

Evaluation of clinical and immunological effects of inactivated influenza vaccine in children with asthma

Chiu W-J, Kuo M-L, Chen L-C, Tsao C-H, Yeh K-W, Yao T-C, Huang J-L. Evaluation of clinical and immunological effects of inactivated influenza vaccine in children with asthma. *Pediatr Allergy Immunol* 2003; 14:429–436. ©2003 Blackwell Munksgaard

Although annual influenza vaccinations are recommended by many authorities, some doctors may be reluctant to vaccinate asthmatic children because of the risk of inducing bronchial reactivity and exacerbating the asthma. In this study, the effect of split influenza vaccine on clinical symptoms, airway responsiveness and its influence on T lymphocytes was evaluated. Twenty-one asthmatic children with stable asthma were recruited and divided into two groups. Eleven patients who received the influenza vaccine formed the vaccination group and 10 patients who received a placebo formed the placebo group. Forced expiratory volume in 1 s (FEV_1), airway response (PC_{20} methacholine, PC_{20} = provocation concentration causing a 20% fall in FEV_1) and the T lymphocyte subset ratio (Th1/Th2) were recorded on day 1 pre-vaccination and day 14 post-vaccination. Patients were also asked to record their peak expiratory flow (PEF) every morning and evening and to complete daily symptom scores over the period of 2 weeks. There were no significant changes in PC_{20} , FEV_1 , PEF variability, symptom scores and the Th1/Th2 ratio between the vaccination and placebo groups between day 1 pre-vaccination and day 14 post-vaccination. Similar results of PEF variability and asthma symptom score were obtained when the analysis was restricted to the day 1 pre-vaccination and day 3 post-vaccination. Immunization with split influenza vaccine does not exacerbate asthma in children either with a clinical or immunological effect. These results suggest that children with stable asthma can safely be immunized with a split influenza vaccine.

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Key words: influenza; vaccine; T helper cells; asthma

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Accepted 15 February 2003

Asthma is a leading cause of chronic illness in childhood. It is the most common cause of hospitalization in children (1, 2), with rates increasing in recent decades (3). Johnston et al. reported that upper respiratory viral infections, including influenza, were associated with 80–85% of asthma exacerbations in school age children (4). In the Tennessee Medicaid study, an estimated 10% to 20% of children younger than 15 years with asthma had an additional outpatient visit during the influenza season (5). A measure such as the influenza vaccination is an effective means of reducing the impact of influenza. Some reports suggest that the influenza vaccination reduces morbidity in patients with

asthma, and annual influenza vaccination for all such patients is recommended (6–8). Unfortunately the overall vaccination rate for patients with asthma is low, less than 10% to 15% (9). Doubts about effectiveness and the fear of side-effects have contributed to the under use of the influenza vaccination by many physicians (9–12).

A previous report using animals has shown that inactivated influenza virus immunization does indeed expand the generation of Th2 cells and this leads to the production of interleukin (IL)-4 (13). In order to examine the effect of the influenza vaccination on asthma patients, children with stable asthma, before and after one dose of inactivated influenza vaccine, were

analyzed for airway reactivity, symptom scores, peak expiratory flow (PEF) variability, forced expiratory volume in 1 s (FEV_1) and airway response (PC_{20} methacholine), as well as changes in the T lymphocyte subset.

