Safety and efficacy of influenza vaccination in a prospective longitudinal study of 31 children with juvenile idiopathic arthritis

N. Toplak[1], V. Šubelj[2], T. Kveder[3], S. Čučnik[3], K. Prosenc[2], A. Trampuš-Bakija[4], L. Todorovski[5], T. Avčin[1]

[1]Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Centre Ljubljana, Slovenia; [2]Laboratory for Virology, National Institute of Public Health, Ljubljana, Slovenia; [3]Immunology Laboratory, Department of Rheumatology, University Medical Centre Ljubljana, Slovenia; [4]Unit of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Slovenia; [5]Faculty of Administration, University of Ljubljana, Slovenia.

Abstract

Objective

Influenza vaccination in children with rheumatic diseases is often recommended, but not frequently performed. Our aim was to assess the safety and efficacy of annual influenza vaccination in a longitudinal follow-up study of an unselected group of children with juvenile idiopathic arthritis (JIA).

Methods

Thirty-one children with stable JIA (10 boys, 21 girls, mean age 11.0 years) receiving various therapies and 14 children in a control group (10 boys, 4 girls, mean age 11.9 years) were vaccinated with the annual influenza vaccine Begrivac® 2008/2009. The children in both groups were followed for adverse events and infections 6 months after vaccination. Autoantibodies production and antibody titers against three vaccine viruses were determined in serial samples taken before, 1 and 6 months after vaccination.

Results

Eleven (35%) children with JIA and 5 (36%) children in the control group reported short-term adverse events. A JIA flare was observed one month after vaccination in 4 (13%) patients, and in the following five months in 7 (23%) patients. The response to vaccination after one month was significant in the control and study groups as a whole, but not in a subgroup of 4 children receiving anti-TNF-α therapy. After six months, no significant differences in the protective titers against vaccine viruses among the patient and control groups were observed. Changes in the mean values of autoantibodies after vaccination were found only for IgG aCL in the JIA group.

Conclusion

No long-term adverse events were reported after influenza vaccination in JIA and control group. Thirty-five percent of children with JIA experienced flare of the disease after vaccination. Protective antibodies against at least 2 vaccine viruses 6 months after vaccination were detected in all patients.

Key words

influenza vaccination, juvenile idiopathic arthritis, autoantibodies

Nataša Toplak, MD
Vesna Šubelj, BSc
Tanja Kveder, PhD
Saša Čučnik, PhD, Assist. Prof.
Katarina Prosenc, MSc
Alenka Trampuš-Bakija, BSc
Ljupco Todorovski, PhD
Tadej Avčin, MD, PhD, Prof.

Please address correspondence to:
Nataša Toplak, MD
Department of Allergology, Rheumatology and Clinical Immunology, University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1000 Ljubljana, Slovenia.
E-mail: natasa.toplak@kclj.si

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Introduction

The safety and efficacy of vaccination in children with rheumatic diseases receiving immunosuppressive medications has been questioned for years. Recommendations on the vaccination of immunocompromised patients were issued by the Advisory Committee of Immunisation Practice (ACIP), but these did not specifically address children with rheumatic diseases (1). Recently, the European League Against Rheumatism (EULAR) has published recommendations for the vaccination of children and adult patients with autoimmune inflammatory rheumatic diseases. Inactivated influenza vaccine should be strongly considered for these patients (2, 3).

In general, there is no contraindication for vaccination with inactivated vaccines. However, vaccination in patients with rheumatic diseases should ideally be administrated during stable disease (3).

Annual influenza vaccination is recommended for all children from 6 months to 18 years of age and for all patients with chronic diseases (4). Influenza vaccine was found to be safe and effective in healthy children. A single dose of vaccine was highly immunogenic in children equal to or over 9 years of age. To produce potentially protective antibody titers, a two-dose regimen is needed in younger children (5). In children with rheumatic diseases, only a few studies have investigated the safety and efficacy of influenza vaccination (6-9). To our knowledge, no study has so far addressed the safety and efficacy of influenza vaccination in children with juvenile idiopathic arthritis (JIA) receiving anti-TNF-α therapy.

The possible appearance of antiphospholipid antibodies (aPL) and their clinical significance in children with JIA after annual influenza vaccination have not been investigated. Only a few studies have assessed the induction of aPL antibodies in adult patients with rheumatic diseases after influenza vaccination (10, 11).

The aim of our study was to assess the safety and efficacy of annual influenza vaccination in a longitudinal follow-up study in children with JIA, and to determine the clinical significance of autoantibodies possibly induced by influenza vaccination.

