

The response to nasal allergen provocation with grass pollen is reduced in patients with chronic rhinosinusitis with nasal polyposis and grass sensitization

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Summary

Background The majority of grass pollen-sensitized rhinitis patients develops allergic symptoms when exposed to the causal allergen and shows a positive nasal allergen provocation test (NAPT). Chronic rhinosinusitis with nasal polyposis (CRSwNP) patients, also characterized by eosinophilic inflammation and local IgE production, can suffer from comorbid inhalant allergy, but may show a different response to allergens.

Objective We aimed to explore the allergic response to grass pollen allergens by NAPT in grass pollen-sensitized CRSwNP patients.

Methods Twelve grass pollen-sensitized CRSwNP patients underwent NAPT with grass pollen and were compared with 12 grass pollen allergic rhinitis patients, 12 control patients and 12 CRSwNP patients without grass pollen sensitization. A positive NAPT was based on change in nasal airflow and symptoms. Further, VAS scores of different symptoms were noted before and after NAPT. Biomarkers such as total IgE, grass pollen-specific IgE and tryptase were measured in serum and nasal secretions.

Results NAPT was positive in 6 of 12 of the grass pollen-sensitized CRSwNP patients, and another four patients developed allergic symptoms not fulfilling the criteria of positivity. In contrast, all patients with allergic rhinitis developed a positive provocation test, whereas in the control group one of the patients and in the non-sensitized CRSwNP group two of the patients developed a positive provocation test.

Conclusion and clinical relevance These results show that allergen exposure induces an attenuated clinical response in patients with CRSwNP and sensitization to grass pollen as compared with grass pollen allergic rhinitis patients.

Keywords allergic rhinitis, chronic rhinosinusitis with nasal polyposis, IgE, nasal allergen provocation test, nasal polyp

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Introduction

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a chronic inflammatory disease of the nose and the paranasal sinuses with development of grape-like structures, often associated with asthma and aspirin sensitivity. CRSwNP appears in 1–4% of the general population and those patients present with symptoms like nasal obstruction, nasal drip and loss of smell [1].

CRSwNP is in most Caucasians characterized by T helper (Th) 2 mediated and eosinophilic inflammation.

Importantly, in nasal polyp tissue, a local polyclonal immunoglobulin E (IgE) production has been identified, which consist of IgE against different allergens [2, 3]. This local IgE concentration is also related to interleukin (IL)-5, eosinophilic cationic protein (ECP) and IgE to *Staphylococcus aureus* enterotoxins (IgE-SAE) [2, 4]. This tissue IgE concentration can be increased irrespective of skin prick test results and is only partially related to the IgE concentration in serum [2, 5]. Furthermore, the local hyperglobulinaemia is not limited to IgE but also includes IgG (IgG₂ and IgG₄) and IgA [5].

The prevalence of allergic sensitization, diagnosed by skin prick test, in patients with CRSwNP has been

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reported to vary from 10% to 54%[1]; however, there is no evidence for a causal relationship [1]. The influence of sensitization on symptoms in CRSwNP patients is under discussion [6]. Keith *et al.* showed that patients with CRSwNP with ragweed allergy experience symptoms, but those symptoms do not increase during the ragweed season, in contrast to patients with allergic rhinitis, who experience a significant increase during the season [7]. In allergic rhinitis (AR), this specific IgE reflects the allergic constitution with an increase of symptoms after exposure to the allergen. However recently, an article by Blomme *et al.* demonstrated that only 81% of grass pollen-sensitized rhinitis patients are symptomatic AR patients [8, 9].

To investigate if local IgE in CRSwNP is functional, Zhang *et al.* recently stimulated nasal polyp tissue with allergens. In this model, they showed that the polyclonal IgE in mucosal nasal polyp tissue is functional. However, the response to specific allergen was limited compared with the response after stimulation with anti-IgE [10].

As patients often observe a lack of seasonal nasal symptoms albeit their sensitization, the goal of this study is to expose grass pollen-sensitized CRSwNP patients to grass pollen allergens (by performing an NAPT) and to measure clinical symptoms and local mediators of allergic reaction. These results were compared with patients with grass pollen AR and healthy controls.

