

MEASUREMENT OF NASAL IgE IN AN EPIDEMIOLOGICAL STUDY: ASSESSMENT OF ITS DIAGNOSTIC VALUE IN RESPIRATORY ALLERGY

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SUMMARY:

Background: Specific questionnaire and skin prick test (SPT) are the most used methods in epidemiological studies on respiratory allergy. SPT, however, can be positive in many subjects without evidence of any allergic disease. Nasal IgE determination has been suggested by some authors as a valuable diagnostic method, which may overcome this lack of specificity.

Objective: The aim of this study was to evaluate sensitivity and specificity of nasal specific IgE for the seven most common inhalant allergens in order to verify its reliability as a screening test.

Methods: 126 children, involved in an epidemiological study on prevalence of respiratory allergic disease, were evaluated. All children were assessed with a specific questionnaire, SPT and nasal specific IgE. Nasal specific IgE were determined with a previously described method modified for screening purposes, in order to test seven allergens at the same time. When discordant results were obtained between questionnaire, SPT and nasal IgE, an allergen specific nasal challenge (ASNC) was performed and nasal tryptase was also determined before and after challenge.

Results: The questionnaire was positive for respiratory allergy in 28/126 children. SPT was positive in 21 of the 28 children, but also in 5/10 children with atopic dermatitis (AD), and in 12/88 children without allergic symptoms. Nasal IgE were positive in 22/28 and also in 2/10 with AD. Nasal challenge and tryptase confirmed the negativity of nasal IgE in 12/17 children with positive SPT but totally negative for allergic respiratory disease. Moreover nasal IgE was found to be positive to dermatophagoides in one of seven children with negative SPT despite a clinical history suggestive for mite respiratory allergy. In this patient and in 2 of the 5 children with AD the positive nasal IgE to mites was confirmed by a positive ASNC and tryptase.

Conclusions: Nasal IgE have shown a specificity significantly higher than SPT (98% vs. 83%) and a good sensibility. This screening test may also be useful to detect the beginning of upper airways sensitization in patients with AD.

Key-words: Nasal IgE - Allergy screening test - Allergen specific nasal challenge - Respiratory allergic disease.

INTRODUCTION

At present, clinical history and skin prick test (SPT) are the most reliable methods used to diagnose respiratory allergy in clinical practice. These methods have, in fact, a high sensitivity although their specificity is not completely satisfactory. It is well known that SPT positivity for inhalatory allergens in the general population can reach 30%. This figure largely exceeds the ascertained prevalence of respiratory allergy (about 10% for asthma and 15% for rhinitis) (1-4). The specific IgE assays, possibly with a panel of the most common allergens (e.g. Phadiatop Pharmacia) bypass some of the problems of SPT, including the subjective evaluation, but they need a blood sample and their specificity is not so far superior to SPT (1-3). Finally, neither blood sampling

nor skin testing are well tolerated and easy to perform in children.

Based upon these considerations we focused our attention on mucosal specific IgE and attempted to verify at what extent their measurement is a valuable diagnostic tool in children. It is well known that allergic subjects carry specific IgE on their mast cells, and that these IgE can be detected in nasal epithelium by mucosal biopsy (5). On the other hand, it has not been fully clarified if IgE are locally produced (6-7). However, mucosal IgE are likely to be directly and primarily involved in the initiation of the allergic reaction, therefore it is expected that their presence has a relevant diagnostic value. The assessment of nasal IgE has been proven to be reliable by several authors, but it has been used so far only for research purpose (8-12). Our nasal IgE measurement, based on a new immunoenzymatic method, is not invasive and therefore it can be easily performed and repeated in children.

In order to test our hypothesis, we studied our test on a population of children screened by a validated ques-

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tionnaire and skin prick test. An allergen specific nasal challenge (ASNC) and tryptase before and after challenge were used in all cases with discordant results.

METHODS

Patients and study design

One hundred and twenty-six children (61 male and 65 female, mean age 9.5 years, age range 6-12 years), who took part to a large epidemiological study on the prevalence of allergic disease in Perugia (13), were studied. Their atopic status was assessed basically using a specific questionnaire and SPT, performed with a standard panel of the most common allergens. Nasal IgE were measured in all children with a new adapted immunoenzymatic-screening assay. An allergen specific nasal challenge (ASNC) with the suspected allergens was performed in those children where clinical history, skin tests and nasal IgE were discordant or the results were equivocal. The level of nasal tryptase was also measured before and after ASNC.