Subjects and methods

Subjects

The study was conducted during September and November 2000 and involved children with stable asthma, who were attending clinics. A placebo-controlled design was used. All medications were continued unchanged during the study (inhaled budesonide 200–400 µg daily). No one had previously received an influenza vaccination. Twenty-one children in total were enrolled in this study. Individuals were excluded if they had a recent acute respiratory illness or a history of egg allergy. There were no patients taking oral steroids at the time of recruitment. All studies on vaccination were performed on an outpatient basis. The Chang Gung Human Research and Ethics Committee had approved the study, and written informed consents were obtained from their parents or guardians of each subject before commencement. The 21 asthmatic children were divided into two groups (vaccination and placebo group). Subjects in the vaccination group ($n = 11$: 9 male and 2 female) with a mean age 10.2 years (range 6–15) received a 0.5-ml intramuscular injection of split antigen trivalent 2000–2001 influenza vaccine [Vaxigrip, Pasteur Merieux (Lyon, France), containing the following: influenza antigens A/WUHAN/359/95 (H3N2) 15 µg HA, A/BAYERN/95 (H1N1) 15 µg HA, B/BEIJING/14/93 15 µg HA]. Subjects in the placebo group ($n = 10$: 6 male and 4 female) with a mean age 10.7 years (range 10–12) received a 0.5-ml intramuscular injection of a saline placebo. The FEV_1 and methacholine challenge test were performed on day 1 pre-vaccination and repeated on day 14 post-vaccination in both groups.

Patients in both groups were requested to record their PEF every morning and every evening and to complete a daily symptom score chart for 2 weeks.

Asthma symptoms, which included night cough, daytime cough, chest tightness, wheeze, shortness of breath, and sleep disturbance were recorded according to the following scores: 0 = absent to 3 = severe. Daily symptoms were 0 = did not occur, 1 = occurred, but not often or severely enough to cause inconvenience, 2 = interfered slightly with daily activities, and 3 = interfered a lot with daily activities. Daily

PEF variability was calculated according to the following formula (7):

Daily variability

$$= \frac{(\text{PEF evening} - \text{PEF morning}) \times 100\%}{1/2 (\text{PEF evening} + \text{PEF morning})}$$

In order to observe the change in the T lymphocyte subset in the asthmatic children and in a healthy group, all three groups were sampled for blood on day 1 pre-vaccination and on day 14 post-vaccination and the Th1/Th2 cells quantified by intracellular staining analysis.

Asthma case definition

All asthmatic children were mite sensitive (*Dermatophagoides pteronyssinus* and *D. farinae*) in terms of having a positive history of asthma attack during house cleaning, a skin test response greater than 2+ (wheal) or 10 mm using the intracutaneous route with mite extract at a concentration of 10^{-6} g/ml and a positive mite RAST result (CAP system, Pharmacia Upjohn Diagnosis, Uppsala, Sweden). The diagnosis and classification of the clinical severity of asthma were made according to the Guidelines of Global Initiative on Asthma (14). Normal controls had no previous clinical history of allergic rhinitis, chronic cough or asthma and no evidence of recent infection.

Methacholine challenge test

The methacholine challenge test was performed as described in previous reports (15, 16). A Devilbiss nebulizer model 646 (APS; Jaeger, Würzburg, Germany) was connected to a dosimeter, which consisted of an input solenoid valve that delivered 20 psi of compressed air generating an aerosol of particle size 5 µm. A timer was set at 0.5 s, which was the duration of each inspiratory breath. The timing of the opening of the valve was triggered manually by the patient with a handheld reset switch and a thermistor breath-sensing unit. The concentrations of methacholine were 0.075, 0.15, 0.62, 1.25, 2.5, 5.0 and 25.0 mg/ml. The methacholine challenge was terminated if the FEV_1 decreased to less than 80% of the baseline value, and a PC_{20} was calculated.

Th1/Th2 cell ratio measurement by intracellular staining analysis (17)

Fresh whole blood was stimulated with 25 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma

Chemical Co., St. Louis, MO, USA), 1 µg/ml ionomycin (Sigma) and 10 µg/ml brefeldin A (Sigma) for 4 h at 37°C. The cells were stained with anti-human CD4 conjugated with fluorescein isothiocyanate (FITC, Becton Dickinson, San Jose, CA, USA), and then the red blood cells were removed with lysis solution (Becton Dickinson). The remaining cells were permeabilized with the solution provided by manufacturer and then stained with intracellular phycoerythrin (PE)-conjugated antibody specific to interferon (IFN)-γ (Becton Dickinson) or specific to IL-4 (Becton Dickinson). After washing with phosphate-buffered saline containing 2% fetal calf serum, the cells were fixed with 1% paraformaldehyde and subjected to flow cytometry (FACScan, Becton Dickinson). The percentage of cytokine-producing CD4⁺ cells was then calculated. A Th1 cell was defined as an IFN-γ producing CD4⁺ cell, and a Th2 cell as IL-4 producing CD4⁺ cell.