Methods

Study population

The study design was a prospective study with a 6-month follow-up after the annual influenza vaccination in the 2008/09 season. The study population consisted of 31 children with stable JIA, who had no progression of disease in the last 6 weeks. Inactive disease was defined as described earlier (12). Erythrocyte sedimentation rate (ESR) values of ≤20 mm/hour were considered normal for all ages and both genders (13). Children with JIA were divided into four groups depending on their treatment at the time of vaccination:

1: patients without therapy or receiving only non-steroidal anti-inflammatory drugs (NSAIDs);
2: patients receiving disease-modifying anti-rheumatic drugs (DMARDs);
3: patients also receiving low-dose steroids;
4: patients also receiving anti-TNF-α agents.

The control group consisted of 14 children who were evaluated at a cardiology outpatient clinic at the time of recommended annual influenza vaccination.

Influenza vaccination

The children in both groups were vaccinated with annual influenza vaccine (Begrivac 2008/2009, Novartis Vaccines and Diagnostics GmbH & Co. KG, Marburg, Germany) from November 2008 to January 2009. Each 0.5 ml dose of the vaccine contained 15 μg of inactivated purified surface fragments from 3 different strains of influenza virus (A/Brisbane/59/2007 (H1N1)-like (A(H1N1)), A/Brisbane/10/2007 (H3N2)-like (A(H3N2)), B/Florida/4/2006 (Yamagata lineage)-like (Influenza B)) according to the latest Recommendations of the World Health Organisation (WHO). Children under the age of 9 who were vaccinated for the first time against influenza received a second dose of vaccine after 1 month.

All children were surveyed and questioned at the time of vaccination about intercurrent infections, past and present

medical history, vaccination history, and history of autoimmune diseases. Children with infections, egg allergy, or history of previous vaccine-adverse events were not included in the study. Children with confirmed or suspected inflammatory or autoimmune diseases were excluded from the control group.

Adverse events and infections after vaccination

All children included in the study were followed for adverse events and infections 6 months after influenza vaccination. Parents were instructed to write a detailed diary about any adverse events and infections after the vaccination period. In the case of febrile illness with body temperature above 37.5°C and/or signs of respiratory tract infection, such as runny nose, throat pain, coughing or hoarseness, the parents took nasal and oral swabs at home according to instructions, and sent samples to the Laboratory for Virology in a specially prepared envelope. Each child received three pairs of swabs with viral transport medium and specific written instructions, including drawings, on how to take swabs at home in case of acute infection. They were also asked to attach a survey form with the swabs. All nasal and oral swabs were tested for influenza and three other respiratory viruses (adenoviruses, enteroviruses, Respiratory Syncytial Virus (RSV)) using the polymerase chain reaction (PCR) method.

JIA disease activity

JIA disease activity was assessed at three time points: 1) before influenza vaccination; 2) 1 month after vaccination; and 3) 6 months after vaccination. Patients were monitored according to 6 variables of the American College of Rheumatology (ACR) paediatric core set (14). A flare was defined as a worsening of 40% in at least 2 disease activity parameters of the ACR paediatric core set, without a simultaneous improvement of 30% or more in at least 2 of the remaining parameters (15). To evaluate the relapse rate in a group of children vaccinated against influenza, we retrospectively reviewed the medical charts of 31 unselected patients with JIA who were not vaccinated against influenza in the 2008/09 season and were followed at our outpatient clinic. The medical data from the group of unvaccinated children were collected for the 6 month observational time from autumn 2008 to spring 2009. The therapy of unvaccinated group of children with JIA was as follows: 6 were treated with methotrexate, 1 with tacrolimus, 1 with cyclosporine A, 2 also received a low dose of methylprednisolone (less than 10 mg per day), and 4 children also received anti-TNF-α agents (2 infliximab and 2 etanercept).

Determination of protective antibodies

The presence of protective antibodies was assessed at three time points: (1) before influenza vaccination; (2) 1 month after vaccination; and (3) 6 months after vaccination. Antibody titers against the 3 vaccine influenza viruses were measured using a virus neutralisation test on Madin-Darby Canine Kidney cells (MDCK) cells (16, 17). Two-fold dilutions of each serum were made and the sera were tested at an initial dilution of 1:4 to 1:128. For sera whose titers were below 4, an arbitrary value of 2 was assigned for subsequent calculation. For sera whose titers were above 128, a value of 256 was established. Serum samples were tested separately and in duplicates; if a result showed a difference by a factor of 2, the sample was retested. For the final result, the mean value of all repeated titers was calculated. Seroprotection was defined as an antibody titer equal as or higher than 40 (18, 19). Reconvalescent titer before vaccination was defined as an antibody titer equal to or higher than 80. One month after vaccination, an adequate immune response to the vaccine was defined as a 4-fold increase in an antibody titer for individuals with antibody titers lower than 40. For individuals with prevaccinal titers higher than 40, at least a 2-fold increase in an antibody titer was defined as an adequate immune response to the vaccine (19).

