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Safe administration of an inactivated virosomal adjuvanted influenza vaccine in asthmatic children with egg allergy

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ABSTRACT

In order to evaluate whether the virosomal adjuvanted influenza vaccine that has been shown to have the lowest egg protein content (Inflexal V, Berna Biotech) could be administered to children with even severe egg allergy without any risk of allergic reactions, we used epicutaneous skin testing with the undiluted vaccine in 88 asthmatic children (44 with and 44 without egg allergy), none of whom had a positive response. They were then vaccinated with the whole dose of Inflexal V intramuscularly in a one-dose protocol, and the occurrence of any immediate or delayed adverse events were actively monitored for 28 days. The results showed the safety of the administration, and demonstrated that Inflexal V can be safely given without performing a vaccine skin test in children with any kind of egg allergy.

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1. Introduction

Egg allergy is one of the most common food allergies in infants and young children. Data collected in 2721 2-year-olds have demonstrated that the prevalence of IgE-mediated reactions to egg can be diagnosed in about 1.6% of cases [1]. Although egg allergy declines with age [2], at the age of 7 years, 0.2% of the children still have a positive skin prick test to egg [3]. The prevalence of egg allergy is even higher in allergic [4] and asthmatic children [5]. Egg is the third most reported culprit food allergen in European children [6].

Influenza is a very common illness that each year may affect more than 30% of the pediatric population [7]. Although it is usually a mild disease in otherwise healthy children, it can evolve negatively in those with a pre-existing severe chronic disease. Asthma is considered a high-risk condition because asthmatic children usually suffer from more severe wheezing episodes during the influenza season, leading to an increasing hospitalisation rates, outpatient visits and drug prescriptions [8,9]. As influenza episodes can

be at least partially prevented by the administration of influenza vaccine, health authorities recommend its use in children with chronic or persistent asthma [10,11].

However, influenza vaccines are derived from the allantoic fluid of chicken embryos inoculated with specific types of influenza viruses, and contain measurable quantities of egg protein allergens [12], which is why a number of adverse allergic events have been reported in patients with egg allergy treated with inactivated influenza vaccines [13–19]; consequently, the administration of influenza vaccine to children with asthma and egg allergy is controversial [20]. Australian authorities indicate that a severe reaction to egg is a contraindication to influenza immunisation [21], as does the Italian Ministry of Health [22]. The American Academy of Pediatrics (AAP) suggests influenza vaccination in children with less severe or local manifestation of allergy to egg without previous vaccine skin testing, but does not recommend it in subjects with a history of systemic anaphylactic symptoms, such as general urticaria, hypotension, or manifestations of upper or lower airway obstruction [22]. If vaccination is indicated, it is suggested that all these children should be skin tested before receiving the vaccine and, if positive, should only be vaccinated using a graded multidose protocol [23].

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The risk of adverse allergic events after the administration of influenza vaccine to children with egg allergy seems to be strictly related to the protein content of the vaccine, and influenza vaccination seems to be generally safe when vaccines with no more than 1.2 µg/mL of egg proteins are used in a two-dose protocol [16]. Ovalbumin is the major allergen contained in both egg white and yolk, and accounts for more than 50% of egg proteins [21]. The European Pharmacopoeia requirement allows up to 1 µg of ovalbumin per dose for split and subunit influenza vaccines as well as for virosomal vaccines, and prescribes to state the maximum amount on the label [24–26]. A comparison of the ovalbumin content in several influenza vaccines present on the market shows that the virosomal adjuvanted influenza vaccine produced by Berna Biotech (Switzerland) contains 1 ng of ovalbumin per 0.5 mL dose [27,28]. This is 500 times less than the amount allowed by European Pharmacopoeia [26].

Inflexal V is a novel virosome-based trivalent influenza vaccine, which has been shown to be highly immunogenic and well tolerated in children as well as young adults and the elderly [28]. Its manufacturing process is comprised of two production blocks: purified and inactivated influenza whole-virus antigen, and the formulation of the influenza glycoprotein subunits [28]. Its process validation is based on the haemagglutinin to phospholipid ratio, the purity of the influenza active ingredient and the consistency of particle size [28]. The high purity of the influenza glycoproteins intercalated into the phospholipid bilayer ensures the low amount of ovalbumin per vaccine dose [28].

