

ALLERGEN SPECIFIC NASAL CHALLENGE TO LATEX IN CHILDREN WITH LATEX ALLERGY: CLINICAL AND IMMUNOLOGICAL EVALUATION

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Received August 22, 2007 – Accepted October 12, 2007

There are no data concerning the significance of allergen specific nasal challenge to latex (ASNCL) in the pediatric population and the effect of mometasone furoate nasal spray [(MFNS), topic corticosteroid exerting a potent anti-inflammatory activity] in children with latex allergic rhinitis. The aims of this study are: to investigate the clinical and immune pathological effects of ASNCL in children with latex allergy; to study the effects of MFNS pre-medication on the clinical and immune pathological effects of ASNCL in children with latex allergy. Thirteen children [6 male and 7 female, mean (SD) age 9.6 (2.9) years] with latex allergy and seven children [3 male and 4 female, mean (SD) age 9.9 (3.8) years] without latex allergy underwent ASNCL. Nasal symptoms were recorded, nasal lavage fluid was collected to measure tryptase, eosinophil cationic protein (ECP), interleukin-5, interferon- γ levels, and spirometric test was performed for each patient without or with pre-medication with MFNS. ASNCL induced a clinical allergic response and increased tryptase levels only in children with latex allergy. No serious adverse events occurred after ASNCL. MFNS pre-medication reduced both tryptase and ECP levels only in children with latex allergy. ASNCL is a simple, reliable and useful tool to make or confirm the diagnosis of nasal symptoms due to latex; it allows us to study both clinical symptoms and local immunological changes. MFNS pre-medication before an ASNCL may prevent some immunological responses induced by ASNCL without clinical allergic modifications.

Latex allergy has clinical and social-economic implications (1). Clinical manifestations may consist of allergic rhinitis, conjunctivitis, asthma, dermatitis, urticaria, angioedema, and anaphylactic shock as has been previously reported (2-4). The prevalence of latex allergy has reached epidemic proportions in highly exposed people(1). The prevalence of latex allergy is less than 1% in the general population

(1-5). Because of several iatrogenic or occupational exposures, rates of latex sensitization and allergy has risen dramatically, mainly when powdered latex gloves are used (2-6). The adoption of preventative measures which reduce latex exposure has been followed by a decrement of latex sensitization and allergy in the population (7).

Diagnostic approaches, e.g. clinical history,

Key words: children, rhinitis, latex, allergen specific nasal challenge to latex

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skin prick test (SPT) to latex, and determination of specific serum immunoglobulin E (IgE) to latex, may be not sufficient to achieve a correct diagnosis of allergic rhinitis due to latex allergy (8). Allergen specific nasal challenge is a diagnostic procedure that can be performed to reach a diagnosis of allergic rhinitis through the identification of the suspected allergen. As previously shown, nasal challenge could be useful not only as a research tool but also for diagnosing occupational rhinitis in latex-sensitized workers (9).

Therefore, the first aim of this study is to investigate the clinical and immune pathological effects induced by allergen specific nasal challenge to latex (ASNCL) in children with suspected latex allergy: there are no studies regarding this issue in pediatric age.

Except for allergen avoidance, intranasal corticosteroids are often an effective choice to treat allergic rhinitis because of their strong anti-inflammatory activity (10). In this regard, mometasone furoate nasal spray (MFNS), (Nasonex, Schering Plough SpA, Segrate, Milan, Italy) has been demonstrated to prevent allergic nasal symptoms due to inhalant allergens (11). In addition, MFNS pre-medication affects the late phase events following allergen specific nasal challenge (12). Therefore, this drug might prevent nasal symptoms in patients with nasal manifestations due to latex exposure.

The second aim of this study is to investigate the clinical and immunological effects of MFNS pre-medication on ASNCL.

MATERIALS AND METHODS

Patients

Table I represents the study design. Thirteen children [6 male and 7 female, mean (SD) age 9.6 (2.9) years] (group A) with clinical manifestations due to suspected latex allergy (rhinitis in all 13, urticaria in 6 patients, asthma in 6 patients, and conjunctivitis in 2 patients) were enrolled in the Pediatric Allergy and Pulmonology Center of the Anna Meyer Children's Hospital of Florence (Italy).

