Paediatric rheumatology

Influenza A H1N1/2009 vaccine in juvenile dermatomyositis: reduced immunogenicity in patients under immunosuppressive therapy

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Abstract

Objectives

The aim of the present paper is to assess the influence of demographic, muscle enzymes, JDM scores and treatment on non-adjuvanted influenza A H1N1/2009 vaccine immunogenicity in juvenile dermatomyositis (JDM) patients.

Methods

Thirty JDM patients and 81 healthy age-matched controls were vaccinated. All participants were evaluated pre- and 21 days post-vaccination and serology for anti-H1N1 was performed by haemagglutination inhibition assay. Muscle enzymes, JDM scores and treatment were evaluated before and after vaccination. Adverse events were reported.

Results

After immunisation, seroconversion rates were significantly lower in JDM patients compared to age-matched controls (86.7 vs. 97.5%, $p=0.044$), whereas seroprotection ($p=0.121$), geometric mean titres (GMT) ($p=0.992$) and factor increase (FI) in GMT ($p=0.827$) were similar in both groups. Clinical and laboratory evaluations revealed that JDM scores and muscle enzymes remained stable throughout the study ($p>0.05$). A higher frequency of chronic course was observed in non-seroconverted compared to seroconverted (100% vs. 27%, $p=0.012$). Regarding treatment, a lower rate of seroconversion was observed in patients under prednisone>20mg/day (50% vs. 4%, $p=0.039$), and in those treated with a combination of prednisone, methotrexate and cyclosporine (50% vs. 4%, $p=0.039$). Local and systemic vaccine adverse events were mild and similar in patients and controls ($p>0.05$).

Conclusion

This study identified that chronic course and immunosuppressive therapy are the major factors hampering seroconversion in JDM, suggesting that a specific protocol may be required for this subgroup of patients. In spite of that, a single dose of non-adjuvanted influenza A/H1N1 2009 vaccine was generally seroprotective in this disease with no evident deleterious effect in disease itself (ClinicalTrials.gov, no. NCT01151644).

Key words

vaccine, immunogenicity, influenza A H1N1/2009, children, juvenile dermatomyositis

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Introduction

Improvements in the diagnosis and management of juvenile dermatomyositis (JDM) have significantly enhanced survival over the last decades (1-4). The treatment used in these patients and disease itself may induce immunosuppression with a consequent increase in infection susceptibility (5-7). Therefore, vaccination emerges as an essential prevention tool in pediatric rheumatologic disease (5, 8).

Recently, the European League Against Rheumatism (EULAR) task force has reinforced the relevance of vaccination in immunosuppressed pediatric rheumatologic patients, due to high risk of severe infection (8). Accordingly, the influenza A H1N1/2009 vaccination was recommended for all immunosuppressed patient (9), due to the high incidence of hospitalisation and death in this particular group of patients reported during the 2009 pandemic (10).

There are scarce data in the literature regarding H1N1 influenza vaccine in JDM patients and all of them are restricted to overall immunogenicity and safety (11-13). Ogimi et al. evaluated the immune response of influenza vaccine in small cohort of juvenile autoimmune rheumatic diseases, including only 6 JDM patients, and reported immune response comparable to controls (11). Only 3 JDM patients were evaluated in the study of Kanakoudi-Tsakalidou et al., thus precluding a definitive conclusion about their findings (12). We have recently assessed immunogenicity and safety of the non-adjuvanted influenza A H1N1/2009 vaccine in 237 juvenile autoimmune rheumatic diseases, including only 18 JDM patients, and showed an overall short-term safety with reduced immune response associated with glucocorticoid use (13), without a specific analysis of this subgroup of patients.

Moreover, the possible role of demographic, disease and therapy factors in vaccine antibody response and the potential impact of vaccine in JDM disease parameters need to be determined. Gender and age are relevant for immunogenicity, since female gender has higher antibodies titers to a large number of viral vaccine (14) and patients younger than 9 years old may induce lesser humoral response to influenza A H1N1(15, 16). Treatment was also identified to contribute to vaccine response in lupus patients (17) and there were reports suggesting that the vaccine may induce flare in systemic lupus erythematosus patients (18).

Therefore, the objectives of this study were to assess the possible association between seroconversion rate with demographic data, muscle enzymes, JDM scores, lymphopenia and treatment in JDM patients, as well as the possible deleterious effect of the non-adjuvanted influenza A H1N1/2009 in the disease itself.

