Short and long-term effects of pandemic unadjuvanted influenza A(H1N1)pdm09 vaccine on clinical manifestations and autoantibody profile in primary Sjögren's syndrome

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A B S T R A C T

Despite WHO recommendations about the A/California/7/2009/H1N1-like virus vaccination, studies evaluating its possible influence on clinical manifestations and autoantibody profile in primary Sjögren's syndrome (SS) are scarce. The aim of this study was to evaluate the possible influence of the unadjuvanted A/California/7/2009/H1N1-like virus vaccination on clinical manifestations and autoantibody profile in SS in the short/long-term. Thirty-six SS patients (The American-European Consensus Group Criteria, 2002) and 36 healthy controls with comparable mean age and gender were evaluated before and 21-days after this vaccination regarding seroprotection/seroconversion, factor increase in geometric mean titer (FI-GMT) and side effects. New onset of disease flares and autoantibody profile [antinuclear antibodies, anti-dsDNA, anti-Ro(SSA)/La(SSB), anti-RNP/anti-Sm, rheumatoid factor, anti-alpha-fodrin, antipolyribonucleoprotein, anti-beta2-glycoprotein-I] were assessed before, 21-days and 1-year after vaccination. Patients and controls had similar rates of seroconversion (77.8 vs. 69.4%, p = 0.42), seroprotection (83.3 vs. 72.2%, p = 0.26) and FI-GMT (p = 0.85). Disease duration, prednisone (2.1 ± 4.9 mg/day), methotrexate and azathioprine did not affect seroconversion (p > 0.05). Regarding short-term, no change in the frequency or levels of autoantibodies was observed (p > 0.05) and only mild side effects were reported in comparable rates to controls (p > 0.05). During 1-year follow-up, the frequency of new disease flares was similar to the previous year (11 vs. 19%, p = 0.51), and four patients developed positivity to one of the following specificities: anti-Ro(SSA)/anti-La(SSB), anti-alpha-fodrin, or IgM antipolyribonucleoprotein. None developed specific lupus autoantibodies. Of note, a significant increase in the mean levels of anti-Ro(SSA) (p = 0.001) and anti-La(SSB) (p = 0.002) was detected after 1-year with no change in the other autoantibodies. This is the first study indicating that influenza A(H1N1)pdm09 vaccine induces long-term changes in autoantibody profile restricted to SS spectrum without a deleterious effect in disease course.

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1. Introduction

Primary Sjögren’s syndrome (SS) is a chronic autoimmune disease characterized primarily by involvement of the exocrine glands, leading to sicca syndrome. Multiple organs may also be affected, causing a highly variable spectrum of clinical manifestations. Most patients have less severe extraglandular symptoms such as fatigue, polyarthralgia and diffuse myalgia, while others develop serious systemic impairments such as pneumonitis, vasculitis, peripheral neuropathy, glomerulonephritis, optic neuritis, multiple sclerosis-like disease and even lymphoma [1]. The etiology is unknown, but the autoimmune nature of SS is supported by the production of multiple circulating autoantibodies [2]. Anti-Ro(SSA) and anti-La(SSB) are detected in up to 90% and 60% of SS patients, respectively [2], and they are included in the disease classification criteria [3].

As a consequence of multiple systemic affections, it is often necessary to use high doses of glucocorticoids, immunosuppressive drugs and biological agents for the treatment of SS patients [4]. Indeed, it is relevant that infections, particularly respiratory, are considered important causes of morbidity and mortality in