Statistical analysis

All statistics were generated using SPSS Statistical Package (SPSS 10.0 for Windows, SPSS Inc.; Chicago, IL, USA). The Wilcoxon signed rank test was used to compare the PC₂₀, FEV₁, PEF variability, symptom scores and the Th1/Th2 ratio either between before and after vaccination in the vaccination group or between before and after placebo injection in the placebo group. The Kruskal–Wallis test was used to compare the PC₂₀, FEV₁, PEF variability, symptom scores or the Th1/Th2 ratio between vaccination and the placebo group on day 1 pre-vaccination (Table 1).

Table 1. Baseline characteristics of children with asthma

	Vaccination group	Placebo group	p-Value
No. of children with asthma	11	10	
Age (year)			
Mean	10.2	10.7	
Range	6–15	10–12	
Sex			
Female	2	4	
Male	9	6	
PC ₂₀ – methacholine			
Mean ± SD (mg/ml)	2.87 ± 1.90	2.38 ± 1.09	0.522
FEV ₁ of predicted value			
Mean ± SD (%)	101.82 ± 13.81	100.70 ± 13.51	0.860
PEF variability			
Mean ± SD (l/s)	5.73 ± 4.38	4.21 ± 2.24	0.438
Asthma symptom score			
Mean ± SD	1.09 ± 1.30	0.50 ± 0.67	0.299
The ratio of Th1/Th2			
Mean ± SD	2.20 ± 1.42	1.97 ± 1.48	0.725

PC₂₀: provocation concentration causing a 20% fall in FEV₁; FEV₁: forced expiratory volume in 1 s; PEF: peak expiratory flow.

Results

Twenty-one asthmatic children were enrolled in this study and divided into two groups, receiving vaccine injections and placebo injections independently (Table 1). According to the grades of asthma severity from the Guidelines of the Global Initiative on Asthma (14), these asthmatic children at baseline had mild-to-moderate persistent asthma.

In the vaccination injection group, five asthma-related parameters including PC₂₀, FEV₁, PEF variability, asthma symptom scores and the ratio of Th1/Th2 cells were assessed (Fig. 1). There was no significant change in mean PC₂₀ between day 1 pre-vaccination (2.87 ± 1.90 mg/ml) and day 14 post-vaccination (2.98 ± 2.07 mg/ml). No significant difference was detected in mean FEV₁ between day 1 pre-vaccination (101.82 ± 13.81% of predicted value) and day 14 post-vaccination (98.95 ± 7.92% of predicted value). Mean PEF variability did not significantly differ between day 1 pre-vaccination (5.73 ± 4.38 l/s) and day 14 post-vaccination (4.99 ± 2.75 l/s). There were no significant differences in mean asthma symptom score from day 1 pre-vaccination (1.09 ± 1.30) to day 14 post-vaccination (1.09 ± 1.22). The difference between day 1 pre-vaccination (2.20 ± 1.42) and day 14 post-vaccination (2.82 ± 1.94) in the Th1/Th2 ratio was also not significant. In the placebo injection group, similar results were observed for the five asthma-related parameters (Fig. 2).

When the PEF variability and asthma symptom scores during day 1 pre-vaccination and day 3 post-vaccination were assessed, there were no significant differences between the two time points, whether for the vaccine (Fig. 3) or placebo (Fig. 4) injection group.

Discussion

Our results indicate that inoculation with inactivated influenza vaccination does not cause clinical exacerbation in children with stable asthma. There was no evidence of a significant deterioration documented by a number of independent parameters such as PC₂₀, PEF variability, FEV₁ and asthma symptom scores. However, some investigators have described adverse effects ranging from decreased PEF or increased bronchial reactivity to methacholine or histamine after inactivated influenza vaccination (18–21).