It is assumed that vaccines with a lower ovalbumin content should induce fewer and less severe allergic reactions. However, the available data concerning the use of influenza vaccines in patients with egg allergy come from just a few studies, all of which were conducted several years ago [12–15,19]. The aim of this study was to verify whether the virosomal adjuvanted influenza vaccine that has been shown to have the lowest egg protein content (Inflexal V, Berna Biotech, Berna, Switzerland) [27] can be administered to asthmatic children with even severe egg allergy, without any risk of allergic reactions.

2. Patients and methods

2.1. Study design

This prospective study was carried out in Italy between 1 November and 10 December 2007, before the beginning of the epidemic influenza period. Informed written consent was obtained from the parents or legal guardians of all of the enrolled children, and the study protocol was approved by the Review Board for Research in Human Subjects of the University of Milan.

2.2. Study population

The study was conducted at the Institute of Pediatrics of the University of Milan, Milan, Italy. The candidates considered for vaccination had to be more than 3 years old and suffering from persistent asthma as defined on the basis of previously described criteria [11,29], never been vaccinated against influenza, regularly followed-up in the outpatient clinic of three of the main allergy centres in Northern Italy, and not receiving systemic steroid therapy. The exclusion criteria were Down's syndrome or other known cytogenetic disorders; a known or suspected immune system disease or more than 14-day administration of immunosuppressive therapy, including systemic corticosteroids (a prednisone-equivalent dose of 2 mg/kg per day, or a total of 20 mg per day for children weighing more than 10 kg); the administration of any blood products,

including immunoglobulins, in the period from 6 months before vaccination to the conclusion of the study; the intention to administer any other investigational vaccine or agent from 1 month before enrolment to the conclusion of the study; or the administration of a dose of any influenza vaccine (commercial or investigational) at any time before enrolment. The following conditions were considered temporarily limiting and the subjects could receive the study vaccine once they had been resolved: a febrile illness (axillary temperature $\geq 37.5^{\circ}\text{C}$ or rectal temperature $\geq 38^{\circ}\text{C}$) or other acute illness in the 36 h preceding vaccination; a respiratory illness in the subject and/or a household member during the 72 h preceding vaccination; wheezing in the 2 weeks before vaccination; the administration of a dose of any commercial or investigational influenza treatment (e.g. specific antivirals for the treatment of influenza) in the 2 weeks before enrolment; antibiotic treatment in the 72 h before vaccination.

The candidates to receive influenza vaccine were screened for egg allergy on the basis of their clinical history, skin prick tests (SPT), the measurement of serum egg white- and yolk-specific IgE antibody levels and a double-blind, placebo-controlled oral food challenge (DBPCFC).

Egg allergy was considered: (1) severe, when a history of anaphylaxis (a severe allergic reaction that may include anaphylactic shock or significant respiratory symptoms and hypotension) [30] after egg ingestion was documented, and when DBPCFC with 0.1–0.3 mL of egg was associated with clear signs and symptoms of allergy; (2) moderate, when clinical manifestations other than anaphylaxis were reported after egg ingestion, and when DBPCFC with 1–3 mL of egg was associated with clinical signs and symptoms of allergy; (3) mild, when there was a history of mild signs and symptoms of allergy after the ingestion of >10 mL of egg.

The candidates underwent standard epicutaneous skin testing with the egg extract in accordance with previously published guidelines [31,32]. Serum egg white- and yolk-specific IgE antibody levels were measured using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Egg allergy was confirmed at DBPCFC in all cases with a positive SPT and/or specific IgE determination with egg white and/or egg yolk, unless a history of anaphylaxis was strongly suspected. Increasing doses of 0.1, 0.3, 1.0, 3.0, 10, 20, and 30 mL of fresh egg, boiled for 30 min at 80°C , were administered at 30-min intervals over 3.5 h using a mixture of potato and saffron as placebo. Identical doses of a placebo sequence were administered on a separate day. The oral food challenge was discontinued upon first appearance of symptoms or after the seventh dose if no symptoms developed [33]. For the purposes of the study, only the reactions to DBPCFC that occurred within 6 h are considered. The children with persistent asthma and egg allergy were considered the study group, whereas gender- and age-matched children with persistent asthma but without egg allergy were assigned to the control group.