Symptoms occurred after exposure to latex products (surgical procedures performed by a dentist in 12 patients, surgical gloves in 9 patients, rubber balloons in 8 patients, household cleaning gloves in 5 patients, and swimming caps in 3 patients).

Seven children [3 male and 4 female, mean (SD) age

9.9 (3.8) years], without clinical manifestations due to latex products were also enrolled as control group (group B).

SPT to latex was performed according to the recommendations of the European Academy of Allergology and Clinical Immunology (13) using a commercial extract supplied by the "Laboratorio Lofarma" (Milan, Italy) (12-13), and the presence of specific serum IgE to latex (UniCAP Pharmacia, Uppsala, Sweden) was detected according to the manufacture's instructions. Positive and negative controls (histamine and saline solution respectively) were additionally performed.

In each of subjects SPTs to common aeroallergens (16) were performed.

SPTs were considered positive if the wheal's diameter was ≥ 3 mm (13). Specific serum IgE to latex were considered positive over 0.35 kU/l levels.

Topical or systemic drugs were avoided during the study. Informed consent was obtained from the parents of all children enrolled. The study was performed according to the Conference of Helsinki.

Nasal lavage fluid

Nasal lavage fluid (NLF) with saline solution was collected before and after the nasal challenge according to the procedure described in a previous study (17). Saline pre-wash was conducted in a single blind manner. Briefly, NLF was performed for approximately 1 minute, 6 ml of normal saline were sprayed into the nose, the fluid collected was filtered (22 micron Millipor filter), centrifuged for 10 minutes and then frozen at -20°C (17).

Allergen specific nasal challenge to latex

Group A and B patients underwent the allergen specific nasal challenge to latex (ASNCL) as previously described (17).

A washout period (drug free) of 4 weeks without nasal symptoms was established before the ASNCL.

In order to determine the latex concentration which could be used to start the ASNCL in group A, an SPT was performed using a latex concentration 500 times lower than that of the commercial latex extract used to confirm the diagnosis of latex allergy. If it was negative (wheal < 3 mm), the SPT was repeated using concentrations 100, 50 and 10 times lower than that of the commercial latex extract.

The undiluted commercial latex extract had a protein concentration of 46 $\mu\text{g/ml}$ (using a previously described method) (14-17). Immediately before the ASNCL, a nasal non-specific provocation test (100 μl in each nostril) with the vehicle used to prepare commercial latex SPT (13-15) was carried out on each child as previously described (17).

The ASNCL was performed by spraying in each nostril

100 µl of the same commercial latex extract used for the SPT at the lowest concentration that had developed a wheal ≥ 3 mm. This method was used to avoid possible adverse and dangerous reactions to high latex concentrations. The device used for spraying the latex extract was similar to that used to perform the NLF as already described (17).

The ASNCL was continued using the next higher latex concentration until the symptom score turned positive (i.e. if the ASNCL was negative at the 1:500 concentration, another ASNCL was performed at the 1:100 concentration after 30 minutes, and so on until the concentration that provoked a symptom score ≥ 5 was reached).

Immunological evaluation

Tryptase (Pharmacia, Uppsala, Sweden), eosinophil cationic protein (ECP) (Pharmacia, Uppsala, Sweden), interleukin-5 (IL-5) (R & D System, Minneapolis, USA), and interferon- γ (INF- γ) (R & D System, Minneapolis, USA) levels were detected according to the manufacturer's instructions. The NFL was collected at the times specified in Table I.

Symptoms

Nasal symptoms were assessed after the ASNCL according to a known symptom score system (positive if ≥ 5). The number of sneezes and the degree of rhinorrhea, mucosal edema, and itching were recorded. The total symptom scores ranged from 0 to 14 and represented the sum of the scores established for sneezing (4 or more sneezes: 1 point), rhinorrhea (mild anterior and posterior: 1 point; abundant anterior and posterior: 3 points), mucosal edema (difficult breathing: 1 point; one nostril block: 2 point; nasal block: 3 points), and itching (itching of the nose: 1 point; itching of the throat: 1 point; itching of the eyes: 1 point). The clinical challenge was considered positive if the total score corresponded to 5 or more points (9-17).