Determination of autoantibodies

The presence of autoantibodies was assessed at three time points: (1) before influenza vaccination; 2) 1 month after vaccination; and 3) 6 months after vaccination.

All participants were screened for the presence of antinuclear antibodies (ANA), antibodies against extractable nuclear antigens (anti-ENA), anti-neutrophil cytoplasmic antibodies (ANCA), IgG/IgM anticardiolipin antibodies (aCL), IgG/IgM/IgA anti-β_2 glycoprotein I antibodies (anti-β_2-GPI), and lupus anticoagulant (LA) before and after vaccination.

ANA were detected by a standard indirect immunoflorescence technique on HEp-2 cells (Immuno Concepts, Sacramento, CA, USA). An ANA of 1:80 or higher was considered positive. Anti-ENA antibodies were detected by standard counter-immunoelectrophoresis using rabbit thymus and human spleen extracts as the antigen substrates (20).

ANCA were detected by indirect immunofluorescence on ethanol-fixed leukocytes (21).

aCL and anti-β_2GPI antibodies were determined by our in-house enzyme-linked immunosorbent assays (ELISAs), as described previously (22).

LA was detected by a one-stage clotting assay using a simplified dilute Russell viper venom time test (dRVVT) and a confirmation dRVVT test with a high phospholipid concentration (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) to neutralise the possible LA effect (23). The normalised ratio of both tests was calculated, and a value of 1.2 or higher indicated the presence of LA.

Statistical methods

Statistical tests were performed using the R software package. The Chi-square test, Student's *t*-test and the Wilcoxon signed-rank test were used where appropriate. The statistical significance of the difference between the intergroup frequency rates before and after vaccination was determined using the Chi-square test. The Student's *t*-test was used to compare the mean values of autoantibodies before and after vaccination. The Wilcoxon signed-rank test was used to compare the values of geometrical mean titers (GMT) of antiviral antibodies before and after vaccination.

For all statistical tests, a significance threshold of 95% was used.

Ethics Committee approval

The parents of each child included in the study were informed about the aim of the study and asked for written informed consent for drawing blood before and after vaccination. The study was approved by the Ethics' Committee of the Slovenian Ministry of Health, and was conducted according to the principles of the Helsinki Declaration.

Results

General characteristics of the study population and control group before influenza vaccination

General characteristics of the study population and control groups of children, including the group of unvaccinated children with JIA, are presented in Table I. The children with JIA were classified according to the classification criteria of the International League of Associations for Rheumatology (ILAR) (24). The therapy of the study group is presented in Table II.

Twenty seven (87%) children with JIA were vaccinated against influenza infection for the first time. Eleven (79%) children in the control group, who were evaluated at a cardiology outpatient clinic, were vaccinated against influenza infection for the first time. Ten children with JIA and two children in the control group received a second dose of influenza vaccine one month after the first vaccination.

Safety of influenza vaccination - Adverse events

Eleven children (35%) in the JIA group and 5 (36%) children in the control group reported short-term adverse events. In the group of children with JIA, 10 (32%) had pain at the injection site lasting 1-4 days, and 1 patient (3%) had a mild systemic reaction (malaise and headache 30 minutes after vaccination). In the control group, 3 children (21%) had pain at the injection site lasting 1–2 days, and 2 children (14%) had a mild systemic reaction (both with malaise). No long-term adverse events were reported during the 6-month follow-up period after vaccination.

Table I. General characteristics of the study group and control groups.

Group of children	Children with JIA vaccinated against influenza	Children with JIA not vaccinated against influenza	Children evaluated at a cardiology clinic, vaccinated against influenza§
Number (M/F)	31 (10/21)	31 (13/18)	14 (10/4)
Mean age ± SD (range)	11.0 ± 4.5 years (3–18)	8.0 ± 4.0.years (2–15)	11.9 ± 4.5 years (4–18)
Inactive disease n. (%), off /on medications	13 (42) 7/6	24 (77) 15/9	
Mean disease duration ± SD (range)	3.4 ± 2.4 years (0.2–9)	3.2 ± 2.7.years (0.1–8.4)	
Disease subtype* POA	15**	16	
EOA	3	3	
PA	3	5	
SJIA	2	3	
PsA	4	4	
ERA	4	0	

JIA: juvenile idiopathic arthritis; M: male; F. female; SD: standard deviation.
*POA: persistent oligoarthritis; EOA: extended oligoarthritis; PA: polyarthritis (RF negative); SJIA: systemic JIA; PsA: psoriatic arthritis; ERA: enthesitis-related arthritis.
**one child was treated for uveitis that was inactive at the time of vaccination.
§six children had congenital heart disease; 3 were operated in the neonatal period and 3 later, in the first years of life. One child had ventricle septum defect and was not operated. Three children had valve disease and one child had concentric hypertrophy of left ventricle. Three children were examined for innocent heart murmur. Three children were treated with angiotensin converting enzyme inhibitors; one among them was also treated with spironolactone, sildenafil and bosentan. Others were without therapy at the time of vaccination.