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this disease [5–7], and vaccination is the most effective preventive measure to control virus dissemination and to reduce associated complications [8]. Pandemic influenza occurs repeatedly, H1N1 (Spanish flu, 1918); A/H2N2 (Asian influenza, 1957); A/H3N2 (Hong Kong flu, 1968); H1N1 (Swine flu, 1976; Russian flu, 1977) strains and A/California/7/2009 [A(H1N1)pdm09] [9], and it is of particular concern for immunosuppressed patients, who may be very exposed to this infection. Indeed, immunocompromised patients have indications to receive vaccine for seasonal and pandemic influenza according to the European League Against Rheumatism [10] and the 2010 Recommendations of the Advisory Committee on Immunization Practices [11], but data regarding its immunogenicity and disease safety in SS are scarce. In fact, the only two available published reports including SS patients focused on an overall analysis of several autoimmune rheumatic diseases (ARD) and evaluated short-term (n = 36) [12] and six months (n = 23) [13] safety and immunogenicity of A(H1N1)pdm09 influenza [12] and seasonal and/or pandemic influenza vaccination [13]. Both studies showed adequate seroconversion rate and mild vaccine side effects in ARD patients [12,13], however without a particular evaluation of the SS group [12,13]. In this regard, a control group of healthy individuals with comparable mean ages and gender is an essential parameter [14] not fulfilled in the previous large cohort study of ARD particularly for SS patients [12]. In addition, the effect of vaccine on disease itself was not evaluated and this is a relevant issue for patients with autoimmune genetic background such as those with SS, since the adjuvanted and non-adjuvanted A/H1N1 vaccines in the general population were associated with the development of various immunological disorders such as Guillain–Barré syndrome, acute encephalomyelitis, thrombocytopenia and vasculitis [15].

In addition, there are no data on the possible influence of the unadjuvanted A/California/7/2009/H1N1-like virus immunization on the autoantibody profile in SS patients. In this regard, increased serum levels of anticardiolipin antibodies (aCL) were observed in patients with systemic lupus erythematosus (SLE) up to one year after seasonal influenza immunization [16]. Likewise, the seasonal and/or pandemic influenza vaccine induced the production of anti-extractable nuclear antigen (anti-ENA) antibodies in ARD patients; nevertheless a SS group was not analyzed [17]. Thus, the aim of the present study was to evaluate short/long-term effects of unadjuvanted influenza A/California/7/2009/H1N1-like virus vaccination on clinical manifestations and autoantibody profile in SS. 

2. Patients and methods

For this study, all SS patients and healthy controls had been 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 center, Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo – HCFMUSP, São Paulo, Brazil, (described in detail elsewhere [12]). The three male SS patients of our cohort did not respond to this call.

The study was approved by the local institutional review board, and all participants signed the informed consent. The trial was registered at clinicaltrials.gov under NCT01151644.

2.1. Patients and healthy individuals

Thirty-six female SS patients (according to The American-European Consensus Group Criteria [3]) aged >18 years (mean age: 55.6 ± 14.7 years) with stable disease (without active neurological or renal affections, and without current vasculitis) were selected and regularly followed for one year at the out-patient Sjögren’s Syndrome Clinic (Rheumatology Division, HCFMUSP). Patients with history of multiple sclerosis-like disease or Guillain–Barré syndrome were excluded. Thirty-six healthy females with comparable age (52.3 ± 11.3 years-old, p = 0.29) to the patients were included.

2.2. Vaccine

The A(H1N1)pdm09 vaccine, a novel, monovalent, unadjuvanted, 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 virus vaccines recommended by the WHO. 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).

2.3. Study design

Participants were assessed immediately before and 21 days after vaccination to determine seroprotection and seroconversion 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. Disease flares were evaluated in all patients retrospectively 1-year prior and prospectively 1-year after vaccination (n = 36). Analysis of autoantibody profile included antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-Ro/SSA, anti-La/SSB, anti-α-fodrin, anti-U1 RNP, anti-Sm, rheumatoid factor (RF), anticardiolipin (aCL) and anti-β2-glycoprotein-I (anti-β2GPI) antibodies, and it was performed in patients immediately before, 21 days (n = 36) and 1-year (n = 20) of vaccination. During the vaccination campaign, healthy volunteers were followed only in the short-term (21 days), thus analysis of autoantibodies 1 year after immunization was performed only for SS patients.

2.4. 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.

2.5. Safety assessments

A 21-day diary card was given to each participant at study entry with a list of 13 solicited adverse reactions. This written card included local reactions (pain, redness, swelling and itching) and systemic adverse events such as arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea and nasal congestion. 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. In addition, all SS patients were also evaluated regarding new onset of disease flares (parotiditis, arthritis, vasculitis, pneumonitis or neurological disorders) retrospectively 1-year prior and prospectively 1-year after vaccination. Medical charts were extensively reviewed for additional clinical and treatment data.
2.6. 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. Sera were stored at −70 °C and the two samples from each patient or control were tested in parallel in the same plate. The immunogenicity of the A/California/7/2009 (H1N1)-like virus vaccine was evaluated with the use of a haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute. Samples were also collected from twenty patients 1 year after vaccination for evaluation of the profile of autoantibodies in the long term. The three samples from each patient (immediately before, 21-days and 1-year) of vaccination were tested in parallel in the same plate for all autoantibody determinations listed below.