Materials and methods

Patients

Twelve patients with CRSwNP and positive skin prick test to grass pollen were compared with 12 patients with AR to grass pollen, 12 control patients and 12 patients with CRSwNP without grass pollen sensitization. All patients underwent a skin prick test with extracts of 18 common inhalant allergens, the standardized pan-European allergen panel, to diagnose grass pollen allergy, as described by Heinzerling [11]. All patients underwent nasal rigid endoscopy prior to NAPT to visualize normal mucosa or nasal polyps. Patients with CRSwNP had bilateral nasal polyposis on nasal endoscopy and fulfilled the definition proposed by the EPOS [1]. Patients with AR were selected based on symptoms during the grass pollen season and based on a positive skin prick test for grass pollen, conform the ARIA guidelines [12]. Control patients were healthy patients without rhinological disease or asthma and with negative skin prick test to the standard panel of allergens. See this article's supplemental information for more detailed information on inclusion and exclusion criteria.

All patients, 18 years or older, gave written informed consent before inclusion. The local ethical committee of Ghent University Hospital, Belgium, approved the study.

Primary end-point: Nasal allergen provocation test

The primary end-point of this study was the nasal allergen provocation test, performed in accordance with the guidelines by Gosepath *et al.* [13]. The NAPT involves the delivery of a small quantity of the grass pollen allergens by spray into both nostrils and it aims to measure the change in airflow and symptoms in the nasal cavity after exposure to grass pollen. The change in airflow is measured by active anterior rhinomanometry (AAR, Atmos, Germany). The sum of the airflow of the left and right side, at transnasal pressure of 150 Pa, was used to perform further analysis. All NAPT were performed outside the grass pollen season [14]. The practical details of the nasal allergen provocation test can be found in the supplemental information.

A positive test result is based on the combination of symptoms and airflow (Table 1). The NAPT was considered positive if one of the following criteria was fulfilled: a decrease in airflow of more than 40% at a transnasal pressure of 150 Pa, an increase in symptom score of more than 3 or an increase in symptom score greater than 2 combined with a decrease in airflow of more than 20%. When the test was positive after provocation with a certain concentration of the grass pollen allergen, the test was ceased.

VAS score

Different symptoms were questioned before and after performing the NAPT using a visual analogue scale (VAS; nasal obstruction, rhinorrhea, sneezing, itching of the nose, running eyes and itching eyes). The patients were asked to score their symptoms between 0 and 10 by putting a mark on the line. The distance from the non-affected end of the line was measured in

Table 1. Criteria for a positive NAPT, based on Gosepath *et al.* [13]

Positive NAPT if	
Increase in symptom score: +	
> 2	Active anterior rhinomanometry decrease in airflow compared with baseline: > 20%
> 3	> 40%
Symptoms evaluated are (score):	
Secretion: no (0), little (1), heavy (2).	
Irritation/sneezing: 0–2 (0), 3–5 (1), > 5 (2).	
Extranasal symptoms: none (0), tearing/itching (1), conjunctivitis/urticaria/coughing/dyspnoea (2).	

centimetres and recorded as a score before and after NAPT.

Serum and nasal secretion collection

Before NAPT, blood was collected by performing a standard venipuncture. After allowing the blood to clot for 15–30 min, serum was obtained by centrifuging for 10 min at 1620 g at room temperature [15]. ECP, specific IgE to SAE, specific IgE to grass pollen (gx3 IgE), house dust mite (hx2 IgE) and tree pollen (tx1 IgE) were measured on serum with UniCAP (Phadia, Uppsala, Sweden). The detection limits were 0.1 kU/l for specific IgE to SAE and other allergens and 2 µg/l for ECP.

Nasal secretions were collected before and 15 min after NAPT by placement of filter discs in the nose [16], and detailed information is available in the supplemental information. Total IgE, gx3 IgE, total IgG, IgG₄ and tryptase (before and after) were measured with UniCAP (Phadia, Uppsala, Sweden). The detection limits were 1.65 kU/l for total and gx3 IgE, 8,275 mg/l for total IgG, 1 mg/l for IgG₄, 8,275 µg/l for tryptase before and 7,317 µg/l for tryptase after NAPT.

Statistical analysis

All statistics were performed using the SPSS 21.0 software. The data are presented as median and interquartile range (IQR). The distributions of baseline characteristics between disease groups were compared using the chi-square test for dichotomous data and the Kruskal–Wallis test for continuous data. Comparisons between two diseases were performed by the Mann–Whitney *U*-test or chi-square test with a Bonferroni correction. For other tests, *P* value < 0.05 was considered statistically significant.