Table II. Data depending on the therapy of children with JIA before vaccination. The number of patients with deterioration 1 and 6 months after vaccination and the number of patients with protective titers against all 3 vaccine subtypes before, 1 and 6 months after vaccination are shown.

Therapy	n. of patients	n. of patients with deterioration		n. (%) of patients with protective titers against all 3 vaccine subtypes		
		1 month after vaccination	6 months after vaccination	Before	1 month after vaccination	6 months after vaccination
Without therapy or NSAIDs	18	1	4	2 (11)	13* (81)	14* (78)
methylprednisolone** (≤8 mg)	7	2	1	2 (29)	5 (71)	4 (67)
methothrexate	8	3	0			
leflunomide	3	0	0	4 (31)	8 (73)	10 (83)
sulfasalazine	2	0	2			
anti TNF-α therapy***	4	0	0	1 (25)	2 (50)	4 (100)
Together	31	4	6	6 (20)	21 (68)	24 (77)
Control group	14			3 (21)	11 (79)	11 (79)

NSAIDs: non-steroidal anti-inflammatory drugs; n: number.
*16 samples were available after 1 month and 18 samples after 6 months.
**Children who received methylprednisolone (mean dosage 0.1 mg/kg body weight (bw), range 0.07–0.4 mg/kg bw) were also treated with disease-modifying anti-rheumatic drugs (DMARDs).
***Children who received anti-TNF-α agents (3 etanercept and 1 infliximab) were also treated with disease-modifying anti-rheumatic drugs (DMARDS).

Disease activity in vaccinated children

The number of patients with deterioration 1 and 6 months after vaccination, depending on the therapy before vaccination, is presented in Table II. A disease flare was observed 1 month after vaccination in 4/31 (13%) patients (2 psoriatic arthritis (PsA), 1 enthesitis-related arthritis (ERA), 1 systemic JIA (SJIA)). In all four patients the number of swollen joints increased. During the

Table III. Infections in a period of 6 months after influenza vaccination in the study group (JIA patients with a flare and without flare) and in the control group. Seven children with JIA and 3 in the control group send more than one swab.

Group	Ch	Sw	Positive PCR for respiratory viruses*					
			Influenza A	Influenza B	Adenovirus	Enterovirus	RSV	All
Study group								
flare	7	11	0	0	1	0	0	1
no flare	10	15	0	1	0	0	0	1
Control group	9	14	2	0	1	1	0	4
All	26	40	2	1	2	1	0	6

Ch: number of children with tested swabs; Sw: number of swabs received; PCR: polymerase chain reaction; RSV: respiratory syncytial virus.
*Time relationship after the vaccination: 10 and 11 days, respectively, for Influenza A virus, 4 months for Influenza B virus, 3 months (study group) and 21 days (control group) for Adenovirus, 20 days for Enterovirus.

Table IV. Children with positive autoantibodies before, 1 and 6 months after influenza vaccination.

Autoantibodies	Study group n. (%)			Control group n. (%)		
	Before	1m	6m	Before	1m	6m
ANA	4 (13)	7 (23)	4 (13)	0	1 (7)	0
Anti-ENA	0	0	0	0	0	0
ANCA	0	0	0	0	0	0
aCL IgG	**3 (10)**	**6 (20)**	**10 (32)**	3 (21)	3 (21)	3 (21)
aβ2-GPI IgG	1 (3)	0	0	0	0	0
LA	1 (3)	3 (10)	0	0	0	0

N: number; m: month; ANA: antinuclear antibodies; Anti- ENA: antibodies against extractable nuclear antigens; ANCA: anti-neutrophil cytoplasmic antibodies; aCL: anticardiolipin antibodies; aβ2-GPI: anti-β_2 glycoprotein I antibodies; LA: lupus anticoagulant.

