

DO TRYPTASE, ECP AND SPECIFIC IgE MEASUREMENT BY NASAL INCUBATION INCREASE THE SPECIFIC NASAL PROVOCATION TEST SENSITIVITY?

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Received November 5, 2003 - Accepted February 23, 2004

The specific Nasal Provocation Test (sNPT) is a third level diagnostic tool. Fitted to reproduce natural exposure condition to pick the responsible allergen for nasal symptoms out, it is applied when prick test and RAST responses are doubtful. SNPT results have been evaluated measuring nasal resistance (anterior rhinomanometry) and nasal symptoms (clinical score), reaching 50 % of sensitivity. This study focused on the determination of allergic response markers, triggered by nasal challenge: tryptase levels in the nose, specific IgE and ECP (Eosinophil Cationic Protein). The aim was to increase sNPT sensitivity. Twenty patients suffering from allergic rhinitis and 16 age-matched-nonallergic subjects were enrolled in the study. Tryptase, specific IgE and ECP were determined in nasal mucosa applying a new method, based on *in situ* incubation, before and after sNPT. The latter was performed following a standardized method. Tryptase levels increased in 13 patients (65 %), were unchanged in four patients (20 %), and slightly decreased in three patients (15 %). The increase recorded was significant in mite allergic patients ($p=0.005$), but not significant ($p>0.05$) in pollen allergic patients. ECP values increased in 13 patients (65 %), were unchanged in two patients (10 %), and highly decreased in five patients (25 %). ECP increase was not significant ($p>0.05$). Specific IgE levels increased in seven patients (35 %), were unchanged in 11 patients (55 %) and decreased in two patients (10 %). The IgE increase was significant in pollen-allergic patients ($p<0.05$), while it was not significant in mite-allergic patients ($p>0.05$). Tryptase, ECP, and specific IgE were not detected in the control group. The data obtained showed a positive sNPT response in 12 patients (60 %). Comparing our results with those derived from classical-parameter employment, we gathered an improvement of 10 %. On the basis of the usual parameters, in fact, we recorded 50 % positivity, while the use of mediators provided an additional 10 % improvement in sNPT sensitivity: taking together the usual parameters and nasal allergic mediators values, we reached an sNPT over-all sensitivity of 85 %.

The specific Nasal Provocation Test (sNPT) is a third level diagnostic method fitted to identify nasal allergic hyper reactivity when prick test and RAST give doubtful responses or when it is necessary to discern the allergen mainly responsible for the clinical manifestations in

presence of multiple sensitization. In fact, nasal challenge with allergen tries to reproduce natural exposure condition and allows monitoring the evolution of nasal allergy (1).

Until now, following a standardized criterion, sNPT used increasing allergen doses. The

Key words: tryptase, ECP, specific IgE, nasal provocation test

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0394-6320 (2004)
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response, measured using anterior active rhinomanometry and nasal symptom score (2), was expressed as nasal resistance.

With the aim of increasing sNPT sensitivity (3), this study focused on the determination of markers of allergic response triggered by nasal challenge, that is nasal levels of tryptase, specific IgE and ECP (Eosinophil Cationic Protein).

Tryptase is a specific marker for mast cell activation; instead, histamine is a marker for both mast cell and basophil activation (4). Several studies suggested that tryptase is a useful indicator of allergic reaction; in fact, it signalizes that mast cell activation is the major event in the immediate nasal-allergic response (5).

Tryptase dosage in nasal lavage fluid has been administered after the application of local allergen challenge (5-7), as prescribed by the nasal lavage protocol, which was developed by Naclerio et al. (8). This method has been used extensively to study inflammatory mediators during nasal challenge experiments and needs some preliminary steps, which consists of four nasal lavages with 5 milliliters of pre-warmed (30° C) physiological saline and of one administration of 0.5 mL oxymetazoline hydrochloride (0.5 mg./mL) spray (9). Furthermore, once nasal fluid is picked, several steps of processing are necessary to obtain the supernatant, which undergoes a complex assessment for the detection of any specific allergic mediator. Finally, nasal blockage cannot be recorded in a reliable way because of the procedure of nasal lavage (10).