All children's parents signed a detailed informed consent and the study was approved by the inner Ethical Committee.

Questionnaire

A trained physician administered a slightly modified version of the American Thoracic Society and National Heart and Lung Institute questionnaire (14) to children. The questionnaire covered several aspects including: family history of allergic diseases, personal history of atopic eczema, rhino-conjunctivitis and asthma, occurrence of symptoms during the different seasons: spring (Sp), autumn-winter (A.W.) or both (Sp.A.W.), and summer (SU); indoor/outdoor symptoms; presence of indoor moulds; correlation with influenza; night awakenings or early morning symptoms; onset or worsening of symptoms in the presence of pets (P.E.). The considered symptoms were:

- asthma: cough, respiratory distress, chest tightness (including nocturnal or exercise-induced), or actual bronchospasm assessed by a physician ;
- rhinitis: itching, rhinorea, blockage, and sneezing ;
- conjunctivitis: itching, edema, flushing, and lacrimation.

Skin prick test

SPT were performed with commercial glycerinated extracts (Alk-Abellö, Milan, Italy). The standard panel of allergens included: *Phleum pratensis* (G6), *Parietaria judaica* (W19), *Olea europaea* (T9),

Dermatophagoides pteronyssinus (D1), *Alternaria tenuis* (M6), cat (E1) and dog (E5) epithelium, plus a positive (histamine 10mg/mL) and a negative (normal saline) control. Wheals were contoured with a soft pen and transferred onto a sticky tape. Skin reactivity was measured as the mean of the major diameter of the wheal plus its orthogonal and graded from 0= negative to ++++= highly positive. A skin reaction of 3 mm was considered the threshold for positivity (15).

Measurement of nasal IgE

Allergen-specific nasal IgE were measured using a previously described method, modified as follows (16). Briefly, seven allergen covalently-coated paper disks plus a negative control disk with chicken albumin (Sigma, Milan, Italy) were fixed on the two sides of a plastic applicator (6 cm length and 0,6 cm width) (fig. n.1). The allergens were the same used for the prick test: G6, W19, T9, D1, M6, E1, and E5 (Alk-Abellö, Milan, Italy). The applicator carrying the disks was covered with a permeable Polyamid monofilament membrane (03-64/45-SEFAR Heiden, Switzerland) (Fig.1). The prepared device was inserted into a nostril and left inside for 20'. The applicator was then removed, put into a tube containing a buffer solution (NaCl 0.9%, Tween 0.1% and NaN₃ 0.05%, w/v) and stored at 4°C until use.

All the samples were run together as follows: after a 10 minutes washing procedure, the applicators carrying the allergen-coated disks were incubated for 30' with a biotinate goat IgG anti-human IgE (VECTOR Laboratories, Milan-Italy). Then, after washing, they were incubated with Streptavidine-Alkaline Phosphatase (VECTOR Laboratories, Milan-Italy) for 30'. Finally, after a further washing, the samples were incubated for 8-10 minutes with BCIP/NBT (SIGMA, Milan-Italy) as a substrate, added with an inhibitor of endogen phosphatase (levamisole-50 mg/ml). The staining on disks was measured by a laser photometer (FAG VIPDENS 111 Densitometer FAG-VRG, Lausanne), and the color intensity was converted in KU/L, as compared to a reference curve obtained with IgE standards (CAP System FEIA IgE Pharmacia, Milan-Italy) and goat IgG anti-human IgE-coated paper disks (VECTOR Laboratoires, Milan-Italy). Values equal or greater than 0.35 kU/L were considered positive (Fig. 1).

The reproducibility of nasal IgE by the method of mucosal incubation has been previously demonstrated (16). The nasal IgE had been repeated 6 times in 10 children at an interval of 15'. Nasal IgE one-way analysis of variance had shown that between-subject variability, i.e. biological variability, was significantly greater than within-subject variability, i.e. measure-

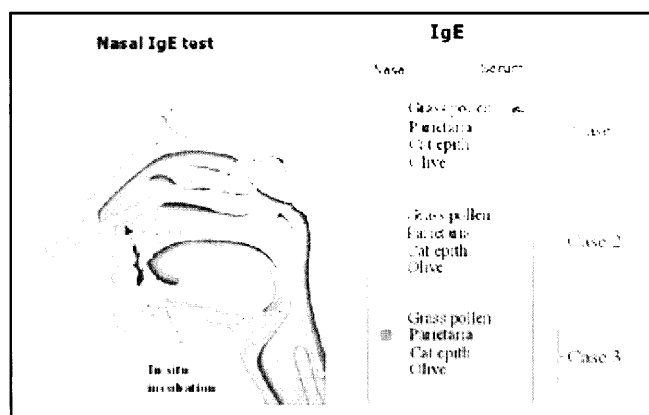


Fig. 1: Nasal IgE test.