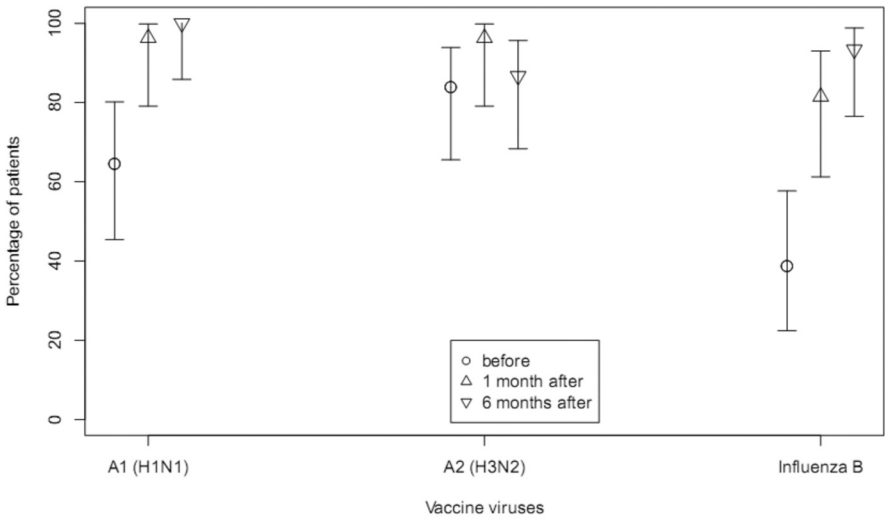

Fig. 1. Percentage of children with JIA with protective titers against 3 vaccine viruses (and 95% confidence intervals of the estimates) before, 1 and 6 months after vaccination.

6-month follow-up period, additional 7/31(23%) patients had a flare – 1 child with persistent oligoarthritis (POA) had a flare after 2 months, and 6/31 (20%) patients (4 POA, 1 ERA, 1 PsA) had a flare after 6 months. In 5/7 children the number of swollen joint increased. All children who had a flare one month after vaccination had at least one criterion for an active disease before vaccination (swollen joint, elevated ESR or physician global assessment indicating disease activity) (12). Five out of 7 children who had a flare during the following five months after vaccination had an active disease before vaccination.

Two children, one with SJIA and one with PsA, started anti-TNF-α treatment one month after vaccination because of a disease flare. One child with ERA started sulfasalazine 3 months after vaccination because of a disease flare. Four children received intra-articular steroid injections during the 6-month follow up period after vaccination.

The numbers of swabs received from patients who had a disease flare and from patients without a flare were not significantly different (Table III). There was no significant correlation between the incidence of infectious episodes and JIA activity.

Disease activity in children with JIA who were not vaccinated

Five children with inactive disease in autumn, 4 without therapy and 1 treated with methothrexate (3 POA and 2 polyarthritis (PA)) developed a swollen joint during the observational period. Two additional children with active oligoarthritis progressed to extended oligoarthritis (EOA). One started infliximab therapy in January 2009 and the other, who had an influenza-like illness at the beginning of February 2009, started methothrexate therapy a few weeks later. According to the medical records, 3 more children had influenza-like infections during the observational period (high fever, malaise, headache or cough). However, no swabs for virus detection were taken. Two among them were on immunosuppressive therapy; 1 was treated with cyclosporine and the other with infliximab. They did not have any relapse of the disease. A relapse of disease after an influenza-like illness was observed in 1 child who had an inactive disease and was without therapy.

All together, in an observational period of 6 months after the vaccination, 35% of vaccinated children and 23% of unvaccinated children with JIA experienced worsening of disease. However, before the winter season the number of children with inactive disease was higher in unvaccinated group of children with JIA (Table I).

Autoantibodies

Autoantibodies before, 1 and 6 months after vaccination are presented in Table IV. A tendency for progressively increased mean values of IgG aCL after influenza vaccination was found, but the difference before and 6 months after vaccination did not reach statistical significance (p=0.05). No other significant changes have been detected.

There was no significant association between infections in the study group and the level of IgG aCL autoantibodies. Two children with a disease flare after 6 months and negative aCL before the vaccination were positive for aCL 6 months after vaccination.

The efficacy of influenza vaccination

The efficacy of influenza vaccination was evaluated by determination of the number of infectious episodes during the 6-month period after the vaccination and by the immunogenicity of the influenza vaccine.

There was no statistically significant difference in the rate of influenza infection between the children with JIA and children in the control group (Table III). Among 3 children who got influenza infection in the observational period of 6 months after vaccination, 2 were vaccinated against influenza for the first time.

In the group of vaccinated children with JIA one was infected with Influenza B virus. In the group of unvaccinated children with JIA no swabs were taken but 4 children had influenza like illness in the observational period.

The number of patients with protective titers against all 3 influenza vaccine viruses before, 1 and 6 months after vaccination depending on the therapy before vaccination is presented in Table II.

The percentages of children with JIA and children in the control group with protective titers against 3 vaccine viruses before, 1 and 6 months after vaccination are presented in Figures 1 and 2, respectively.