To evaluate tryptase nasal level after allergen challenge, a simple and new method has been chosen (11,12), so the determination in nasal fluid was replaced by local incubation.

Eosinophils are considered the main players of the late-phase reaction together with basophils; their activation is associated with the release of granule proteins, among which ECP acts as a valuable marker after nasal challenge (13). ECP measurement in nasal lavage has been performed as marker of eosinophilic activation in the target organ.

We have applied the same new method of nasal incubation (11) both to the ECP assays and to the determination of specific IgE.

MATERIAL AND METHODS

Patients and control subjects

Twenty patients (10 men and 10 women, mean age 30 years, range: 13-61) suffering from allergic rhinitis were enrolled. Sixteen age-matched, nonallergic subjects (nine men and seven women) were also recruited, as control group.

The inclusion criteria for patients were as follows:

- 1) clinical history of allergic rhinitis
- 2) sensitization confirmed by skin-prick tests with glycerinated extracts (Lofarma SpA Milan, Italy). In particular, 13 patients (65%) had a multiple sensitization, while seven (35%) had a monosensitization; more precisely, eight (40%) of them were allergic to dust mites and 12 (60%) to pollens. Besides, eight (40%) members belonging to the latter group were allergic to grass pollen mix, two (10%) to olea europea and two (10%) to parietaria judaica and officinalis.

The patients were nasal-symptom free, none had received immunotherapy and no medication was taken at the time of investigation. All patients and control-group subjects gave their written informed consent to the study, which was approved by the Regional Ethics Committee of Tuscany, Italy.

Study design

The study was carried out from October 2000 to July 2001. During this period, all patients underwent the following tests:

- 1) direct nasal dosage of ECP, tryptase and specific IgE;
- 2) specific nasal provocation test (sNPT), by challenging the nasal mucosa with the allergen considered as the main responsible for the rhinitis symptoms;
- 3) nasal dosage of tryptase and specific IgE, 20 minutes after the end of sNPT;
- 4) nasal levels of ECP, 24 hours after the sNPT;
- 5) recording of rhinitis symptoms according to a clinical score on a four-point scale from 0 to 3, where 0 represents no symptoms, 1 mild, 2 moderate and 3 severe symptoms.

Nasal levels of tryptase, specific IgE and ECP were also evaluated in 16 nonatopic-subjects.

Nasal dosage

Nasal levels of specific IgE (11,14), tryptase (15) and ECP (12,16) were determined before and

after sNPT by direct incubation on nasal mucosa, using the new method previously described (26,27). CAP System Sponges covalently coupled with specific allergen, together with anti-ECP antibody or with anti-tryptase antibody, were utilized.

Special plastic sticks were employed to determine the mediators at nasal mucous-membrane level, which in addition acted as substrates in the solid phase for the dosages of antibodies, specific anti-IgE, anti-tryptase and anti-ECP.

Stick conditions of employment and development:

1) the subject blew his/her nose and raised his/her head; then the nasal applicator was inserted into the nose at the level of the anterior part of the inferior turbinate;

2) after 20 minutes of nasal incubation, the substrates were drawn out and washed in NaCl 0.9% (w/v) solution containing Tween 20 at a concentration of 0.1 ml/l;

3) then the substrates were put in test tubes with 1 ml of 0.9% (w/v) NaCl solution containing 0.02% sodium azide (w/v) and stored at -20°C , until their development;

4) the processing for IgE, tryptase and ECP was carried out by Unicap IgE, Tryptase and ECP System Feia (Pharmacia), respectively.

The concentrations of tryptase and ECP were expressed as $\mu\text{g/L}$, and the specific IgE levels were expressed as KiloUnits/l.

Specific NPT

This test was performed following a previously standardized method (17):

1) basal anterior active rhinomanometry (RynoMenfis 3.2)

2) lactose powder control applied into the nostril with the highest patency, through the means of a specially designed apparatus (Nasal insufflator; Lofarma SpA, Milan, Italy);

3) dry-powdered allergenic extract administration (Lofarma SpA, Milan) at the lowest concentration (20 U.A.) into the same nostril;

4) rhinomanometry after 10-15 minutes;

5) administering of increasingly higher dosages (40-60-80 U.A.), tested until an increase in resistance equal or greater than 100% was reached.

sNPT was considered negative if the nasal resistance remained the same level, even at the highest dosage, or when the typical nasal allergic crisis was not recorded.