Spirometric indices

Spirometric indices were evaluated before and after the ASNCL (Table I) using a commercial spirometer (Quark PFT1, Cosmed, Rome, Italy) by measuring Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and Forced Expiratory Flow between 25% and 75% of FVC (FEF_{25-75}). FEV_1/FVC ratio lower than 88% predicted was used to define a spirometric bronchial obstruction. A decrease in FEV_1 higher than 12% was considered to be a significant change after the ASNCL (18).

Pre-medication with MFNS

NLF, ASNCL, and spirometry were repeated 35 days later, following a 5 day pre-medication with MFNS (100 µg

daily in each nostril: total dose 200 µg daily) and about 12 hours after the last administration of this drug (Table I).

Statistical analysis

All data were analyzed with a commercial statistical package (SPSS for Windows). The Student t test for independent or paired samples as appropriate was used to compare means. *P* values < 0.05 were considered significant.

RESULTS

Specific IgE in patients

In group A the wheal's mean (SD) value of SPT to latex and the UniCAP mean (SD) value of specific IgE to latex was respectively 6.3 mm (3.4) and 29.2 kU/l (27.3).

Five out of thirteen (38.5%) children were IgE sensitized to one or more of the common aeroallergens.

In group B SPT to latex was negative and specific serum IgE to latex were absent. Two children (28.5%) were IgE sensitized to one or more of the common aeroallergens.

Allergen specific nasal challenge to latex

There was no appearance of symptoms after a nasal non-specific provocation test in all children.

In group A, ASNCL induced nasal and other symptoms (symptoms score ≥ 5) both without (13/13, 100%) or with (12/13, 92.3%) an MFNS pre-medication (Tables II and III). In the entire group B, ASNCL did not induce nasal or other symptoms. ASNCL was well tolerated in all children and no adverse events were present.

Immunological evaluation

In Fig. 1 and Table IV the mean values of INF- γ , tryptase, ECP and IL-5 levels without and with MFNS treatment (group A) are reported. No changes in INF- γ , tryptase, ECP and IL-5 mean values were detectable in group B (data not shown). The basal mean levels of INF- γ , ECP, tryptase and IL-5 were not significantly different among the two groups. There were no significant changes of IL-5 and INF- γ levels before and after a positive ASNCL without and with MFNS treatment (group A).

However, in group A we found:

- 1) significantly higher levels of tryptase

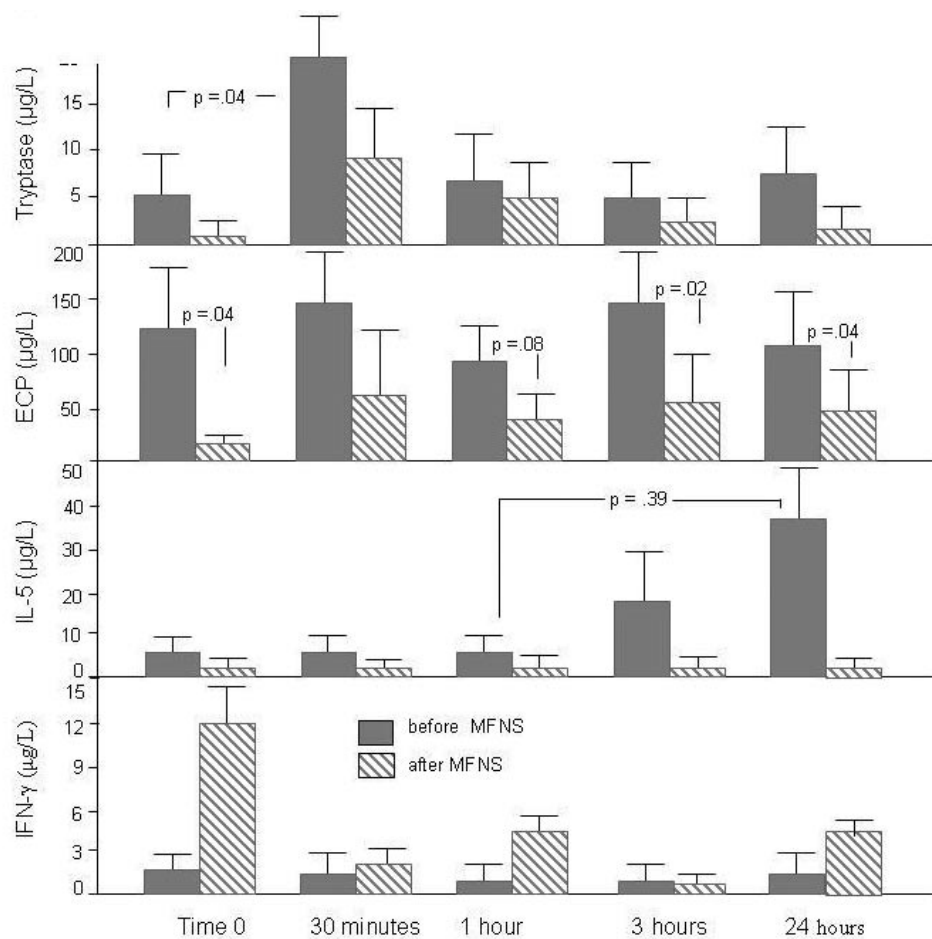


Fig. 1. Nasal levels of tryptase, ECP, IL-5, and INF- γ before and 30 minutes, 1 hour, 3 hours and 24 hours after an ANSCL: effect of MFNS.

30 minutes (Fig. 1) after a positive ASNCL in comparison to basal values; after MFNS treatment this observation was undetectable;

2) significantly ECP basal lower levels before MFNS treatment if compared with ECP basal levels after MFNS treatment (Fig. 1).

In group B no significant modifications of these (tryptase, ECP) and other (IL-5, INF- γ) parameters were observed after ASNCL.

Symptoms and Spirometric indices

There were no significant changes in the symptom score (Tables II and III) and spirometric indices (FEV_1 , FVC and FEV_{25-75} values) (data not shown) either before or after a positive ASNCL without and with MFNS treatment (group A). In group B there were no symptoms or changes in spirometric indices after ASNCL.

DISCUSSION

Specific nasal challenge test is used to confirm the causal relationship between allergen exposure and symptom appearance (19). Various allergens are used for specific nasal challenge (e.g. birch, grass, rye, mugwort and plantain) (20). However, specific nasal challenge with latex has been used only in adult patients (9-21).

Kurtz and co-workers have developed a hooded exposure chamber system to allow evaluation of rhino-conjunctivitis and asthma, especially in health care workers after latex exposure (20). This is a method for masked semi-quantitative latex aeroallergen challenges that mimic occupational latex exposure to powdered latex gloves. The patients presented only respiratory symptoms after the latex provocation test and did not experience

Table I. Study design (without or with a pre-medication with MFNS). Symptom score was performed during 30 minutes, during 30 minutes-1 hour; during 1-3 hours and during 3-24 hours after a positive (group A) and a negative (group B) ASNCL.

Patients group A (patients with latex allergy)

1) Latex SPT (from 1:500 to 1:1);	1) Measures of FEV ₁ ,	1) NLF (tryptase, ECP,
2) Measures of FEV ₁ , FVC and FEF ₂₅₋₇₅ ;	FVC and FEF ₂₅₋₇₅ .	INF- γ and IL-5).
3) NLF (tryptase, ECP, INF- γ and IL-5);		
4) ASNCL.		
▲ Time 0	▲ 10 and 40 minutes after a positive [or the last concentration (1:1) if negative] ASNCL	▲ 30 minutes, 1 hour, 3 hours and 24 hours after a positive [or the last concentration (1:1) if negative] ASNCL

Patients group B (patients without latex allergy)

1) Latex SPT (from 1:500 to 1:1);	1) Measures of FEV ₁ ,	1) NLF (tryptase, ECP,
2) Measures of FEV ₁ , FVC and FEF ₂₅₋₇₅ ;	FVC and FEF ₂₅₋₇₅ .	INF- γ and IL-5).
3) NLF (tryptase, ECP, INF- γ and IL-5);		
4) ASNCL.		
▲ Time 0	▲ 10 and 40 minutes after ASNCL (1:1)	▲ 30 minutes, 1 hour, 3 hours and 24 hours after ASNCL (1:1)

systemic or late-phase reactions.

Palczynski and co-workers evaluated the usefulness of a nasal challenge test in the diagnosis of allergic rhinitis in subjects occupationally exposed to natural rubber latex products (9). The nasal provocation test with natural rubber latex provoked nasal symptoms in subjects who presented respiratory symptoms related to natural rubber latex. To our knowledge, there are no relative studies in pediatric age.