GMTs of protective antibodies and the p-values for the JIA group as a whole, a subgroup receiving DMARDs and the control group before, 1 and 6 months after vaccination are presented in Table V.

Before vaccination, 6 (20%) JIA patients and 3 (21%) of the controls already had neutralisation antibodies against all three vaccine viruses. Two children in the JIA group and 2 in the control group had reconvalescent titers to all three vaccine viruses; 1 child in the JIA group and both children in the control group had also been vaccinated against influenza in previous seasons.

One month after vaccination, 27/31 samples in the JIA group and all samples in the control group were available for testing.

In general, protective titers against all three vaccine viruses one month after vaccination were detected in 21 (68%) children in the JIA group and 11 (79%) children in the control group (Table II). GMTs for all vaccine viruses were significantly elevated 1 month after vaccination in the whole group of children with JIA including children receiving DMARDs and in the control group (Table V). However, the group of 4 children also receiving anti-TNF-α therapy did not respond significantly to any of the vaccine viruses one month after vaccination.

In the whole group of children with JIA, an inadequate response (below 4-fold increase in patients with prevaccinal titers below 40, or less than 2-fold increase in patients with prevaccinal titers equal to or higher than 40) to vaccination was mainly observed for the Influenza B vaccine virus; 9 children in the JIA group (one among them was vaccinated also in previous seasons) and 7 in the control group (one among them was vaccinated also in previous seasons) did not respond adequately or had values below the prevaccinal titers. Six children in the JIA group (3 among them were vaccinated also in previous seasons) and 3 in the control group did not respond adequately to the A (H3N2) vaccine virus. The best response was detected for the A (H1N1) vaccine virus - only 2 children in the JIA group and 1 in the control group did not respond adequately. However, the protection titers were reached in the majority of children in both groups. Only 5 children in the JIA group and 3 in the control group did not reach the protective titers against the Influenza B vaccine virus.

All children receiving anti-TNF-α therapy were vaccinated against influenza for the first time. However, one of them already had protection titers against all three vaccine viruses. One 6 years old child on anti-TNF-α therapy did not develop a protection titer against the A (H1N1) and A (H3N2) vaccine viruses. He received a second dose of vaccine after 1 month.

Six months after vaccination, 30/31 samples in the JIA group and all samples in the control group were available for testing.

Protective titers against all three vaccine viruses were detected in 24 (77%) children in the JIA group and 11 (79%)

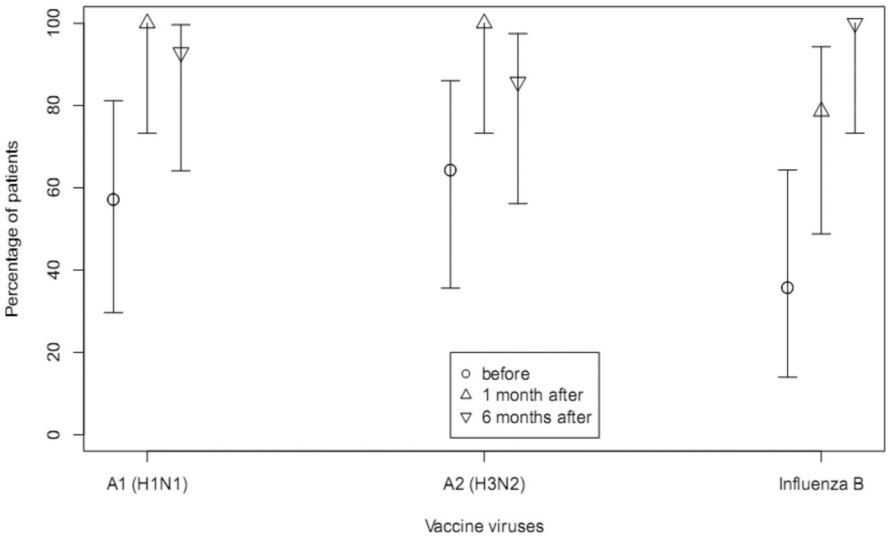

Fig. 2. Percentage of children in the control group with protective titers against 3 vaccine viruses before (and 95% confidence intervals of the estimates), 1 and 6 months after vaccination.

Table V. Geometrical mean values of protective antibody titers for three vaccine viruses in the control and JIA groups, separately presented for children receiving DMARDs, before, 1 and 6 months after vaccination. The reported *p*-values correspond to the Wilcoxon signed-rank test of the statistical difference between the concentrations observed before and after vaccination.