Methods

Thirty consecutive JDM outpatients, including 18 JDM patients of our previous study (13), routinely followed at the Pediatric Rheumatology Unit and the Rheumatology Division of Clinics Hospital, São Paulo, Brazil, were included in this study. All patients fulfilled the international classification criteria for JDM (19). A total of 81 age-matched healthy subjects were concomitantly included in the control group. All participants were ≥9 and ≤21 years old, and exclusion criteria included previous proven infection by influenza A H1N1/2009, anaphylactic response to vaccine components or to egg, previous vaccination with any live vaccine four weeks before or any inactivated vaccine two weeks before the study, 2010 seasonal influenza vaccination, acute infection resulting in fever over 38°C at the time of vaccination, Guillain-Barré syndrome or demyelinating syndromes, blood transfusion within six months, and hospitalisation (13).

Study design

This was a prospective, open study conducted between March 2010 and April 2010. All JDM patients were invited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the Immunisation Centre of the same hospital. Healthy volunteers who came to this centre seeking vaccination in response to the Public Health National Campaign were included as control group. This protocol was ap-

proved by the Local Institutional Review Board, and informed consent was obtained from all participants or their legal guardian. The study was registered at clinicaltrials.gov under no. NCT01151644.

A single intramuscular dose (0.5 ml) of H1N1 A/California/7/2009-like virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur) was administered to all participants. Patients and controls were evaluated on the day of vaccination (from March 22nd to April 2nd) and after three weeks. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Vaccine

A novel monovalent, non-adjuvanted, inactivated, split-virus vaccine was supplied by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The vaccine contained an inactivated split influenza virus with 15 μg of haemagglutinin 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. Embryonated chicken eggs were employed using the same standard techniques for the production of seasonal, trivalent, inactivated influenza vaccine. The vaccine was presented in 5-ml multi-dose vials with thimerosal (45 μg per 0.5-ml dose) as a preservative.

Haemagglutination inhibition assay

The antibody levels against H1N1 A/California/7/2009-like virus were evaluated using the haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute. Sera were tested for antibodies to the H1N1 A/California/7/2009 influenza strain supplied by Butantan Institute at an initial dilution of 1:10, and at a final dilution of 1:2560. For calculation purpose, negative titers had an assigned value of 1:5, and titers greater than 1:2560 a value of 1:2560. Samples were tested in duplicate, and geometric mean values were used in the analysis. Virus concentrations were previously determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally oc-

curring nonspecific inhibitors from the sera as previously described (20).

The immunogenicity end-points after vaccination were the seroprotection (SP) rate (antibody titre ≥1:40), seroconversion (SC) rate (pre-vaccination titre <1:10 and post-vaccination HIA titre ≥1:40 or pre-vaccination titre ≥1:10 and ≥4-fold increase in post-vaccination titre), geometric mean titres (GMTs), and factor increase in GMT (GMT of the ratio of antibody titres after and before vaccination).

Safety assessment

On the day of vaccination, patients or parents were given a 21-day personal diary card containing the following list of pre-defined adverse events: local reactions (pain, redness, swelling, and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea, and nasal congestion). Participants were asked to give 'yes/no' responses to each side effect and to return their diary cards at the second evaluation day (21 days after vaccination). Adverse events that were not on the list were also reported. All local reactions were considered related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analysed by the investigators to determine their causality. Severe adverse events were defined as those requiring hospitalisation or death.

Disease activity, JDM clinical course, muscle strength and treatment in JDM patients

JDM activity was assessed by disease activity score (DAS) (21) (range 0–20), and muscle strength was evaluated by childhood myositis assessment scale (CMAS) (22) (range 0–52) and manual muscle testing (MMT) (23) (range 0–80). The JDM clinical course was classified in monophasic, recurrent and chronic (24). The serum muscle enzymes performed were aspartate aminotransferase (AST) (normal value <41 IU/L), alanine aminotransferase (ALT) (normal value <37 IU/L), lactate dehydrogenase (LDH) (normal range 240–480 IU/L), creatine phosphokinase (CK) (normal range 39–308 IU/L) and aldolase (nor-

mal value <7.6 IU/L). Data concerning the current JDM treatments included: prednisone, methotrexate, azathioprine, chloroquine, cyclosporine, cyclophosphamide, mycophenolate mofetil, intravenous immunoglobulin and rituximab.