Case 1: patient sensitized to grass pollen, evaluated during winter.

Case 2: patient sensitized to cat epithelium, after exposure.

Case 3: patient sensitized to parietaria, evaluated during spring.

ment error ($p < 0.001$). The coefficient of variation computed using the within-subject standard deviation was 37-6% (16).

Allergen-specific nasal challenge

A severity score was attributed to nasal symptoms (itching, sneezing, rhinorrhea, and blockage) plus lacrimation, as follows: 0= absent, 1= mild, 2= moderate, 3= severe. The symptom score was evaluated before and after challenge. The specific nasal challenge was performed spraying into a nostril the saline solution as negative control and then increasing concentrations of the standardized allergen extract: 2, 4 and 8 BU/mL (ALK Abello, Milan, Italy) at 20-minute intervals. An increased score of 3 or greater was used to define the threshold dose for the positive challenge.

Measurement of Nasal Tryptase

Nasal mucosal tryptase was measured, before nasal challenge and 30 minutes after, by means of the the same method used for nasal IgE (16-17). In this case, the solid phase was a sponge covalently coupled with an anti-tryptase antibody (Unicap System FEIA, Pharmacia, Upssala, Sweden). The antibody-coated sponges were repeatedly washed with NaCl 0.9%, air-dried, fixed to the nasal applicator and then covered with a permeable membrane. The applicator was left into a nostril for 10 minutes and then stored at -20°C in a buffer solution, as described above. The samples were run at the same time using the Unicap Tryptase System FEIA (Pharmacia, Upsala, Sweden). Results were expressed in mcg/L according to the standard reference curve

included in the system. An increased value after ASNC of 100% or more was considered positive.

RESULTS

The questionnaire gave positive results for respiratory allergy in 22% of children. Out of the 98 children without respiratory allergy, 10 resulted to have atopic eczema. Family history (at least one parent with respiratory allergy) was positive in 64% of children with respiratory symptoms and in 70% of children with atopic eczema (table 1). Four of the asthmatic children had seasonal symptoms and the remaining 8 had perennial disease. Out of the 16 children with rhinitis, 7 had seasonal symptoms and 9 had almost perennial symptoms (table 1). Skin tests provided at least one positive result in 30% of children: in 9/12 with asthma, in 12/16 with rhinitis, in 5/10 with atopic eczema and in 12/88 with negative history for the mentioned clinical manifestations (table 1-2). Nasal IgE were negative in the 3 patients with clinical history of asthma but negative to skin tests. The negativity of ASNC supported by no increase of Tryptase confirmed these results (table 3). Nasal IgE were negative also in 3/4 children with history of rhinitis and negative SPT. Also in these cases the results of nasal IgE were confirmed by a negative ASNC and negative response of Tryptase (table n.4). In the patient with positive rhinitis history and negative SPT, nasal IgE to mite resulted positive and, in fact the patient reacted to the ASNC and also tryptase increased significantly after challenge (table 4). Nasal IgE, ASNC, and tryptase increase after challenge were negative in 15/17 subjects with negative questionnaire for respiratory symptoms and positive SPT (table 5). Two subjects of the five with atopic dermatitis and positive prick test for Dermatophagoides (table 5) showed positive nasal IgE for the same allergen according to ASNC and increased triptase after challenge, despite negative respiratory symptoms (table 5). No significant change in nasal tryptase between baseline and after challenge could be detected in the other children which underwent ASNC (2.06 ± 0.7 VS 2.56 ± 1.3 ; $p = \text{NS}$).