	Control group (n=14)			Study group* (n=31)			DMARDs (n=13)		
Vaccine virus	before	1m	6m	before	1m	6m	before	1m	6m
A (H1N1)	53.55	240.70 *p*=0.0039	72.68 *p*=0.58	32.8	174.9 *p*<0.001	90.93 *p*=0.07	23.02	139.4 *p*=0.0059	105.9 *p*=0.17
A (H3N2)	60.58	158.7 *p*=0.017	88.47 *p*=0.36	62.16	153.2 *p*<0.001	86.34 *p*=0.36	65.53	118.7 *p*=0.044	95.37 *p*=0.44
Influenza B	29.44	92.15 *p*=0.011	113.5 *p*=0.021	16.75	100.2 *p*<0.001	98.32 *p*<0.001	23.69	118.7 *p*=0.032	113.8 *p*=0.022

n: number of patients; m: month; DMARDs: disease-modifying anti-rheumatic drugs. *including children receiving DMARDs.

children in the control group (Table II). All 4 children on anti-TNF-α therapy had protective titers to all three vaccine viruses 6 months after vaccination although, as a group, they did not respond significantly. All children in the study had protective antibodies against at least 2 vaccine viruses 6 months after vaccination. However, we observed significantly lower GMTs 6 months after the vaccination for the A (H1N1) and A (H3N2) vaccine viruses compared to the values after 1 month. Compared to the GMTs before vaccination, the values after 6 months were still significantly elevated for the Influenza B vaccine virus in both study groups including children treated with DMARDs, but not in a subgroup of 4 children also receiving anti-TNF-α therapy.

Discussion

Vaccinations in children with JIA are often postponed or refused by parents or doctors because of safety and efficacy issues, especially in children receiving immunosuppressive drugs. In both groups included in our study, we found a very high percentage of children who received influenza vaccination for the first time (87% in the study group and 79% in the control group) regardless of the ACIP recommendations on influenza vaccination for all children from 6 month to 18 years, and especially for chronically ill and immunocompromised patients (1). However, we observed a high percentage of children with protective antibodies to at least one vaccine virus before vaccination, probably due to infections encountered in previous years.

One month after vaccination, a flare of disease was observed in 4 patients. They had a stable disease before vaccination but none of them had inactive disease. In the observational period of 6 months, another 7 children had a flare. Two among them had inactive disease before vaccination; both developed a swollen joint in a period of 6 months after vaccination. The infections did not have any influence on disease activity, and we found no association between disease activity and autoantibodies after vaccination.

To obtain a better insight on the impact of influenza vaccination on JIA disease activity, we retrospectively reviewed the medical charts of 31 unselected children with JIA who had not been vaccinated against influenza in the 2008/09 season. Unvaccinated JIA control group had a higher percentage of patients with inactive disease as the study group, which may reflect a possible selection bias that parents of children with milder disease were not concerned regarding the influenza infection and refused vaccination. In the 6 months observational period, 5 children who had had inactive disease before the winter season developed a swollen joint, and 2 with active arthritis showed worsening of their condition. In 2 of these 7 children influenza-like infection could have been the trigger for disease flare. According to the medical records, 2 additional children had influenza-like illness, but did not experience a disease relapse. It is, however, interesting to note that both of them were treated with immunosuppressive drugs, *i.e.* cyclosporine A and infliximab, respectively. The number of children with disease relapses or disease progression was not significantly different among vaccinated and unvaccinated groups. However, there are limitations in comparison of both groups. The unvaccinated group of children was included retrospectively and not all parameters for ACR disease activity score were available. We counted the number of swollen joints and if we do the same for the study group the percentage of patients with disease flare in a 6 months period in a study group is 29, comparing to 24 in a control group.

The safety of influenza vaccination in children with rheumatic diseases was first studied by Olson *et al*. (6). It was noticed that some patients with juvenile rheumatoid arthritis may have clinically significant exacerbations of disease activity after influenza vaccination. A study by Malleson *et al*. followed 3 years later (7). The number of patients included in that study was similar to the number in the present study but the children were followed for only 4 weeks after vaccination. No child with inactive arthritis developed a swollen joint after vaccination. Deterioration was observed in 3 patients by global assessment and in 7 patients by increased joint count. In a study published in 2001 which included children with several rheumatic diseases, no flares were observed (8). In a recently published study focusing on the immunogenicity of influenza vaccine in children with different rheumatic diseases, including Kawasaki and Crohn disease, two children (one with JIA and one with Takayasu arteritis) had a flare of disease 2 weeks after vaccination, but

the disease activity before vaccination was not described (9).