Statistical analysis

The immunogenicity and safety analyses were descriptive, and the two-sided 95% confidence intervals (CI) were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for haemagglutination inhibition titres. The analysis of continuous variables was based on distributional assumptions. The GMTs and FI in GMT were compared between JDM patients and the healthy controls using a two-sided Student's t-test or Mann-Whitney U-test on the log$_{10}$-transformed titres. Mann-Whitney U-test was also used to compare demographic data, muscle enzymes, JDM scores and prednisone current dose between patients with and without seroconversion. For categorical variables, statistical summaries included the rates of seroconversion that were compared using Fisher's exact test. All tests were two-sided with a 0.05 significance levels.

Results

Demographic data

JDM patients and healthy controls had similar current age (15.5 [9–21] *vs.* 15 [9–21] years, p=0.511) and frequencies of female gender (63% *vs.* 41%, p=0.286). The median disease duration of JDM was 5.5 (2–17) years.

Response to immunisation in JDM patients and controls

Table I illustrates seroprotection, seroconversion, GMTs and factor increases in the GMTs in JDM patients and controls before and after influenza A H1N1/2009 vaccination. Prior to immunisation, the seroprotection rate and GMT were comparable between JDM patients and healthy controls (p=0.457, p=0.817; respectively). After immunisation, the seroconversion rate was significantly lower in JDM patients compared to healthy controls (86.7%, 95% CI 74.9% to 99.3% *vs.* 97.5%, 95% CI 94.1% to 100.9%, p=0.044),

whereas the seroprotection rate was similar in both groups (90%, 95% CI 79.6% to 101.1% *vs.* 97.5%, CI 94.1% to 100.9%, *p*=0.121). In addition, GMT after immunisation and factor increase in GMT were alike in the two groups (*p*=0.992 and *p*=0.827 respectively). None of the JDM patients and three (3.7%) healthy controls received previous immunisation with seasonal 2008/2009 influenza vaccine (*p*=0.562).

Immunisation response and disease parameters in JDM patients

Demographic data, muscle enzymes, JDM scores, lymphopenia and treatment at vaccination according to presence or absence of seroconversion in JDM patients after influenza A H1N1/2009 vaccination are shown in Table II.

Demographic data were comparable in the two groups (*p*>0.05) (Table II). The clinical courses of 19 JDM patients under any immunosuppressive agents were monophasic in 3 (15.8%), recurrent in 7 (36.8%) and chronic in 9 (47.4%). A higher frequency of chronic course was observed in non-seroconverted compared to seroconverted patients (100% *vs.* 27%, *p*=0.012). None of the patients had moderate or severe clinical activity or muscle weakness and seroconverted and non-seroconverted groups had comparable levels of JDM scores (*p*>0.05). Lymphopenia was not observed in patients that did not seroconverted. Muscle enzymes were also alike in both groups, except for a higher median level of aldolase in the non-seroconverted patients (7.4 [4.9–9.1] *vs.* 4.4 [2.1–7.2] IU/L, *p*=0.026). Regarding therapy, the four JDM patients that did not seroconvert had chronic course of disease and were more often under higher dose of prednisone (>20 mg/day) compared to those that seroconverted (50% *vs.* 4%, *p*=0.039). Likewise, a higher frequency of methotrexate (100% *vs.* 38%, *p*=0.036) and combination of prednisone, methotrexate and cyclosporine use (50% *vs.* 4%, *p*=0.039) was observed in patients that did not seroconvert (Table II).

Further analysis of the possible effect of vaccine in disease parameters revealed that the median of pre- and post-vaccination DAS (0 [0–11] *vs.* 0 [0–14], *p*=0.954), CMAS (52 [45–52] *vs.* 52 [41–52], *p*=0.803) and MMT (80 [74–80] *vs.* 80 [79–80], *p*=0.987) remained largely unchanged. Likewise, no significant differences were observed in muscle enzymes before and after immunisation: AST (20 [10–45] *vs.* 23 [11–36] IU/liter, *p*=0.246), ALT (32.5 [12–72] *vs.* 31 [11–63] IU/liter, *p*=0.825), LDH (187 [93–469] *vs.* 179 [83–446] IU/liter, *p*=0.906), CK (124 [49–533] *vs.* 102 [33–481] IU/liter, *p*=0.339) and aldolase (4.8 [2.1–9.1] *vs.* 4.8 [0–7.5] IU/liter, *p*=0.333). Frequencies of lymphopenia before and after immunisation were comparable (7% *vs.* 0%, *p*=0.492). Fur-

Table I. Seroprotection (SP), seroconversion (SC), geometric mean titers (GMT) and factor increases in the GMT (FI in GMT) in juvenile dermatomyositis (JDM) patients and controls before and after influenza A/H1N1/2009 vaccination.