2.3. Procedures

Before enrolment, the medical history of each subject was reviewed in order to ensure compliance with the inclusion and exclusion criteria. Demographic data and medical history (including current medications and recent vaccinations) were recorded at the time of enrolment, and the children underwent a physical examination including rectal temperature. All of them underwent epicutaneous skin testing with the undiluted influenza vaccine Inflexal V (Berna Biotech, Berna, Switzerland), using the procedures previously used for the egg extract [32].

All of the children intramuscularly received the whole influenza vaccine (one-dose protocol) in the deltoid region of the upper arm in a physician-supervised clinical setting equipped with appro-

priate emergency treatment materials (AMBU bag, adrenalin and antihistamines). In order to be able to document any immediate adverse reactions to the vaccine, all of the recipients were observed for 4 h after the injection. In particular, cardiorespiratory parameters (heart and breath rate, blood pressure, SatO₂) were regularly monitored, and the children were observed for the development of vomiting, diarrhea, complaints of abdominal pain, coughing, wheezing, sneezing, rhinitis, ocular signs, skin rashes, angioedema, itching, throat tightness, shortness of breath or anaphylaxis.

Subsequently, follow-up phone calls were made to the parents of each participant 1, 2, 7, 14 and 28 days after immunisation in order to assess any delayed adverse events (AEs). The monitored AEs included fever, local skin swelling, systemic allergic reactions, and urgent medical visits or hospitalisations attributed to the vaccine. In order to evaluate the occurrence of any AEs, the parent(s) or legal guardian(s) were given a digital thermometer and asked to keep a daily record on a diary card of the subject's rectal temperature and the occurrence of specific symptoms (including local skin swelling, urticaria, decreased activity, irritability, cough, sore throat, headache, muscle aches, chills, vomiting, runny nose or nasal congestion, and sneezing). They were also asked to record any other AEs, unscheduled physician visits, or the use of concomitant prescription and non-prescription medication at any time during the month after vaccination, and to contact the investigator immediately if any significant illness or hospitalisation occurred.

2.4. Statistical analysis

The data were analysed using SAS Windows v.12 software. The continuous variables are shown as mean values $\pm$ standard deviation (S.D.), and the categorical variables as numbers and percentages. A *p*-value of <0.05 was considered significant for all of the statistical tests. The Kruskal–Wallis test was used when the data were not normally distributed or were non-parametric. Categorical data were analysed using contingency tables and the chi-squared or Fisher's test.

3. Results

Table 1 shows the demographic characteristics and medical history of the 88 children with persistent asthma, 44 with and 44 without egg allergy. The two groups were comparable in terms of gender, age, asthma severity, allergies other than to egg, and the number of subjects with a chronic severe underlying disease. None of them had ever been vaccinated against influenza. A skin test with undiluted vaccine was negative in all cases.

Eleven of the children with egg hypersensitivity were considered as having severe allergy (nine with mild-moderate anaphylaxis after egg ingestion and clear signs and symptoms of allergy at DBPCFC with 0.1–0.3 mL of egg, and two with a history of severe anaphylaxis after egg ingestion contraindicating diagnostic challenge), nineteen moderate allergy (ten with dermatitis, six with urticaria, two with vomiting and diarrhea, and one with bronchospasm after egg ingestion, all with clinical signs and symptoms of allergy at DBPCFC with 1–3 mL of egg), and fourteen mild allergy (all with dermatitis or urticaria after egg ingestion, with clinical signs and symptoms of allergy at DBPCFC after >10 mL of egg).