Results

Demographic and clinical features

Twelve patients in each group were included in this study, of which the demographics are shown in Table 2. All AR patients and patients with CRSwNP with sensitization had a positive skin prick test for grass pollen. Ten AR patients and 11 sensitized CRSwNP patients showed positivity to other allergens like tree pollen, house dust mites or others. Asthma was present in four of the sensitized CRSwNP patients, nine of the non-sensitized CRSwNP patients and three patients of the group with AR (respectively, *P* < 0.05, *P* < 0.05 and *P* < 0.05 compared with controls). Aspirin intolerance was present in two of the sensitized and in four of the non-sensitized CRSwNP patients.

Primary end-point: Nasal allergen provocation test

None of the subjects who underwent NAPT had a positive response to the saline challenge. After performing the nasal provocation with grass pollen, 50% (6 out of 12 patients) of the grass pollen-sensitized CRSwNP patients responded positive. Three patients developed a positive result after provocation with 0.1 IR of grass pollen, and two patients and one patient after concentration of 1 IR and 10 IR, respectively (Table 3). Next to the six of 12 patients developing clear positive response after NAPT, we identified four patients in the sensitized CRSwNP group who developed some symptoms (increase of symptom score ≤ 2) or had a decrease of airflow (decrease in % airflow ≤ 20%), but those reactions were not sufficient to evaluate them as positive (marked in light grey) (Table 3).

All AR patients (2 at 0.1 IR; 5 at 1 IR and 5 at 10 IR) and one control patient (at 0.1 IR) had a positive response after NAPT (Fig. 1).

To be complete, we performed NAPT in 12 CRSwNP patients without grass pollen sensitization (negative skin prick test and negative gx3 IgE in serum and nasal secretions). Two of 12 patients of the non-sensitized CRSwNP group developed a positive NAPT.

Symptom score before and after NAPT

At baseline, there is a significantly higher symptom burden observed in the grass pollen-sensitized CRSwNP patients based on nasal obstruction (vs AR *P* < 0.05; vs controls *P* < 0.05) and rhinorrhea (vs controls *P* < 0.05). After NAPT, there was a significant increase in nasal obstruction, rhinorrhea, sneezing and itching of the nose in patients with AR, measured by VAS. Patients with CRSwNP with grass pollen sensitization also showed a significant increase in nasal obstruction, rhinorrhea and itching of the nose after NAPT (Fig. 2).

Biomarkers measured in serum

In serum, total IgE and specific IgE to grass pollen were higher in AR (*P* < 0.001) and sensitized CRSwNP patients (*P* < 0.001) compared with controls (Fig. 3a).

Looking at the grass pollen-sensitized CRSwNP group, there was no significant difference in serum parameters (total IgE, SAE IgE, gx3 IgE, ECP) between positive and negative NAPT.

Serum measurements after NAPT did not significantly changed compared with those before NAPT.

Biomarkers measured in nasal secretion

Total IgE in nasal secretions was significantly higher in AR patients (*P* < 0.05) and CRSwNP with sensitization

Table 2. Clinical characteristics and inflammatory measurements in serum and nasal secretions in controls, allergic rhinitis based on grass pollen allergy and CRSwNP with and without grass pollen sensitization

Groups	Control	Allergic rhinitis	CRSwNP with sensitization	CRSwNP without sensitization
Subjects (n)	12	12	12	12
Age (years), median (IQR)	28 (25–42)	26 (24–31)	41.5 (34–51)	50 (40–62)
Gender female/male, <i>n/n</i> (%)	6/6 (50/50)	5/7 (42/58)	2/10 (17/83)	7/5 (58/42)
BMI (kg/cm ²), median (IQR)	22.72 (21.01–23.64)	21.61 (20.91–27.04)	21.97 (20.99–23.23)	24.82 (22.08–27.44)
Total nasal polyp score, median (IQR)	NA	NA	6 (4–6)	6 (5–7)
Graspollen allergy, <i>n</i> (%)	0	12 (100)	12 (100)	0
Mono/polysensitive, <i>n/n</i>	NA	2/10	1/11	NA
Asthma, <i>n</i> (%)	0	3 (25)	4 (38.5)	9 (75)
Aspirin sensitivity, <i>n</i> (%)	0	0	2 (17)	4 (30)
Serum, median (IQR)				
Serum total IgE (kU/l)	19.40 (8.90–49.88)	101.25 (62.88–469.75)	222.50 (138.63–438.25)	89.75 (35.58–125.88)
Serum SAE IgE (kU/l)	0.02 (0.15–0.45)	0.13 (0.06–0.73)	0.30 (0.09–1.56)	0.15 (0.07–0.50)
Serum gx3 IgE (kU/l)	0.02 (0.02–0.03)	12.85 (5.03–23.90)	3.02 (0.51–10.05)	0.03 (0.02–0.05)
Serum hx2 IgE (kU/l)	0.03 (0.02–0.12)	4.38 (1.29–15.25)	4.33 (0.31–14.25)	0.03 (0.03–0.06)
Serum tx1 IgE (kU/l)	0.04 (0.04–0.05)	0.35 (0.07–2.96)	0.08 (0.07–0.82)	0.06 (0.04–0.06)
Serum ECP (µg/l)	7.27 (4.16–14.15)	15.08 (5.70–27.40)	28.00 (7.81–44.70)	21.30 (14.98–36.50)
Nasal secretion, median (IQR)				
Nasal total IgE (kU/l)	1.65 (1.65–3.20)	35.23 (1.65–63.89)	23.00 (5.60–115.68)	1.65 (1.65–38.39)
Nasal gx3 IgE (kU/l)	1.65 (1.65–1.65)	14.11 (2.30–24.56)	3.53 (1.65–7.60)	1.65 (1.65–1.65)
Nasal gx3 IgE/total IgE	1.00 (0.51–1.00)	0.42 (0.29–0.92)	0.19 (0.03–0.50)	1.00 (0.04–1.00)
Nasal total IgG (mg/l)	256.17 (192.67–457.25)	182.62 (86.05–314.56)	173.51 (35.01–809.30)	212.01 (53.58–602.39)
Nasal IgG4 (mg/l)	16.75 (3.36–33.26)	12.87 (4.79–53.48)	8.65 (3.50–61.61)	10.19 (3.17–64.55)