Clinical score

Patients were asked to assess the intensity of sneezing, itching and ocular tearing, which occurred after nasal challenge. Each of these symptoms was rated from 0 to 3, according to the following scoring:

Score: 0 = absent

1 = mild

2 = moderate

3 = severe

A clinical score ≥ 3 was considered sign of positive sNPT, even if the nasal resistance remained the same.

Statistical analysis

Statistical analysis was made using t-Student test for paired samples.

RESULTS

The sNPT was positive in 10 patients (50%) referring to the rhinomanometric evaluation and/or to the symptomatological score.

Tryptase nasal dosage

Fig. 1 shows the comparison between the tryptase levels before and after the sNPT.

The tryptase levels resulted increased in 13 patients (65%), unchanged in four patients (20%), and slightly decreased in three patients (15%).

If we consider the pollen-allergic patients separately from the mite-allergic patients, we may affirm that the increase recorded is not significant for the former ($p > 0.05$), while it is significant for the latter ($p = 0.05$).

ECP nasal dosage

Fig. 2 shows the comparison between the ECP levels before and after the sNPT.

The ECP values increased in 13 patients (65%), were unchanged in two patients (10%), and greatly decreased in five patients (25%).

On the whole, the ECP increase was not statistically significant ($p > 0.05$); instead, if we consider the pollen-allergic patients separately from the mite-allergic patients, we may point out that there was a decrease in the former, while the recorded increase was not significant in the latter ($p > 0.05$).

Fig. 1. Tryptase levels ($\mu\text{g/L}$) before and after sNPT.

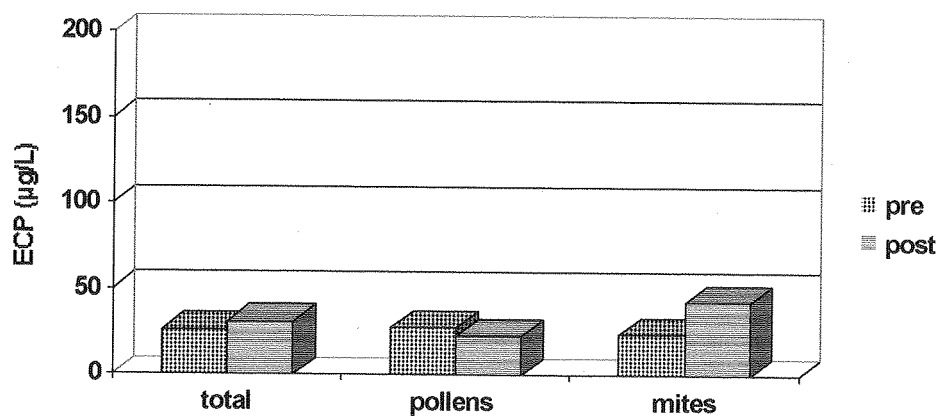
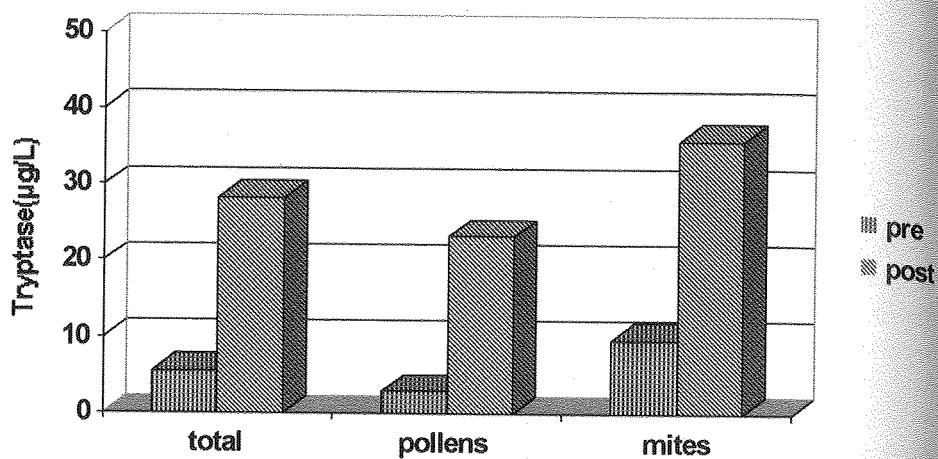
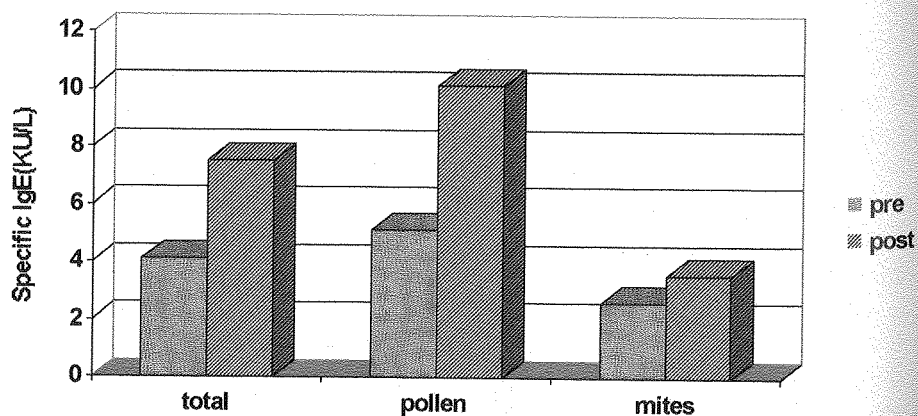