2.7. Haemagglutination inhibition 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 [18]. The A(H1N1)pdm09 vaccine immunoresponse was evaluated by determining levels of antibodies by haemagglutination inhibition. Anti-H1N1 titers were determined by influenza HIA. The percentages of seroprotection (SP) (titer ≥1:40) and seroconversion (SC) (a pre-vaccination titer <1:10 and a post-vaccination HIA titer ≥ 1:40 or pre-vaccination titers ≥1:10 and a ≥4-fold rise post-vaccination), geometric mean titers (GMTs) and factor increases (FIs) in GMTs were calculated [12].

2.8. Autoantibodies

For the evaluations of several autoantibodies, three samples from each patient (immediately before, 21-days and 1-year after vaccination) were tested in the same assay and applied in duplicates. Antinuclear and anti-dsDNA antibodies were detected by indirect immunofluorescence using as substrates Hep-2 cells or Crithidia lucilie, respectively (INOVA Diagnostics Inc., San Diego, USA) [19]. Serum levels of anti-Ro/SSA, anti-La/SSB, anti-U1RNP and anti-Sm antibodies were determined by ELISA with the purified antigens according to manufacturer's instructions (INOVA Diagnostics Inc., San Diego, USA) [20]. Concentrations of anti-alpha-fodrin antibodies (IgG and IgA) were also measured by ELISA with the purified protein (ORGENTEC Diagnostika GmbH, Mainz, Germany) [21]. Rheumatoid factor (IgM-RF) was measured by ELISA (INOVA Diagnostics Inc., San Diego, USA). Serum antiphospholipid antibodies were also evaluated. IgG and IgM anticardiolipin antibodies (aCL) were tested by ELISA as previously described (INOVA Diagnostics Inc., San Diego, USA) [22]. IgG and IgM anti-beta2-glycoprotein-I (anti-β2GP1) were measured by ELISA (ORGENTEC Diagnostika GmbH, Mainz, Germany) as previously outlined [23].

2.9. Statistical analysis

Comparison between two groups (SS patients vs. healthy controls and SS patients with A(H1N1)pdm09 seroconversion vs. SS patients without seroconversion) was conducted using Student's t test or Mann–Whitney U test for continuous variables and Chi-squared test or Fisher's exact test for categorical variables when applicable. For analysis comparing multiple data of the same group, one-way repeated measures analysis of variance (ANOVA) for continuous data and McNemar’s test for categorical data were performed. Results are shown as a proportion, or mean ± standard deviation (SD). Only two-tailed tests were applied. p-values <0.05 were considered to be statistically significant.

3. Results

3.1. Short-term evaluation

3.1.1. Immunogenicity and safety of A(H1N1)pdm09 vaccine in patients and controls

All SS patients and controls were female, with comparable mean ages (55.6 ± 14.7 vs. 52.3 ± 11.3 years-old, p = 0.29). Pre-vaccination seroprotection rates (11.1 vs. 8.3%, p = 1.00), seroconversion (77.8 vs. 69.4%, p = 0.42), and seroconversion after immunization (83.3 vs. 72.2%, p = 0.26) were similar in patients and controls, respectively (Table 1). GMT before [8.9 (6.5–12.3) vs. 8.1 (6.2–10.5), p = 0.96] and after immunization [95.1 (60.4–149.8) vs. 89.8 (57.9–139.2), p = 0.791], and Fi-GMT [10.7 (6.7–16.9) vs. 11.1 (7.1–17.3), p = 0.85] were also comparable in patients and controls, respectively. Regarding short-term (21-days period) side effects, only mild reactions related to the vaccine were observed in comparable rates in patients and healthy individuals (p > 0.05) (Table 1).

3.1.2. Influence of clinical features and therapy on A(H1N1)pdm09 vaccine seroconversion (SC)

In SS patients, seroconversion rate was not affected by disease duration (p = 0.59), use of glucocorticoid (p = 0.16), methotrexate (p = 0.65), antimalarial (p = 0.68), azathioprine (p = 0.21), or concomitant use of glucocorticoid and immunosuppressive drugs (p = 0.31) (Table 2).