Administration of the whole vaccine was safe and well tolerated by all of the children, with no differences between those with and without egg allergy, or between those with egg allergy of different severity. Table 2 summarises the cardiorespiratory and AE data during the 4 h after influenza vaccination: the cardiorespiratory parameters were good and similar in all of the subjects, and acute AEs were demonstrated in one child with mild egg allergy (bron-

chospasm) and one with severe egg allergy (erythema), as well as in one control subject (bronchospasm). All three children had mild symptoms that promptly disappeared after routine therapy.

Table 3 shows the local AEs occurring during the week after influenza vaccination. As expected, local AEs (mainly tenderness) were diagnosed in 15–18% of the children, with no between-group difference in their frequency and marginal use of topical drugs for their treatment.

In the 28 days after influenza vaccination, late systemic AEs (mainly fever) were demonstrated in about 40% of the children, once again with no differences between the groups and subgroups. A marginal number of children experienced fever of $>39^{\circ}\text{C}$, and a limited number were treated with drugs. None of the children experienced a serious AE, and none was hospitalised.

4. Discussion

This study shows for the first time the safety of an inactivated virosomal adjuvanted influenza vaccination administered in a one-dose protocol to children with chronic asthma and egg allergy. Although influenza vaccination is recommended throughout the world for children with persistent asthma and previous studies have already demonstrated that up to 1.2 $\mu\text{g/mL}$ of ovalbumin content influenza vaccination is safe in all the patients [13–23], there is still controversy and ambiguity concerning the use of inactivated trivalent influenza vaccine in children with egg allergy [10,21–23]. Until the end of the last century, the problem of influenza vaccination in children with egg allergy only regarded those with asthma and those with chronic underlying diseases, who are considered at risk of influenza complications. However, the recent endorsement by some national health authorities of influenza vaccination in healthy children aged 6–59 months considerably increases the number of subjects for whom clarifying the safety of influenza vaccination in the presence of egg allergy may be important [10]. Consequently, data from studies specifically planned to verify whether and how inactivated trivalent influenza vaccines should be administered to children with egg allergy may be significantly more clinically relevant than previously thought.

The current recommendations concerning the use of influenza vaccine in children with severe egg allergy indicate the need for a vaccine skin test to decide whether the vaccine should be administered by means of multiple grade injections or directly with the whole dose [10,15,20–24,34,35]. Our findings seem to suggest that this may no longer be necessary, at least when using a vaccine containing 1 ng of ovalbumin per dose. In all of our subjects with or without egg allergy, skin tests with undiluted vaccine were always negative. It seems reasonable to attribute this finding to the fact that the vaccine we used has a significantly lower egg protein content than previously used vaccines [36,37]. In this regard, an important study tested a vaccine with an egg protein concentration that was hundreds of times higher than that of the one used in the current study [15]. Removing the need for a vaccine skin test from the protocol for the administration of influenza vaccine in children with severe egg allergy seems to be important not only because it simplifies the procedure, but also because it makes it possible to avoid the risk that eligible patients may be denied vaccinations simply because of non-specific irritant skin test reactions. James et al. [15] found that children without egg allergy can show positive intradermal skin test responses even when vaccine administration is perfectly tolerated.

The safety and the good tolerability of the vaccine we used is further demonstrated by the absence of any significant early or late allergic reactions. Immediately after administration, there were transient adverse events (one episode of wheezing and the

Table 1
Characteristics of the study population

Characteristic	Children with persistent asthma and egg allergy (<i>n</i> = 44)	Children with persistent asthma but without egg allergy (<i>n</i> = 44)
No. of males (%)	31 (70.5)	31 (70.5)
Mean age $\pm$ S.D., years	6.03 $\pm$ 3.33	6.34 $\pm$ 3.56
Asthma severity		
Controlled, No. (%)	34 (75.0)	32 (72.7)
Partially controlled, No. (%)	9 (20.5)	11 (25.0)
Uncontrolled, No. (%)	2 (4.5)	1 (2.3)
Asthma prophylaxis, No. (%)	16 (36.4)	18 (40.9)
Egg allergy		
Mild, No. (%)	14 (31.8) ^a	0 (0.0)
Moderate, No. (%)	19 (43.2) ^a	0 (0.0)
Severe, No. (%)	11 (25.0) ^a	0 (0.0)
Other allergies		
None, No. (%)	4 (9.1)	6 (13.6)
Other food, No. (%)	5 (11.3)	4 (9.1)
Inhalants, No. (%)	15 (34.1)	16 (36.4)
Other food plus inhalants, No. (%)	20 (45.5)	18 (40.9)
Underlying chronic diseases other than asthma, No. (%)	2 (4.5) ^b	3 (6.8) ^c
Previous influenza vaccination, No. (%)	0 (0.0)	0 (0.0)
Skin test with Inflexal V		
Negative, No. (%)	44 (100.0)	44 (100.0)