gx3 IgE: IgE to mix of grass pollen; hx2 IgE: IgE to mix of house dust mite; Tx1 IgE: IgE to mix of tree pollen; NA: not applicable.

($P < 0.01$) compared with control patients (Fig. 3c). Specific grass pollen IgE (gx3 IgE) was significantly higher in AR compared with controls ($P < 0.01$; Fig. 3d) and measurable in 9 of 12 patients. In nasal secretions of sensitized CRSwNP, the gx3 IgE was measurable in 6 of 12 patients. This nasal gx3 IgE was only a small portion of the nasal total IgE in sensitized polyp patients as shown by the ratio gx3 IgE/total IgE (0.19 [0.03–0.50]). Additionally, the amount of IgG and IgG₄ was measured in nasal secretions. There were no significant differences between groups in IgG and IgG₄ (Fig. 3e and f).

In the grass pollen-sensitized CRSwNP group, there was no significant difference in nasal secretion parameters (total IgE, gx3 IgE, IgG and IgG₄) between positive and negative NAPT. Additionally, there was no significant change in nasal biomarkers before versus after NAPT.

As a marker of mast cell degranulation, tryptase was measured in nasal secretions. The nasal tryptase was significantly higher in AR compared to control at baseline (Fig. 3g). Nasal tryptase was increased after NAPT in 8 of 12 AR patients, whereas it was increased after NAPT in 3 of 12 patients with CRSwNP sensitized to grass pollen. However, no statistical significant increases in nasal tryptase concentrations were found after NAPT in AR ($P = 0.136$) and in the grass pollen-sensitized CRSwNP ($P = 0.525$).

Discussion

This is the first study performing NAPT in grass pollen-sensitized CRSwNP patients; we compared the response to NAPT to the responses in healthy controls and AR patients. Half (6/12) of the CRSwNP patients with grass pollen sensitization, diagnosed by skin prick test, experienced a positive response after allergen exposure and another 4 of 12 patients experienced complaints or reductions in airflow, which did not fulfil the criteria of a positive NAPT. In contrast, all patients (12/12) in the AR group developed a positive NAPT. Thus, CRSwNP patients with a positive skin prick test to grass pollen responded to allergen exposure, but this response was attenuated compared with AR subjects.

When ragweed allergic CRSwNP patients were naturally exposed to ragweed pollen during the season, in a study by Keith *et al.*, a non-significant increase of symptoms was observed, whereas in ragweed AR, a significant increase in symptoms was observed [7]. Similar to our results, there was an increase in nasal blockage and nasal itch in ragweed allergic CRSwNP patients, which, however, appeared to be attenuated compared with AR subjects.