3.1.3. Short-term A(H1N1)pdm09 vaccine effects on disease flares and autoantibody profile

None of the SS patients had disease flares and no patient had new positive tests or increased titers of ANA, anti-dsDNA, anti-U1RNP/Sm, anti-Ro(SSA)/La(SSB), RF, anti-α-fodrin, aCL or aβ2GP1 (p > 0.05).

3.2. Long-term A(H1N1)pdm09 vaccine effects on disease flares and autoantibody profile

3.2.1. Disease flares 1-year prior and 1-year after vaccination

The prospective long-term evaluation of SS patients revealed that 11% had new disease flares during the 1-year period, but the rate of new flares was similar to the previous year (19%, p = 0.51) (Table 3).

### Table 1

<table>
<thead>
<tr>
<th>Age, years [mean ± SD]</th>
<th>Controls n=36</th>
<th>SS n=36</th>
<th>p-value</th>
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<tr>
<td>55.6 ± 14.7</td>
<td>52.3 ± 11.3</td>
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<td>0.29</td>
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<tr>
<td>Female [n (%)]</td>
<td>36 (100)</td>
<td>36 (100)</td>
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*SS = primary Sjögren’s syndrome; n = number of patients; SD = standard deviation.*
2.2. Autoantibody profile prior and 1-year after vaccination

In the long-term, 4/20 (20\%) SS patients presented new positive tests: one patient became positive for anti-Ro/SSA, another for anti-La/SSB, one became positive for IgA anti-alpha-fodrin, and the last patient for IgM anticardiolipin (with levels >40 MPL). None developed lupus specific autoantibodies. Of note, comparing antibody levels at study inclusion, at 21-days and 1-year after vaccination, significant increases in the levels of anti-Ro/SSA (100.0±40.8 vs. 100.9±40.3 vs. 114.1±40.1U, respectively; p = 0.0001) and anti-La/SSB (60.8±42.0 vs. 60.5±41.9 vs. 72.9±47.2, respectively; p = 0.002) were observed after 1 year, whereas no change was detected for other antibodies specificities investigated (Table 4).

4. Discussion

This is the first study to indicate that influenza A(H1N1)pdm09 vaccine induces long-term changes in autoantibody profile restricted to SS spectrum without a deleterious effect in disease course. The long-term evaluation was a relevant aspect of this study, since increasing levels of autoantibodies induced by vaccination was a late phenomenon. In fact, a three months evaluation of autoantibody profile in A(H1N1)pdm09 immunization has not observed increased antibody levels in SLE patients [24], as also demonstrated herein for the 21-days assessment. We did not detect the occurrence of serious vaccine short-term adverse events and demonstrated adequate seroconversion and seroprotection rates particularly in SS. Our finding is reinforced by the comparable mean ages and gender in the groups of SS patients and healthy individuals, an essential parameter [14] not fulfilled in our previous large cohort study of autoimmune rheumatic diseases for these particular group of patients [12]. Glucocorticoid did not have a deleterious effect in vaccine humoral response probably related to the use of low doses of this drug. Indeed, doses higher than 20 mg/day were associated with a diminished A(H1N1)pdm09 vaccine antibody production in rheumatoid arthritis (AR) and systemic lupus erythematosus (SLE) [25,26]. Unlike evaluations of AR and SLE patients [25–27], immunosuppressive agents did not reduce vaccine immunogenicity, but the small representation of patients under this therapy in the present study hampers the interpretation of this finding. Vaccine did not trigger disease's exacerbations, since the frequencies of parotitis, arthritis, vasculitis, and pneumonitis at 1-year follow-up were similar to the previous year. Additionally, our data suggest long-term vaccine safety in an autoimmune rheumatic disease, with no cases of neurological disorders such as Guillain–Barré syndrome, which was described months after immunization mainly with adjuvanted pandemic influenza vaccine in the general population [15].

The main limitation of our study is the evaluation of a group of SS patients with peculiar features, i.e. with stable disease and using low doses of glucocorticoids and immunosuppressant drugs. In contrast, in systemic lupus erythematosus (SLE), there are published data indicating that the unadjuvanted A(H1N1)pdm09 vaccine is safe in patients with active disease and using high doses of prednisone and immunosuppressants, but with significantly impaired immunogenicity [26].