Fig. 2. ECP levels ($\mu\text{g/L}$) before and after sNPT.

Fig. 3. Specific IgE levels (UI/L) before and after sNPT.



PATIENTS	sNPT	PRE	POST
3	Grass-pollen	W21=<0.35	W21=6
4	Mites	E1=<0.35	E1=4
6	Grass-pollen	E1=<0.35	E1=2
7	Mites	M6=6 G6=<0.35 T3=6 T9=9	M6=6 G6=4 T3=<0.35 T9=2
9	Grass-pollen	D1=2 E1=10 T3=6 T9=3	D1=1.5 E1=6 T3=<0.35 T9=2
10	Grass-pollen	E1=<0.35	E1=3
11	Parietaria	G6=5 D1=1.5	G6=10 D1=2
13	Grass-pollen	M6=2	M6=2
14	Mites	E1=1 T9=<0.35	E1=<0.35 T9=1.5
16	Parietaria	G6=0.8	G6=1
17	Mites	T9=<0.35	T9=3

Tab. I. The 11 patients with specific IgE different from those expected from sNPT.

D1 =

Dermatophagoides pteronyssinus

M6 = *Alternaria alternata*

E1 = *Cat epithelium*

G6 = *Grass pollen mix*

W21 = *Parietaria judaica*

T3 = *Betulla verrucosa*

T9 = *Olea europea*

PATIENTS	AAR/Symptoms	TRYPTASE	sIgE
1	+	↑	↑
2	-	=	↓
3	-	↑	↑
4	+	↑	↑
5	-	↓	↑
6	-	↑	↑
7	-	↑	↓
8	+	↑	=
9	+	=	=
10	-	↓	=
11	-	↑	↑
12	+	=	=
13	-	↑	=
14	+	↑	↑
15	-	=	=
16	+	↑	=
17	+	↓	=
18	-	↑	=
19	+	↑	=
20	+	↑	=

Tab. II. results of sNPT according to previously standardized parameters (nasal resistance and/or symptomatological score), and nasal levels of allergic mediators.

Specific IgE nasal dosage

Fig. 3 shows the comparison between the specific IgE levels before and after the sNPT.

The IgE levels increased in seven patients (35%), were unchanged in 11 patients (55%), and decreased in two patients (10%).