Our study mostly provides evidence that ASNCL is a safe and reliable test to make diagnosis of latex allergy in children with suspected nasal symptoms due to latex exposure. Neither bronchial nor systemic reactions were observed after the ASNCL.

We show that all children sensitized to latex and with suspected latex allergy had clinical responses to ASNCL, whereas children without latex allergy did not respond to the challenge. Therefore ASNCL may

be performed in children with suspected latex allergy as an additional and confirming diagnostic test.

In the nasal lavage Palczynski and co-workers (9) noted a significant increase of ECP levels 24 hours after a challenge with natural rubber latex while we did not find any statistically significant change in ECP concentration. We detected significantly higher levels of tryptase 30 minutes after a positive ASNCL in comparison to basal values. Bellussi and co-workers showed a significant increase of tryptase levels after a specific nasal provocation test in mite allergic patients without a significant ECP increase (22).

Other authors studied the changes of mediators (cytokines: IL-3, IL-4, IL-5, IL-8, IL-10, IL-13) and cells (eosinophils and macrophages) in nasal lavage fluid after a specific nasal provocation test using grass pollen (23-24).

Topical corticosteroids (e.g. mometasone furoate,

Table II. Symptom score after an ASNCL performed in 13 children.

ASNCL at various concentrations (from 1:500 to 1:1) without a pre-medication with mometasone furoate nasal spray					
	1:500	1:100	1:50	1:10	1:1
No. Patients					
1	2	3	6		
2				2	7
3	7				
4				3	8
5	3	3	6		
6		3	2	9	
7				2	8
8	2	2	6		
9				2	7
10				3	10
11		2	1	3	9
12	6				
13	2	7			

ASNCL at various concentrations (from 1:500 to 1:1) with a pre-medication with mometasone furoate nasal spray					
	1:500	1:100	1:50	1:10	1:1
No. Patients					
1	3	3	0	1	4
2				2	6
3	6				
4				3	5
5	7				
6		4	6		
7				2	6
8	2	4	6		
9				2	6
10				2	7
11		0	1	1	6
12	4	7			
13	0	2	4	7	

Table III. Symptom score (mean $\pm$ SD) after an ASNCL in 13 children at various concentrations (from 1:500 to 1:1) both before (b.) and after (a.) MFNS.

	ASNCL (1:500)	ASNCL (1:100)	ASNCL (1:50)	ASNCL (1:10)	ASNCL (1:1)
No. Patients	6	6	5	7	6
Symptom score b. MFNS (mean $\pm$ SD)	3.67 $\pm$ 2.25) (a*)	3.40 ($\pm$ 2.07) (b*)	3.75 ($\pm$ 2.63) (c*)	2.50 ($\pm$ 0.55) (d*)	7 ($\pm$ 3.58) (e*)
	ASNCL (1:500)	ASNCL (1:100)	ASNCL (1:50)	ASNCL (1:10)	ASNCL (1:1)
No. Patients	6	6	5	8	7
Symptom score a. MFNS (mean $\pm$ SD)	3.63 ($\pm$ 2.58) (a)	2.60 ($\pm$ 1.67) (b)	3.25 ($\pm$ 3.20) (c)	2.17 ($\pm$ 0.75) (d)	6 ($\pm$ 0.63) (e)

Table IV. Levels [mean ($\pm$ SD)] of ECP, tryptase, IL-5, and INF- γ in NLF before (time 0) and 30 minutes, 1 hour, 3 hours and 24 hours after an ASNCL before (b) and after (a) MFNS.