Variables	JDM (n=30)	Controls (n=81)	*p*-value
SP			
Before immunisation	30 (12.5–45.5)	22.2 (13.1–31.3)	0.457
After immunisation	90 (79.6–101.1)	97.5 (94.1–100.9)	0.121
SC	86.7 (74.9–99.3)	97.5 (94.1–100.9)	**0.044**
GMT			
Before immunisation	13.8 (9.1–21)	13 (10.1–16.9)	0.817
After immunisation	259.9 (155.5–434.4)	260.6 (204.4–332.2)	0.992
FI in GMT	18.8 (11.4–31.1)	20 (15.2–26.3)	0.827

Values expressed in % (95% confidence interval).

Table II. Demographic data, muscle enzymes, juvenile dermatomyositis (JDM) clinical courses and scores, lymphopenia and treatment at vaccination according to seroconversion (SC) to influenza A H1N1/2009 vaccine in JDM patients.

Variables at vaccination (reference values)	Without SC (n=4)	With SC (n=26)	*p*-value
Demographic data			
Current age, years	15 (12–16)	15.5 (9–21)	0.646
Disease duration, years	4.9 (4–12)	7.2 (2–17)	0.806
Female gender	2 (50)	17 (65)	0.611
Muscle enzymes			
AST, IU/liter (<41)	26 (10–35)	19 (10–45)	0.471
ALT, IU/liter (<37)	41 (32–57)	31 (12–72)	0.155
LDH, IU/liter (240–480)	196 (168–211)	183 (93–469)	0.858
CK, IU/liter (39–308)	223 (65–533)	124 (49–387)	0.647
Aldolase, IU/liter (<7.6)	7.4 (4.9–9.1)	4.4 (2.1–7.2)	**0.026**
JDM clinical course			
Monophasic	0 (0)	11 (42)	0.267
Recurrent	0 (0)	8 (31)	0.550
Chronic	4 (100)	7 (27)	**0.012**
JDM Scores			
DAS (0–20)	3 (0–11)	0 (0–7)	0.126
CMAS (0–52)	51.5 (48–52)	52 (45–52)	0.894
MMT (0–80)	80 (80–80)	80 (74–80)	0.621
Lymphopenia (<1000/mm^3)	0 (0)	2 (7.7)	1.0
Treatment			
Prednisone	4 (100)	11 (42)	0.097
Current dose, mg	5.8 (2.5–12.5)	4 (1–35)	0.646
Prednisone > 20mg/day	2 (50)	1 (4)	**0.039**
Immunosuppressor (any)	4 (100)	15 (58)	0.267
MTX	4 (100)	10 (38)	**0.036**
Cyclosporine	2 (50)	4 (15)	0.169
Prednisone, MTX and cyclosporine	2 (50)	1 (4)	**0.039**
Azathioprine	0 (0)	2 (8)	1.0
Chloroquine	3 (75)	4 (15)	0.169

Values expressed in median (range) or n (%). AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; DAS: disease activity score; CMAS: childhood myositis assessment scale; MMT: manual muscle testing; MTX: methotrexate.

Table III. Adverse events of influenza A/H1N1/2009 vaccination in juvenile dermatomyositis (JDM) patients and controls.

Variables	JDM (n=30)	Controls (n=81)	*p*-value
Local reactions			
Pain	9 (30)	19 (23)	0.472
Redness	0	2 (2)	1.0
Swelling	0	2 (2)	1.0
Itching	0	1 (1)	1.0
Systemic reactions			
Arthralgia	1 (3)	2 (2)	1.0
Fever	1 (3)	3 (4)	1.0
Headache	5 (17)	18 (22)	0.606
Myalgia	1 (3)	6 (7)	0.671
Sore throat	0	5 (6)	0.321
Cough	0	5 (6)	0.321
Diarrhea	0	2 (2)	1.0
Rhinorrhea	4 (13)	3 (4)	0.083
Nasal congestion	0	3 (4)	0.561

Values expressed in n (%).

thermore, therapy was stable throughout the study in all patients.

Adverse events

Local and systemic vaccine adverse events were mild and had similar frequencies in JDM and controls (*p*>0.05) (Table III). None of them had severe adverse events.

Discussion

This study revealed that the non-adjuvanted influenza A H1N1/2009 virus immunisation is effective in JDM patients and identified that JDM chronic course and immunosuppressive therapy may hamper the vaccine induced antibody production.