If we consider the pollen-allergic patients separately from the mite-allergic patients we may stress that the recorded increase was significant for the former ($p < 0.05$), but not for the latter ($p > 0.05$).

Tab. I shows that, in 11 patients, the specific IgEs obtained were different from what was expected using a sNPT.

Non-allergic control group

Tryptase, ECP, and specific IgE were not detected by the nasal incubation method in the control group.

DISCUSSION

The lavage technique, developed by Naclerio et al. (8), allows measurement of mediators in nasal lavage fluid: tryptase (marker of mast cell activation) and ECP (marker of eosinophil activation) have been previously assessed after nasal provocation in nasal lavage fluid (18). However, symptoms can only be recorded as sneezing, since pre-treatment with a vasoconstrictor spray precludes measurements of nasal resistance (10).

The aim of this study was to identify further parameters to increase the sensitivity of the sNPT. We used an alternative technique to obtain the nasal dosage of tryptase, IgE and ECP.

The sNPT positivity, as evaluated by previously standardized parameters (rhinomanometric nasal resistance and/or symptomatological score) and stressed in this study, overlapped on the results obtained in previous personal researches: in fact, the sNPT was positive in 50% of the subjects positive to prick test.

The tryptase increase pointed out by nasal dosage is the most significant parameter for the sNPT positivity evaluation: in fact tryptase increased in 65% of the subjects, that is 15% more than the positivity values measured applying the usual parameters.

Compared with the tryptase levels, the specific IgE increase showed a lower significance, in fact this parameter remained unchanged in 11 patients: these data could be consistent with the hypothesis of a delayed increase at an intermediate stage, between the early tryptase release and the late ECP production. Another side of the local IgE response needs some comments, that is the presence of specific IgE against allergens different from those used during the sNPT. This could be explained in a double way:

- cross reactivity between the IgE specific against the challenging allergen and other IgE with similar epitopes;
- in patients with multiple sensitization the allergen tested could challenge the release of specific IgE, together with the release of IgE against other allergens among those positive at the Prick-test: for example, the patient no. 3 showed a high skin-test positivity to grass pollen and to parietaria; after the sNPT to grass pollen, the nasal dosage revealed the increase of both IgE-G6 and IgE-W21. In a few words, the sNPT acted as a specific challenge for the tested allergen, but also as an aspecific stimulus for other allergens responsible for the nasal symptoms.

The increase in ECP was not statistically significant, hence confirming the heterogeneous results obtained in other studies (19): in fact, kinetics of ECP release after nasal allergen challenge are not well defined and patients might have eosinophils with different activation levels in their nasal mucosa (13). We can hypothesize that repeated dosages might help to find the ECP release peak.

Since the tryptase levels showed the highest significance in mite-allergic patients, while the IgE concentrations were more significant in pollen-allergic subjects, we can consider positive the sNPT in the following cases:

- o tryptase and specific IgE both increased;
- o tryptase increased and IgE unchanged;
- o tryptase unchanged and IgE increased.

Referring to these parameters, we have obtained sNPT positive in 12 patients (60%). Besides, comparing the values of previously standardized parameters (nasal resistance and/or symptomatologic score) and the nasal levels of allergic mediators (Tab. II) we found an overlapping of positivity in 7/20 (35%) of patients.

In 3/20 (15%) subjects an increase of nasal resistance and symptom score were observed, on the contrary significant-negative-mediator values were not found; finally, in 7/20 (35%) patients increased levels of allergic-nasal mediators and unchanged-nasal resistances were observed, thus reaching an over-all sensitivity of 85%.

Since we have recorded a 50% positivity on the basis of the usual parameters, we can conclude that the nasal dosage of tryptase and specific IgE provided a 35% improvement in the sensitivity of the nasal provocation test without any loss in specificity (none of the subjects considered as positive had increased resistances after lactose powder control inhalation).

Furthermore, the method of nasal incubation proved to be simple, well tolerated (20) and suitable for the sNPT; while the dosage of tryptase, IgE and ECP in nasal lavage are not compatible with the challenge test. Thanks to its tolerability, this updated form of sNPT could be used as a monitoring method of the treatment of allergic rhinitis, specifically concerning pediatric patients (20).

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