An earlier study showed that the inactivated vaccine did increase sensitivity to methacholine in 9 out of 10 asthmatic adults for 3 days after polyvalent influenza vaccination (18). Additionally, Banks et al. found that 47% of

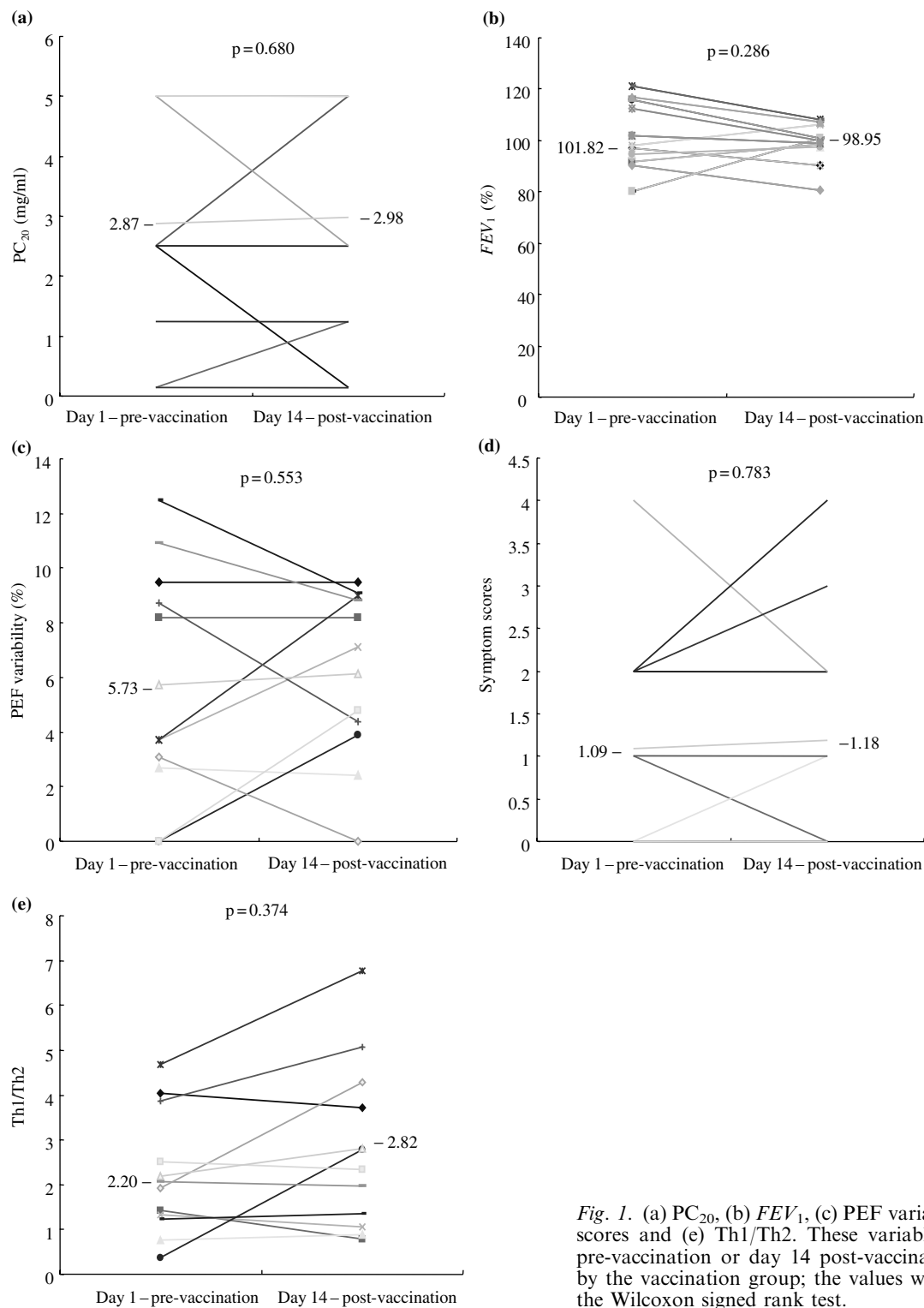


Fig. 1. (a) PC_{20} , (b) FEV_1 , (c) PEF variability, (d) symptom scores and (e) Th1/Th2. These variables either on day 1 pre-vaccination or day 14 post-vaccination were recorded by the vaccination group; the values were compared using the Wilcoxon signed rank test.