Vaccine efficacy assessment is based on the decline in infection rates and the production of protective antibodies after vaccination. Influenza vaccine efficacy was evaluated in several studies. It was estimated that annual influenza vaccination reduced laboratory confirmed influenza A by 77–91%. Lower protection rates were found for the B influenza virus, especially among children aged 2-6 years, where the vaccine efficacy against laboratory confirmed influenza B infection was only 22% (4). The advantage of our study compared to previously published reports is that infections were followed by laboratory determinations of viruses in the case of an illness after vaccination. One 8-year-old child with JIA who had received two doses of vaccine was infected by the Influenza B virus 4 months after vaccination in spite of developing a seroconversion for Influenza B vaccine virus (Yamagata lineage) already 1 month after vaccination. However, the PCR analyses of the virus detected in the swab revealed that the child had been infected with Influenza B virus of Victoria lineage, which was not included in the vaccine. None of the children with JIA got an influenza A virus infection in the 6-month follow up period. Two children in the control group got an influenza A virus infection 10 and 11 days after vaccination, respectively. Interestingly, both had had reconvalescent titers against A (H1N1) and A (H3N2) viruses even before vaccination.

In the present study, the response to vaccination after 1 month was significant in both groups for all vaccine viruses, and in the subgroups of children receiving different therapies except for those who also received anti-TNF-α therapy. Six months after vaccination, the mean values of GMTs were still significant for the Influenza B vaccine virus, except in a group of 4 children receiving also anti-TNF-α therapy. In three so far published studies investigating the efficacy of influenza vaccination in children with rheumatic diseases, the immunogenicity of the vaccine was comparable to the controls regardless of immunosuppressive therapy (6-8).

In other studies a lower response rate to the Influenza B virus one month after the vaccination was found, including studies of adult patients with rheumatoid arthritis (RA) who were treated with anti-TNF-α drugs (8, 25). However, the responses to vaccination in adult RA patients were estimated only 6 weeks after vaccination.

In our study group, only one 6 years old child was treated with infliximab at the time of vaccination. The time span from infliximab infusion until vaccination was at least 2 weeks for both vaccine doses. The child developed an adequate immune response to the A (H1N1) and A (H3N2) viruses only after the second vaccine dose. In the study published by Elkayam *et al*. of a group of patients with RA who were vaccinated 3 weeks after infliximab infusion, but not in the group of patients who received influenza vaccination on the same day as infliximab infusion, an insignificant response 4 weeks after vaccination was observed for A (H1N1) and A (H3N2) viruses. It was suggested that the administration of a vaccine on the day of infliximab infusion seems to produce a better humoral response than vaccination 3 weeks later (26).

In the present study, we observed a tendency for progressively increased mean values of IgG aCL after influenza vaccination, but the difference before and 6 months after vaccination did not reach statistical significance. The induction of IgG aCL did not have any clinical significance and was not associated with disease activity. Accurate information on the induction of autoantibody synthesis following routine immunisations in humans is scarce. Recently, our group published the first study investigating the induction of autoantibodies following influenza vaccination in apparently healthy adults. Changes in autoantibody values were most frequently observed for aCL and anti-β_2-GPI (27). A few studies investigating the production of autoantibodies following influenza vaccination in patients with systemic lupus erithematosus (SLE) were published; the most inducible autoantibodies were again aPL, further supporting the infectious and/or vaccination-induced production of aPL (10, 11). The possible mechanisms for this autoimmune phenomenon after vaccination, probably in persons with genetic predisposition, include molecular mimicry (28).

A limitation of our study is the small number of children enrolled. In a study published by Malleson, the number of children was almost the same (7). Seventy children were included in a study published in 2001, but this study group also included children with other auto-immune diseases (8). Data on the response to influenza vaccination in children treated with anti-TNF have not been published so far. Unfortunately, the number of children receiving anti-TNF-α therapy in our study was too low to enable us to draw any conclusions. Nevertheless, we recorded a good response 6 months after vaccination in 4 children on anti-TNF-α therapy who showed no change in disease activity. Further studies on larger groups are needed to support this finding.

In conclusion, no long-term adverse events were reported during the 6-month follow-up period after influenza vaccination in JIA and control group. Thirty-five percent of children with JIA experienced flare of the disease after vaccination. Based on our data, we can not exclude a temporal rather than causal relation between vaccination and disease flares. Protective antibodies against at least 2 vaccine viruses 6 months after vaccination were detected in all patients.

Authors' contributions

NT and TA participated in the design of the study. NT performed clinical evaluations of children included in the study. VŠ and KP performed and commented on laboratory tests for protective antibodies before and after influenza vaccination, and PCR tests for nasal and oral swabs. TK, SČ and ATB performed and commented on laboratory tests for autoantibodies. LT performed statistical analyses of obtained data. NT, TA and LT drafted the manuscript. All authors read and approved the final manuscript.

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