Glucocorticoid: Major Factor for Reduced Immunogenicity of 2009 Influenza A (H1N1) Vaccine in Patients with Juvenile Autoimmune Rheumatic Disease. *J Rheumatol*. 2012;39(1):167-73. <http://dx.doi.org/10.3899/jrheum.110721>.
- Wiesik-Szewczyk E, Romanowska M, Mielnik P, Chwalińska-Sadowska H, Brydak LB, Olesińska M, et al. Anti-influenza vaccination in systemic lupus erythematosus patients: an analysis of specific humoral response and vaccination safety. *Clin Rheumatol*. 2010;29(6):605-13. <http://dx.doi.org/10.1007/s10067-010-1373-y>.
- Holvast B, Huckriede A, Kallenberg CG, Bijl M. Influenza vaccination in systemic lupus erythematosus: safe and protective? *Autoimmun Rev*. 2007;6(5):300-5. <http://dx.doi.org/10.1016/j.autrev.2006.09.012>.
- Abu-Shakra M, Press J, Varsano N, Levy V, Mendelson E, Sukenik S, et al. Specific antibody response after influenza immunization in systemic lupus erythematosus. *J Rheumatol*. 2002;29(12):2555-7.
- Shoenfeld Y, Agmon-Levin N. 'ASIA' - autoimmune/inflammatory syndrome induced by adjuvants. *J Autoimmun*. 2011;36(1):4-8. <http://dx.doi.org/10.1016/j.jaut.2010.07.003>.
- Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. *Vaccine*. 2009;27(49):6959-65. <http://dx.doi.org/10.1016/j.vaccine.2009.08.101>.
- Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. *Best Pract Res Clin Rheumatol*. 2005;19(5):685-708. <http://dx.doi.org/10.1016/j.berh.2005.03.010>.
- Swaak AJ, Groenwold J, Aarden LA, Statius van Eps LW, Feltkamp EW. Prognostic value of anti-dsDNA in SLE. *Ann Rheum Dis*. 1982;41(4):388-95. <http://dx.doi.org/10.1136/ard.41.4.388>.
- Lu CC, Wang YC, Lai JH, Lee TS, Lin HT, Chang DM. A/H1N1 influenza vaccination in patients with systemic lupus erythematosus: Safety and immunity. *Vaccine*. 2011;29:444-50. <http://dx.doi.org/10.1016/j.vaccine.2010.10.081>.
- Urowitz MB, Anton A, Ibanez D, Gladman DD. Autoantibody response to adjuvant and nonadjuvant H1N1 vaccination in systemic lupus erythematosus. *Arthritis Care Res (Hoboken)*. 2011;63(11):1517-20. <http://dx.doi.org/10.1002/acr.20599>.
- Mathian A, Devilliers H, Krivine A, Costedoat-Chalumeau N, Haroche J, Huong DB. Factors influencing the efficacy of two injections of a pandemic 2009 influenza A (H1N1) nonadjuvanted vaccine in systemic lupus erythematosus. *Arthritis Rheum*. 2011;63(11):3502-11. <http://dx.doi.org/10.1002/art.30576>.
- Bickel M, von Hentig N, Wieters I, Khaykin P, Nisius G, Haberl A, et al. Immune response after two doses of the novel split virion, adjuvanted pandemic H1N1 influenza A vaccine in HIV-1-infected patients. *Clin Infect Dis*. 2011;52(1):122-7. <http://dx.doi.org/10.1093/cid/ciq003>.
- Borba EF, Saad CGS, Pasoto SG, Calich AL, Aikawa NE, Ribeiro AC, et al. Influenza A/H1N1 vaccination of patients with SLE: can anti-malarial drugs restore diminished response under immunosuppressive therapy? *Rheumatology (Oxford)*. 2012;51(6):1061-9. <http://dx.doi.org/10.1093/rheumatology/ker427>.
- Ribeiro AC, Guedes LK, Moraes JC, Saad CG, Aikawa NE, Calich AL, et al. Reduced seroprotection after pandemic H1N1 influenza adjuvant-free vaccination in patients with rheumatoid arthritis: implications for clinical practice. *Ann Rheum Dis*. 2011;70(12):2144-7. <http://dx.doi.org/10.1136/ard.2011.152983>.
- Gabay C, Bel M, Combescure C, Ribi C, Meier S, Posfay-Barbe K, et al. Impact of synthetic and biologic disease-modifying antirheumatic drugs on antibody responses to the A/503-adjuvanted pandemic influenza vaccine: a prospective, open-label, parallel-cohort, single-center study. *Arthritis Rheum*. 2011;63:1486-96. <http://dx.doi.org/10.1002/art.30325>.