19 patients with asthma had an increase in bronchial reactivity to histamine after influenza vaccination (19). Bell et al. found a significant fall (12% from baseline) in morning PEF at 48 h after killed influenza vaccination in children with chronic severe asthma (age 6–16 years) compared with a control group that received

no vaccination (20). Campell and Edwards evaluated 28 patients with asthma who received influenza vaccination and placebo injection over a period of 3 weeks and reported a mean reduction in PEF of 168 l/min during the week after vaccination (21). One double-blind placebo-controlled study by Nicholson et al.

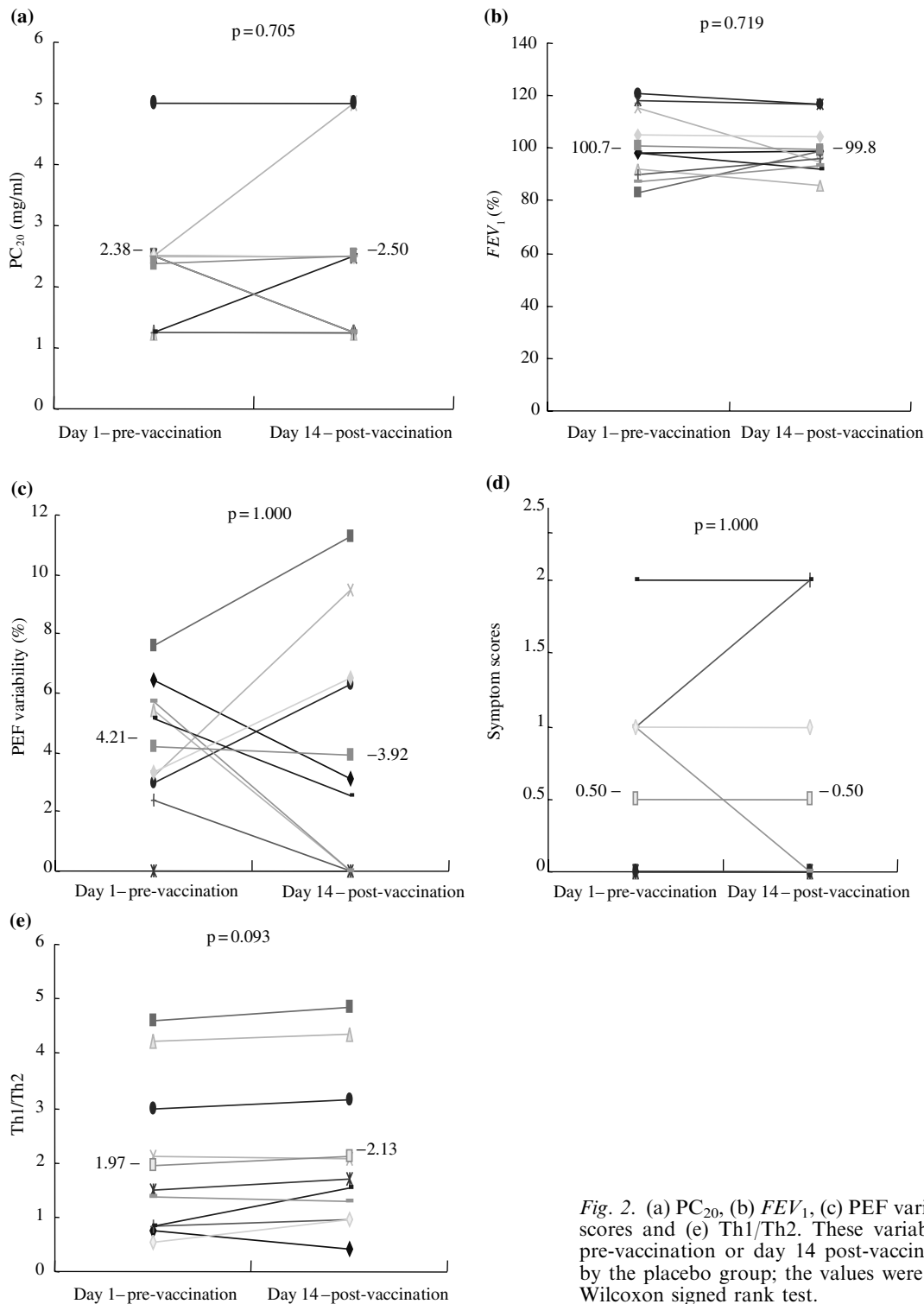


Fig. 2. (a) PC_{20} , (b) FEV_1 , (c) PEF variability; (d) symptom scores and (e) Th1/Th2. These variables either on day 1 pre-vaccination or day 14 post-vaccination were recorded by the placebo group; the values were compared using the Wilcoxon signed rank test.

revealed that 11 patients with stable asthma had a fall in PEF of more than 20% in the 72 h after inactivated split-virus or surface-antigen vaccination (22).

The discrepancy between these studies can be explained in several ways. First, the duration of observation between before and after vaccin-