^a *p* < 0.0001 vs. asthmatic children without egg allergy; no other significant difference between the groups.^b One child with mild egg allergy and celiac disease, one child with severe egg allergy and congenital cardiopathy (interventricular defect).^c One child with celiac disease, one with congenital cardiopathy (interatrial defect), and one with epilepsy.**Table 2**
Cardiorespiratory parameters and adverse events in the 4 h after influenza vaccination

Parameter	Children with persistent asthma and egg allergy				Children with persistent asthma but without egg allergy (<i>n</i> = 44)
	Mild (<i>n</i> = 14)	Moderate (<i>n</i> = 19)	Severe (<i>n</i> = 11)	Total with egg allergy (<i>n</i> = 44)	
Cardiorespiratory parameters					
Median SatO ₂ (range), %	99 (97–100)	99 (96–100)	99 (98–100)	99 (96–100)	99 (96–100)
Median heart rate (range) per min	82 (60–105)	78 (68–111)	76 (68–125)	78 (60–125)	80 (69–103)
Median breath rate (range) per min	24 (22–27)	23 (20–27)	23 (18–28)	23 (18–28)	24 (18–30)
Median blood pressure (range), mmHg					
Systolic	90 (86–105)	90 (84–120)	95 (88–109)	90 (84–120)	90 (85–110)
Diastolic	65 (63–70)	60 (50–70)	65 (54–85)	65 (50–85)	65 (55–70)
Adverse events					
No. (%)	1 (7.1)	0 (0.0)	1 (9.1)	2 (4.5)	1 (2.25)
Erythema, No. (%)	0 (0.0)	0 (0.0)	1 (9.1)	1 (2.25)	0 (0.0)
Bronchospasm, No. (%)	1 (7.1)	0 (0.0)	0 (0.0)	1 (2.25)	1 (2.25)
Drug use for adverse events					
Aerosol with salbutamol, No. (%)	1 (7.1)	0 (0.0)	0 (0.0)	1 (2.25)	1 (2.25)
Oral prednisone, No. (%)	1 (7.1)	0 (0.0)	0 (0.0)	1 (2.25)	1 (2.25)

No significant between-group difference.

Table 3
Local adverse events in the week after influenza vaccination

	Children with persistent asthma and egg allergy				Children with persistent asthma but without egg allergy (<i>n</i> = 44)
	Mild (<i>n</i> = 14)	Moderate (<i>n</i> = 19)	Severe (<i>n</i> = 11)	Total with egg allergy (<i>n</i> = 44)	
Redness >3 cm, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.25)
Swelling, No. (%)	1 (7.1)	1 (5.3)	1 (9.1)	3 (6.8)	3 (6.8)
Tenderness, No. (%)	2 (14.3)	2 (10.5)	2 (18.2)	6 (13.6)	7 (15.9)
Total with local adverse events, No. (%)	2 (14.3)	3 (15.8)	2 (18.2)	7 (15.9)	8 (18.2)
Drug use for local adverse events					
Topical drugs, No. (%)	1 (7.1)	1 (5.3)	0 (0.0)	2 (4.5)	3 (6.8)
Systemic drugs, No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

No significant between-group difference.

onset of erythema) in two children with egg allergy: however, as one episode of bronchospasm also occurred in a control subject, it is impossible to be sure that the episode of wheezing was attributable to egg hypersensitivity. Similarly, no differences were found between the subjects with egg allergy and controls in terms of the incidence of late local or systemic reactions.