DISCUSSION

A recent editorial (18), supported by another recent study (19), concludes that the most reliable method for the diagnosis of respiratory allergy, rhinitis in particular, is the assessment of typical symptoms with adequate statistic standardization. The editorial underlines that prick test and serum specific IgE can be essential to confirm symptoms, but they do not add information to clinical evaluation and they are of scarce relevance in the absence of symptoms themselves (18). It was

	Asthma 12 (13.5%)	Rhinitis 16 (17.8%)	Atopic dermatitis 10 (10.7%)	No allergic symptoms 88 (69.5%)
Comorbidity	Rhinitis: 12/12 Conjunctivitis: 5/12	Conjunctivitis: 8/16	Negative	-
Seasonality	4 seasonal	7 seasonal	10 perennial	-
Family history for allergic diseases	9/12 (75%)	9/16 (56%)	7/10 (70%)	11/88 (12%)

Table 1: Prevalence of asthma, rhinitis, and atopic dermatitis in the 126 children studied, according to clinical history.

	Asthma positive	Rhinitis positive	Atopy** positive	No allergic diseases
Positive Skin test	9/12	12/16	5/10	12/88
Positive Nasal IgE	9/12	13/16	2/10	0/88

Table 2: Skin test and nasal IgE positivity for inhalant allergens in relation to clinical history for asthma, rhinitis and atopic dermatitis (according to questionnaire).

Pt	Symptoms*	Skin test	Nasal IgE	Tryptase (µg/L)**	
				B.	A.
1	Sp. P.E.	G6 +++ W19 ++ E1 +++ E5 ++	30 3.5 10.5 3.5		
2	A.W.	D1 +++ M6 ++	35 Neg	1 2	<1 <1
3	A.W. P.E.	G6 ++ D1 +++ E1 +++	Neg 25.5 35	2 2	10 9.5
4	Sp.A.W.	G6 ++++ D1 +++ E1 ++	20.5 40 Neg	2 3	6.5 7.4
5	S	G6 +++ T9 ++	15.5 8.2		
6	S	G6 ++++ T9 ++	18 3.5		
7	A.W.	D1 Neg	Neg	2 2	<1 <1
8	Sp.A.W.	G6 +++ D1 +++ M6 ++	10.5 17 6.5		
9	A.W.	D1 +++	28.5		
10	A.W.	D1 Neg	Neg	1 1	2.5 1.8
11	Sp.A.W.	G6 Neg T9 Neg D1 Neg	Neg Neg Neg	3 2 2 2 2 2	2.6 1.6 1.5 2.2 1.2 2
12	Sp	G6 ++++ T9 ++	17 2.5		

Table 3: Children with positive questionnaire for asthma (and associated rhinitis).

*Sp = Spring;
S = Summer;
A.W = Autumn-
Winter;
P.E = pet exposure.
**B = baseline;
A = after challenge.

Pt.	Symptoms*	SPT	Nasal IgE (kU/L)	ASNC**		Tryptase (µg/L)	
				B.	A.	B.	A.
1	Sp.A.W.	G6 +++ D1 +++ E1 ++	16 22 8.5				
2	Sp.	G6 +++ T9 +++ M6 ++	32 3.5 2.8				
3	Sp	G6 ++++ T9 +++ W19 ++	27 10.5 2.5				
4	A.W. P.E.	G6 +++ D1 +++ E1 ++	Neg 15.6 4.5	1	2	2.2	2.8
5	A.W.	D1 Neg T9 ++	1.8 8.2	3	7	15	35.5
6	Sp.A.W.	G6 ++ D1 +++ E1 ++	11 26.5 Neg	2	2	1.8	2.5
7	A.W.	D1 ++++	35.5				
8	Sp.A.W.	G6 +++ T9 ++ D1 ++	14.5 2.7 3.5				
9	Sp.A.W.	G6 +++ D1 ++ M6 ++	25.6 7.8 10.5				
10	Sp	G6 ++	4.5				
11	A.W.	D1 Neg	Neg	3	3	2.5	3.8
12	Sp	G6 ++ T9 ++ D1 ++	5.5 0.7 Neg	2	2	3.2	2.5
13	Sp	G6 +++	8.4				
14	Sp	G6 +++ T9 ++	3.5 3.2				
15	A.W.	D1 Neg	Neg	3	3	<1	<1
16	A.W.	D1 Neg	Neg	2	3	2.5	1.3

Table 4: Children with positive questionnaire for rhinitis with or without conjunctivitis.
 * Sp = Spring;
 S = Summer;
 A.W = Autumn-Winter;
 P.E = pet exposure.
 ** B = baseline;
 A = after challenge.