ation differed among these reports. Moldoveanu et al. investigated the human response to inactivated influenza vaccine administered by systemic routes (23). Antigen-specific IgG increased significantly in sera beginning on day 13. The length of observation in our study was 14 days, thereby enabling us to detect potentially delayed

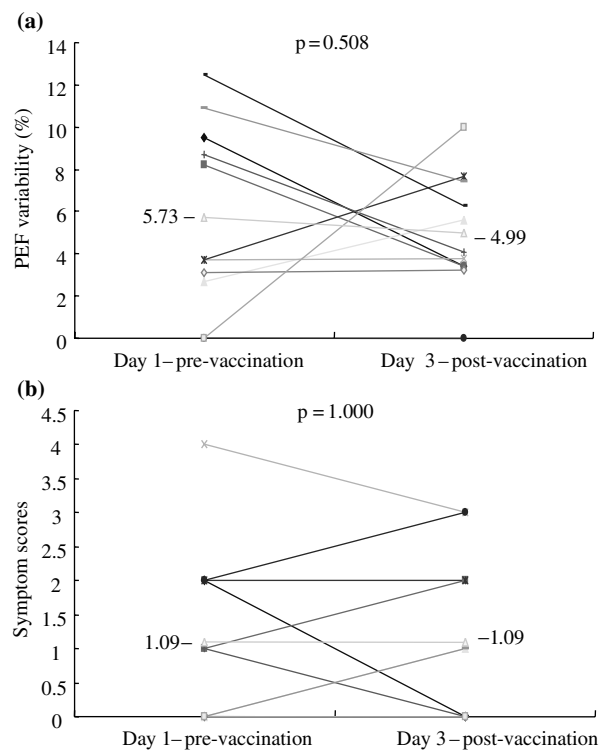


Fig. 3. (a) PEF variability and (b) symptom scores. These variables either on day 1 pre-vaccination or day 3 post-vaccination were recorded by the vaccination group; the values were compared using the Wilcoxon signed rank test.

reactions to the vaccine. We also noted no difference in PEF variability and FEV_1 in the 3 days after influenza vaccination and placebo injection. Second, the whole virus vaccines are more likely to cause side-effects than modern purified split vaccines (24). In our study, a split vaccine was used and no serious side-effects were found. Third, it is possible that the clinical deterioration in the asthma symptoms can be detected only in children with poorly controlled asthma. Our patients were stabilized prior to enrollment, which may have diminished their sensitivity to any adverse effects of the influenza vaccination. Because the patients in this study had mild-to-moderate persistent asthma, these finding cannot be extrapolated to severe asthma. Fourth, whether changes to the vaccine components from year-to-year to allow for antigenic drift of virus may change the effect of vaccination is still not clear.