The absence of any significant early or late AEs after vaccine administration also seems to be attributable to the low egg protein content of the vaccine. A comparison of the ovalbumin protein content of six inactivated influenza vaccines currently marketed in Europe has demonstrated large differences [27], although all of the studied vaccines had an ovalbumin protein content lower than that permitted by the European Pharmacopeia [24–26]. As the vaccine we used had the lowest ovalbumin content, our results show that Inflflexal V can be safely administered in a one-dose protocol without performing a vaccine skin test in children with any kind of egg allergy, but they cannot be extrapolated to vaccines with a higher egg protein content. Further specific studies in children with severe egg allergy are needed in order to decide whether it is possible to administer other influenza vaccines without performing a skin test with the undiluted product and without multiple graded doses. Moreover, despite a second influenza vaccine dose should not be associated with an increased risk for allergic reactions, further researches are required on the safety evaluation of the two-dose schedule recommended for previously unvaccinated young children as well as of the annual vaccination of asthmatic children with egg allergy [24–26]. However, considering that the cell culture-derived influenza vaccines are coming [38,39], it is possible that in the future the problem of residual egg proteins will be minimal.

Disclosure statement

Appropriate informed consent was obtained, and the study was conducted in accordance with the guidelines for human experimentation specified by the authors' institution.

Conflict of interest

None declared.