Another important concern regarding vaccines is that they can occasionally stimulate autoimmune production or even a recently defined syndrome known as autoimmune/inflammatory syndrome induced by adjuvants (ASIA) [28]. In this regard, a case of primary Sjögren's syndrome following adjuvant influenza A(H1N1)pdm09 vaccination was described [29]. Symptoms started seven days after the immunization, with development of polyarthralgia, dry mouth, dry eyes, positive antinuclear antibodies, positive anti-Ro/SSA antibody and the presence of lymphocytic infiltrates in the salivary glands [29]. Analysis of samples from labial tissues using polymerase chain reaction method (PCR) showed the absence of the influenza A(H1N1)pdm09 RNA, indicating that there was no direct viral presence in the salivary glands [29]. The authors hypothesize that vaccine antigens could trigger the immune system by different mechanisms such as molecular mimicry, epitope spreading and polyclonal activation, leading to the outbreak of Sjögren's syndrome manifestations and the appearance of antinuclear and anti-Ro/SSA antibodies [29]. In this regard, we chose to use solely the non-adjuvanted vaccine because SS patients have theoretically a genetic predisposition for the development of autoimmune phenomena [1]. Furthermore, according to a systematic review on vaccination in patients with autoimmune rheumatic diseases carried by European League Against Rheumatism (EULAR), the safety regarding adjuvants in these diseases remains to be defined [30].

Importantly, the changes in autoantibody profile observed herein were restricted to SS spectrum and mainly in the levels of anti-Ro/SSA/La(SS-B), raising the hypothesis that this antibody production may be a consequence of molecular mimicry. In this aspect, it is interesting that it was observed molecular mimicry between the antigen Ro and the Epstein–Barr virus (EBV) protein EBNA-1 [31,32].

Increased serum levels of these antibodies observed herein were not, however, associated with disease flares, as shown by clinical evaluation. Indeed, titers of anti-Ro antibodies may fluctuate during the course of the illness in both SLE and SS patients, but these changes were not associated with disease flares, with the exception of some patients with skin vasculitis [33]. A similar phenomenon was observed in SLE patients six months after non-adjuvanted A(H1N1)pdm09 immunization, with significantly higher levels of

<table>
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<th>Table 2: A(H1N1)pdm09 vaccine seroconversion (SC) and clinical and therapeutic features of SS patients.</th>
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<tr>
<td>SS H1N1 SC+</td>
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<tr>
<td>Age, years [mean ± SD] (n = 28)</td>
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<tr>
<td>Disease duration, years [mean ± SD] (n = 28)</td>
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<td>Treatment</td>
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<tr>
<td>Prednisone [n (%)]</td>
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<td>-dose, mg/day [mean ± SD]</td>
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<td>-range, mg/day</td>
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<tr>
<td>Antimalarial drugs [n (%)]</td>
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<td>Immunosuppressive agents</td>
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<td>Azathioprine [n (%)]</td>
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<td>-dose, mg/day [mean ± SD]</td>
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<td>-range, mg/day</td>
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<tr>
<td>Methotrexate [n (%)]</td>
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<td>-dose, mg/week [mean ± SD]</td>
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<td>-range, mg/week</td>
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<td>Mycophenolate mofetil [n (%)]</td>
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<td>Prednisone + immunosuppressive agents</td>
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<td>Without any of the above medications [n (%)]</td>
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SS = primary Sjögren's syndrome; SC = seroconversion; n = number of patients; SD = standard deviation.

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<tr>
<th>Table 3: Disease flares 1-year prior and 1-year after A(H1N1)pdm09 vaccination in SS patients.</th>
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<tr>
<td>1-Year prior vaccination</td>
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<tr>
<td>Parotitis [n (%)] (n = 36)</td>
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<tr>
<td>Arthritis [n (%)]</td>
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<td>Cutaneous vasculitis [n (%)]</td>
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<td>Pneumonitis [n (%)]</td>
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<td>Neurological disorders [n (%)]</td>
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SS = primary Sjögren's syndrome; n = number of patients.
anti-Sm compared to baseline, but without clinical consequences [25].

In conclusion, influenza A(H1N1)pdm09 vaccine stimulates long-term production of SS related autoantibodies without a relevant change in clinical course. The additional observation of an adequate seroconversion and seroprotection rates supports its recommendation in this disease.

Acknowledgements

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