Viral infection can induce the production of virus-specific antibodies and elicit cellular immunity including the expansion of both CD4+ and CD8+ T cells (25). Considerable evidence has shown that influenza virus replicates in epithelial cells and leukocytes resulting in the production of chemokines and cytokines, which

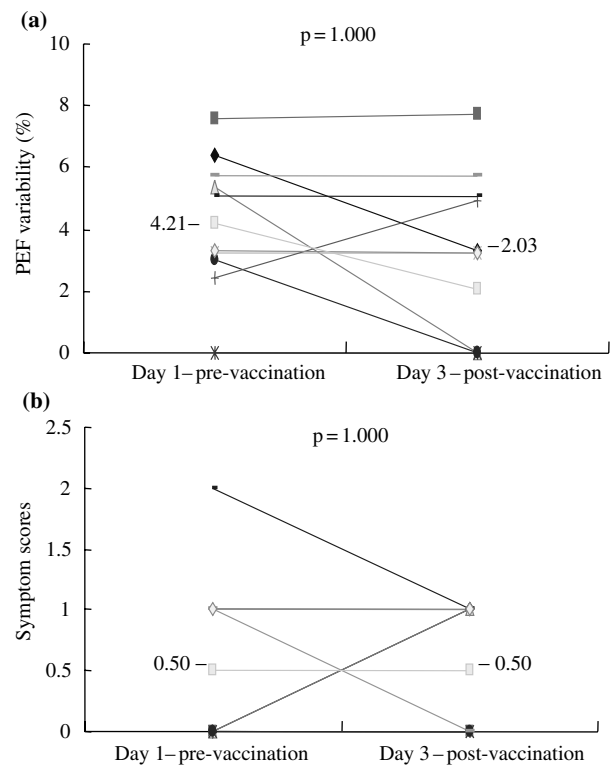


Fig. 4. (a) PEF variability and (b) symptom scores. These variables either on day 1 pre-vaccination or day 3 post-vaccination were recorded by the placebo group; the values were compared using the Wilcoxon signed rank test.

favor the development of a Th1-type immune response (26, 27). This is in contrast to asthma, which is a disorder characterized by the predominance of Th2 cells that secrete mainly IL-4 and IL-5 (28, 29). Some reports indicated that influenza virus infection may be linked to an increased rate of repeated wheezing episodes or contribute to the precipitation of an attack of asthma (30–32). Kondo and Abe found that a decrease in FEV_1 of 20% from baseline was detected in 20 children with serologically proven influenza in a residential treatment center for asthma (30). Lin et al. also reported that 12 asthmatic children (age 4–6 years) had attacks of asthma associated with influenza infection (31). Moreover, recent data have provided further support that subjects with an allergy have an intrinsic inability to regulate IL-10 production in response to influenza virus infection (33). But it is not clear whether inactivated influenza vaccination, as well as live influenza virus, can exacerbate asthma symptoms. In an animal study, Moran et al. demonstrated that inactivated influenza virus immunization primed a Th2-type response, whereas live virus elicited a Th1 response (13). Hence, a study to examine the immunological changes in the T lymphocyte

subset (Th1/Th2) before and after influenza vaccination was carried out. From the result of this study, it was shown that there was no statistical difference between the pre-vaccination and post-vaccination Th1/Th2 ratios, for both the vaccine and placebo groups. Thus, the effect of the immunological response would seem to be compatible with the clinical effect of vaccination on the asthma symptoms.

The potential benefit from the prevention of the morbidity associated with influenza virus infection has been emphasized at all times. Immunization with an inactivated influenza vaccine does not seem to induce exacerbation of poor lung function and asthma symptoms measured by changes in airway responsiveness; neither does it cause any changes in the T lymphocyte subsets. Therefore, we recommend that children with stable asthma can safely be immunized with inactivated influenza vaccine.

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