noticed that these tests could provide (especially for some allergens) a low specificity, thus giving positive results in healthy controls (19). The author suggests the measurement of mucosal IgE as a valuable method to be possibly used in cases of uncertain diagnosis because they are produced and excreted locally (20). The hypothesis of local production of IgE has received strong support by significant studies in these last years (8-11). Anyway a better specificity of IgE detected in nasal biopsies, compared to serum IgE, has been clearly shown (5). A tight correlation between specific nasal IgE and allergenic exposure was indeed confirmed by our group in mite allergic patients living at high altitude (21-22). Based on these results, we have

tried to evaluate the measurement of specific nasal IgE as a screening method. This was done in a population of schoolchildren diagnosed by means of a specific questionnaire and skin test, taking into account the most frequent allergens in our country. According to questionnaire results, the prevalence of asthma and rhinitis in our population did not differ from other epidemiological studies. As compared to the results of questionnaire and skin tests, detection of nasal IgE resulted to be sensitive and specific. The investigated test could correctly diagnose sensitization in all the cases with discordant clinical history and skin test, as demonstrated by nasal challenge with the suspected allergen and tryptase. It was not surprising to find a

Pt	Symptoms*	SPT	Nasal IgE (kU/L)	ASNC**		Tryptase (µg/L)**	
				B.	A.	B.	A.
1		G6 +++ T9 ++	Neg Neg	1 1	2 1	<1 <1	<1 <1
2	AE	G6 +++ D1 +++	Neg Neg	0 1	0 1	<1 1.5	<1 1.7
3		G6 +++ T9 ++	Neg Neg	1 1	2 2	<1 <1	<1 <1
4	AE	D1 +++	2.5	2	6	3.7	33.5
5		G6 +++ D1 +++	Neg Neg	1 2	1 1	<1 <1	1.8 1.8
6		D1 +++	Neg	2	1	1.5	2
7		D1 +++ E1 +++	Neg Neg	2 2	2 2	1.5 1.5	<1 <1
8		M6 +++	Neg	2	3	<1	<1
9		G6 +++ E1 +++	Neg Neg	1 1	2 1	<1 <1	<1 <1
10	AE	D1 +++	6.5	4	9	15	105
11	AE	D1 +++	Neg	0	0	<1	<1
12		D1 +++ E1 +++	Neg Neg	1 1	1 2	1.5 1.5	<1 <1
13		G6 +++ T9 +++	Neg Neg	1 1	2 2	1.2 1.5	1.5 1.2
14	AE	D1 +++ G6 +++	Neg Neg	1 1	1 1	1.2 1.2	1.5 1.5
15		D1 +++	Neg	1	2	<1	<1
16		G6 +++ D1 +++	Neg Neg	2 1	2 1	<1 2.5	<1 1.6
17		G6 +++ T9 +++	Neg Neg	1 0	2 1	2.5 <1	2.7 <1

Table 5: Children with negative questionnaire for respiratory allergy and with positive SPT.
* AE: atopic eczema.
** B: baseline; A: after challenge.

patient with perennial rhinitis without cutaneous response and positive nasal IgE to mites. Other authors have previously reported patients with a suggestive clinical history, who had positive local IgE despite negative skin test and RAST (8-11). Of notice was the concordant positivity of nasal IgE and skin test in 2 children with atopic eczema and negative for allergic respiratory symptoms. This finding suggests that specific mucosal IgE in patients with atopic eczema may outline the respiratory sensitisation, and can probably be considered as a marker of allergic march. Nasal IgE assay was more specific than SPT, as evidenced by the positivity of this test only in 2 of 17 children without respiratory symptoms but positive to prick test. As proposed in an other study (5), further evidence of a better specificity is supported by negative nasal IgE for 5/6 allergens positive to prick tests without a correlation with clinical

history, ASNC and tryptase in patients with asthma and rhinitis.

Direct detection of nasal Tryptase was useful to objectively confirm the specific ASNC results, as evidenced also in other studies performed in nasal lavage fluid (23-24). The negativity of this mediator, in fact, showed the low specificity of prick test in the group of children without respiratory symptoms. Furthermore, according with the results of our screening test, it was useful to show airways inflammation in three cases, one child with clinical history of perennial rhinitis but negative to prick tests and 2 children with atopic eczema, negative to the questionnaire for respiratory allergic disease.

In conclusion, the measurement of nasal mucosal IgE has shown to be easy to perform, not invasive and well

tolerated. Moreover, it proved to be helpful in cases where usual procedures provide controversial results. This test could be also used for early detection of airway sensitisation in children with positive skin test without actual evidence of respiratory symptoms, if proper longitudinal studies will confirm this finding.

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