The advantage of the present study was the inclusion of a homogenous group of patients that fulfilled the criteria for JDM (19) and the comparison with an age-matched control group, since vaccine efficacy has a distinct pattern in pediatric population (15). We also included only patients over 9 years of age, excluding the group of infants and children who had reduced humoral response to influenza A H1N1/2009 vaccine and required two doses of this vaccine (15, 16). Additionally, the use of non-adjuvant vaccine was chosen to avoid an autoimmune-related disease (25). The prospective design of this rare disease resulted, however, in a limited number of participants, and to our knowledge our study encompasses the

largest JDM population that received influenza vaccine (11-13).

After immunisation with influenza A H1N1/2009 vaccine, the immunoresponse was impaired in JDM patients, as also observed in our recent report for the same vaccine in adult DM (26). Similarly, we evidenced reduced seroconversion rates for the same vaccine in a cohort of 99 of juvenile systemic lupus erythematosus (JSLE) and 93 juvenile idiopathic arthritis (JIA) patients (13). Further studies will be performed to assess the influence of influenza A H1N1/2009 vaccine in disease parameters and the potential deleterious effect of therapy in immunoresponse treatments in each of these diseases.

In contrast, previous studies in juvenile rheumatic diseases (27), including a very limited number of JDM populations (11, 12), demonstrated satisfactory immunogenicity with seasonal and pandemic influenza vaccination, independent of glucocorticoid and immunosuppressive therapies. In addition, the lower seroconversion rates in JDM patients cannot be explained by previous immunisation with seasonal influenza vaccine.

The four patients without seroconversion had chronic course of JDM and therefore, they were still under immunosuppressants combination in spite of mild disease activity parameters. Glucocorticoid was the major factor for the reduced overall immune response of

pandemic vaccine in our recent study with juvenile autoimmune rheumatic diseases, mainly comprised by JSLE, JIA and 18 JDM also included in the present evaluation (13).

We have identified that immunosuppressive therapy may hamper vaccine antibody response in JDM patients. In our previous study including several pediatric autoimmune diseases, lymphopenia and immunosuppressants did not influence seroconversion against the same vaccine (13). Likewise, previous studies reported no effect of immunosuppressants in immunogenicity with seasonal (12, 27) and pandemic influenza vaccine (11) in patients with rheumatic diseases. In contrast, glucocorticoid and/or immunosuppressant use was associated with lower humoral and cell-mediated responses against the H1N1 strain of seasonal influenza vaccine in adult systemic lupus erythematosus (28, 29) and rheumatoid arthritis patients (30). In a recent study on pandemic influenza A H1N1/2009 vaccine in adult lupus, immunogenicity was improved in those under antimalarials therapy (17).

As regards the possible influence of other clinical and laboratorial parameters, lymphopenia was not a relevant finding in these patients and does not seem to interfere with immunoresponse to vaccine in JDM. Of note, in lupus, pandemic vaccination failure was significantly associated with reduced lymphocyte count (31).

The evaluation of the potential relevance of disease activity, as determined by JDM score, in pandemic vaccine antibody response was impaired by the small representation of patients with moderate or severe flares in our cohort that excluded hospitalised patients. Disease safety is reinforced by our findings of stable JDM scores and laboratorial muscle evaluation parameters throughout the study, including the borderline higher levels of aldolase in the non-seroconverted group. In this regard, studies with adult SLE have demonstrated no effect of seasonal influenza immunisation on disease flares (18).

Of note, influenza A H1N1/2009 vaccine was well tolerated and safe in JDM patients, as no serious short-term

adverse event was observed, as also reported previously in a limited number of JDM patients that received influenza vaccine (11, 12). In our large study with 237 pediatric autoimmune rheumatic diseases patients, only arthralgia was more frequently observed, comparing patients to healthy controls (13).

Notably, for pandemic influenza vaccines to be licensed, all children, adolescents and adults must meet all three current immunologic standards established: a percentage of seroprotection >70%, seroconversion >40%, and a factor increase in GMT >2.5 (29-31). JDM patients and healthy controls evaluated herein fulfilled all of the three criteria, indicating that the vaccine, while being less immunogenic, was effective.

In conclusion, this study identified that in JDM patients, chronic course and immunosuppressive therapy may hamper seroconversion, suggesting that a specific vaccination protocol may be required for this subgroup of patients. In spite of that, a single dose of non-adjuvanted influenza A H1N1/2009 vaccine was generally seroprotective and had no evident deleterious effect in disease itself.

Acknowledgments

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