		Time 0	30 minutes	1 hour	3 hours	24 hours
ECP (μ g/L)	b. MFNS	124.4 ($\pm$ 82.2)	141.0 ($\pm$ 57.6)	73.0 ($\pm$ 40.0)	141.0 ($\pm$ 61.5)	127.4 ($\pm$ 74.6)
	a. MFNS	6.2 ($\pm$ 2.0)	51.0 ($\pm$ 70.0)	26.8 ($\pm$ 18.6)	53.4 ($\pm$ 37.9)	43.0 ($\pm$ 40.0)
Tryptase (μ g/L)	b. MFNS	5.7 ($\pm$ 11.7)	20.4 ($\pm$ 25.0)	7.1 ($\pm$ 10.6)	5.0 ($\pm$ 6.9)	8.2 ($\pm$ 14.0)
	a. MFNS	0.6 ($\pm$ 0.8)	11.6 ($\pm$ 18.8)	6.2 ($\pm$ 10.2)	2.8 ($\pm$ 5.7)	1.2 ($\pm$ 2.1)
IL5 μ g/L	b. MFNS	4.3 ($\pm$ 6.1)	4.6 ($\pm$ 4.5)	3.3 ($\pm$ 3.6)	15.6 ($\pm$ 14.9)	41.1 ($\pm$ 45.1)
	a. MFNS	0.0 ($\pm$ 0.0)	0.6 ($\pm$ 1.3)	0.8 ($\pm$ 1.1)	0.6 ($\pm$ 1.3)	2.0 ($\pm$ 2.1)
INF γ μ g/L	b. MFNS	1.83 ($\pm$ 2.2)	1.5 ($\pm$ 1.2)	1.0 ($\pm$ 0.8)	1.0 ($\pm$ 1.2)	1.6 ($\pm$ 2.6)
	a. MFNS	12.0 ($\pm$ 25.7)	2.3 ($\pm$ 2.3)	3.6 ($\pm$ 4.9)	0.6 ($\pm$ 0.8)	4.0 ($\pm$ 5.0)

budesonide, fluticasone propionate) are effective in the treatment of allergic rhinitis (25), acting on both the early and late phases of allergic inflammation (26), therefore, a pre-treatment with these drugs seems to be recommendable.

In our study a 5 day pre-medication at a dosage of 100 μ g daily in each nostril did not prevent nasal symptoms resulting from ASNCL. But there are other studies about the efficacy of a pre-treatment with nasal steroid on the avoidance of rhinitis appearance due to other allergens. The mean nasal symptom scores and sneezing frequency were consistently lower with mometasone furoate pre-treatment compared with placebo after a nasal challenge with ragweed antigen in patients with allergic rhinitis to ragweed (26).

A prevention of nasal symptoms has been described in patients with seasonal allergic rhinitis pre-treated with fluticasone propionate (100 μ g bd into each nostril for 7 days), and receiving a nasal allergen challenge with timothy grass pollen after the treatment, far from the hay fever season (27).

It is possible that higher dosages or a longer period of administration of mometasone furoate might determine a clinical protective effect in children with nasal symptoms due to latex products. This protection has been demonstrated with a longer period of administration of mometasone furoate in presence of nasal symptoms due to seasonal inhalant allergens (11). In our study a pre-treatment longer than 5 days was not possible because of a poor compliance.

Moreover, our study demonstrates no correlation between the presence of immunological changes (e.g. ECP and tryptase) in NLF and clinical manifestations before or after ASNCL with or without mometasone furoate treatment. Fieri and co-workers found a reduction of IL-6 and IL-8 levels in nasal lavage fluid of patients treated with mometasone furoate after a nasal challenge with ragweed antigen compared to the levels obtained before the treatment with mometasone furoate (26). Erin and co-workers have shown that a single dose of nasal steroid (budesonide) could abolish IL-5 and IL-13 responses following a nasal allergen challenge (27). In patients with grass pollen seasonal-allergic rhinitis out of season receiving specific nasal challenge, fluticasone propionate (100 μ g bd into each nostril for 7 days in 13 patients) induced a significant reduction of some cytokines (IL-4, IL-5, IL-6, IL-13) and chemokines (eotaxin, RANTES, MCP-1, MIP-1 α , IL-8 and IP-10) in nasal secretions in the late phase if compared with the levels before the start of the nasal therapy while INF- γ levels were not modified (28). The absence of modifications of IL-5 levels in our study compared to the Erin and co-workers' study (28) may be due to: 1) different efficacy between fluticasone propionate and mometasone furoate, 2) different response to one allergen (e.g. timothy grass pollen) versus another allergen (e.g. latex).

In conclusion, ASNCL, in patients with suspected nasal symptoms to latex, is a simple, reliable and useful tool to confirm a diagnosis of allergic rhinitis

to latex. In patients with nasal symptoms to latex exposure and with specific IgE to latex, ASNCL is a useful tool to study immunological modifications in NLF (e.g. tryptase, ECP, INF- γ , IL-5 and e.g. other mediators not evaluated in this report). Finally, MFNS pre-medication before an ASNCL may prevent some immunological responses (e.g. tryptase and ECP level modifications) induced by ASNCL.

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