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References

- [1] Eggesbo M, Botten G, Halvorsen R, Magnis P. The prevalence of allergy to egg: a population-based study in young children. *Alergy* 2001;56:403–11.
- [2] Boyano-Martinez T, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. *J Allergy Clin Immunol* 2002;110:304–9.
- [3] Roberts G, Peckitt C, Northstone K, Strachan D, Lack G, Henderson J, et al. Relationship between aeroallergen and food allergen sensitization in childhood. *Clin Exp Allergy* 2005;35:933–40.
- [4] Ratner B, Untracht S. Egg allergy in children: incidence and evaluation in relation to chick-embryo-propagated vaccines. *Am J Dis Child* 1952;83:309–16.
- [5] Simpson AB, Glutting J, Yousef E. Food allergy and asthma morbidity in children. *Pediatr Pulmonol* 2007;42:489–95.
- [6] Steinke M, Fiocchi A, the REDALL group. Food allergy in children and potential allergy medicine users in Europe. A randomised telephone survey of children in 10 European nations. *Int Arch Allergy Immunol* 2007;143:290–5.
- [7] Neuzil KM, Mellen BG, Wright PF, Michel EF, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med* 2000;342:225–31.
- [8] Glezen WP, Greenberg SB, Atmar RL, Piedra PA, Couch RB. Impact of respiratory virus infection on persons with underlying conditions. *JAMA* 2000;283:499–505.
- [9] Neuzil KM, Wright PF, Michel EF, Griffin MR. The burden of influenza illness in children with asthma and other chronic medical conditions. *J Pediatr* 2000;137:856–64.
- [10] Centers for Disease Control Prevention (CDC). Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;56(RR06):1–54.
- [11] WHO. Global Strategy for Asthma Management and Prevention (revised 2007). Available at <http://www.ginasthma.com>.
- [12] O'Brien TC, Malonet C, Tauraso MN. Quantitation of residual host protein in chicken-embryo-derived vaccines by radial immunodiffusion. *Appl Microbiol* 1971;21:780–2.
- [13] Miller JR, Orgel HA, Meltzer EO. The safety of egg-containing vaccines for egg-allergic patients. *J Allergy Clin Immunol* 1983;71:568–73.
- [14] Murphy KR, Strunk RC. Safe administration of influenza vaccine in asthmatic children hypersensitive to egg proteins. *J Pediatr* 1995;106:931–3.
- [15] James JM, Zeiger RS, Lester MR, Fasano MB, Gern JE, Mansfield LE, et al. Safe administration of influenza vaccine to patients with egg allergy. *J Pediatr* 1998;133:624–8.
- [16] Ratner B, Untracht S. Allergy to virus and rickettsial vaccines. I. Allergy to influenza A and B vaccines in children. *JAMA* 1946;132:899–905.
- [17] Bierman CW, Shapiro GG, Pierson WE, Taylor JW, Foy HM, Fox JL. Safety of influenza vaccination in allergic children. *J Infect Dis* 1977;136:S68–73.
- [18] Davies R, Pepys J. Egg allergy, influenza vaccine, and immunoglobulin E antibody. *J Allergy Clin Immunol* 1976;57:373–83.
- [19] Kawahara H, Morisawa Y, Katunuma T, Ohya Y, Saito H, Akasawa A. Immediate adverse reactions after administration of the influenza vaccine to patients with positive CAP-RAST to egg white. *Alerugi* 2002;51:559–64.
- [20] Anolik R, Spiegel W, Posner M, Jakabovics E. Influenza vaccine testing in egg sensitive patients. *Ann Allergy* 1992;68:69.
- [21] Allen CW, Campbell DE, Kemp AS. Egg allergy: are all childhood food allergies the same? *J Pediatr Child Health* 2007;43:214–8.
- [22] Italian Ministry of Health. Circular No. 1 9/8/2007. Prevention and control of influenza. Recommendations for the influenza season 2007–2008 [Ministero della Salute Italiano. Circolare n. 1-9/8/2007. Prevenzione e controllo dell'influenza. Raccomandazioni per la stagione 2007–2008].
- [23] American Academy of Pediatrics. Influenza. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. Red book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006. p. 401–11.
- [24] European Pharmacopoeia 01/2008:0158. Influenza vaccine [split virion, inactivated].
- [25] European Pharmacopoeia 01/2008:0869. Influenza vaccine [surface antigen, inactivated].
- [26] European Pharmacopoeia 01/2008:2053. Influenza vaccine [surface antigen, inactivated, virosome].
- [27] Kürsteiner O, Moser C, Lazar H, Durrer P. Inflflexal® V—the influenza vaccine with the lowest ovalbumin content. *Vaccine* 2006;24:6632–5.
- [28] Mischler R, Metcalfe IC, Inflflexal. Inflflexal® V, a trivalent virosome subunit influenza vaccine: production. *Vaccine* 2002;20:B17–23.
- [29] Bateman ED, Hurd SS, Barnes PJ, Bousquet JJ, Drazen JM, Fitzgerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143–78.
- [30] Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G, et al. The management of anaphylaxis in childhood: position paper of the European Academy of Allergy and Clinical Immunology. *Allergy* 2007;62:857–71.
- [31] Dreborg S. Diagnosis of food allergy: tests in vivo and in vitro. *Pediatr Allergy Immunol* 2001;16(Suppl. 14):24–30.
- [32] Bock SA, Buckley J, Holst A, May CD. Proper use of skin tests with food extracts in diagnosis of food hypersensitivity. *Clin Allergy* 1978;8:559–64.
- [33] Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergy and Clinical Immunology. *Allergy* 2004;59:690–7.
- [34] Zeiger RS. Current issues with influenza vaccination in egg allergy. *J Allergy Clin Immunol* 2002;110:834–40.
- [35] Sugai K, Shiga A, Okada K, Iwata T, Ogura H, Maekawa K, et al. Dermal testing of vaccines for children at high risk of allergies. *Vaccine* 2007;25:3454–63.
- [36] Principi N, Esposito S. Are we ready for universal influenza vaccination in paediatrics? *Lancet Infect Dis* 2004;4:75–83.
- [37] Mark C. Large variations in the ovalbumin content in six European influenza vaccines. *Pharmeur Sci Notes* 2006;1:27–9.
- [38] Gregersen JP. A risk-assessment model to rate the occurrence and relevance of adventitious agents in the production of influenza vaccines. *Vaccine* 2008. April 15 Epub.
- [39] Gregersen JP. A quantitative risk assessment of exposure to adventitious agents in a cell culture-derived subunit influenza vaccine. *Vaccine* 2008. April 18 Epub.