We presume that there are different reasons why not all of the sensitized polyp patients showed a fully

Table 3. Detailed results of grass pollen allergic CRSwNP ($N = 12$) patients

Disease	SPT	Serum [kU/l]				Nasal secretions				Nasal provocation test								Symptom score	
		Total IgE	gx3 IgE	SAE	Total IgE	gx3 IgE	Total IgG	IgG4	Provocation	% airflow	Symptom score	% airflow	Symptom score	% airflow	Symptom score	% airflow	Symptom score		
		(kU/l)	(kU/l)	(kU/l)	(kU/l)	(kU/l)	(mg/l)	(mg/l)		0.1 IR	1 IR	0.1 IR	1 IR	0.1 IR	1 IR	0.1 IR	1 IR	10 IR	10 IR
CRSwNP with allergy	Grass,	136.00	8.09	0.36	BDL	BDL	BDL	3.39	0	96	0	101	0			81	2		
	Grass and tree	176.00	3.23	BDL	10.12	BDL	346.74	7.75	1	33	2								
	Grass and HDM	1220.00	6.66	BDL	103.65	BDL	488.58	73.29	1	68	2								
	Grass and tree	274.00	2.80	1.70	36.45	7.939	126.31	13.14	0	74	0	138	0			104	2		
	Grass	269.00	0.97	3.03	33.29	BDL	220.70	26.59	0	121	1	93	0			95	0		
	Grass and tree	146.50	10.70	1.12	4.09	BDL	40.87	3.85	0	122	1	120	1			129	1		
	Grass and tree	83.00	2.33	BDL	BDL	BDL	33.05	2.06	1	92	0	71	1			59	2		
	Grass and tree	448.50	51.90	BDL	119.69	18.434	1310.14	97.02	1	81	1	5	2						
	Grass, tree, HUM	148.50	0.36	BDL	246.98	5.975	916.20	159.34	1	117	1	21	2						
	Grass and HDM	407.50	0.55	1.82	12.50	5.406	BDL	1.69	1	34	2								
CRSwNP	Grass and tree	50.00	0.37	BDL	12.71	6.575	65.74	9.55	0	136	0	114	2			0			
	Grass and tree	1550.00	60.20	0.77	4271.41	51.516	2210.83	73.83	0	418	0	247	2			0		3	

The results of the skin prick test (SPT), serum (total IgE, gx3 IgE and SAE IgE) and nasal secretion (total IgE, gx3 IgE, total IgG and IgG₄) measurements are presented. The individual measurement of airflow (presented as % of baseline measurement) and symptoms after each provocation (concentration 0.1 IR, 1 IR and 10 IR) are disclosed. Marked in dark grey are the patients with a positive NAPT and marked in light grey are the patients with some response but not a positive result.

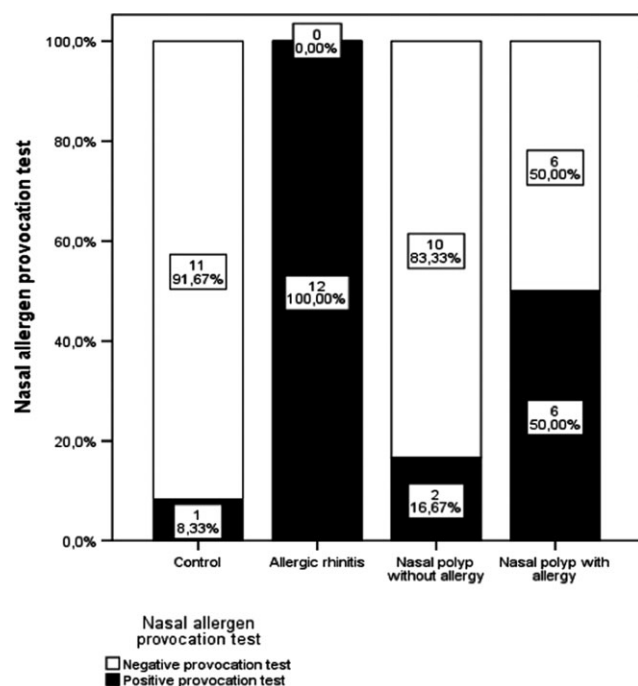


Fig. 1. Nasal allergen provocation test performed in the four different groups: control ($n = 12$), allergic rhinitis ($n = 12$), CRSwNP with allergy ($n = 12$) and CRSwNP without allergy ($n = 12$). The negative results are marked in white and the positive results are shaded. The numbers represented are the percentage and absolute count in each group.

positive NAPT. First, CRSwNP patients were included on the basis of nasal polyps observed by nasal endoscopy. The presence of these polyps gave rise to reduction of airflow and nasal obstruction, as shown by the VAS scores before NAPT. This may have decreased the baseline airflow to an extent that made further changes difficult to appreciate. It has to be pointed out, however, that all patients, even those with a high nasal polyp score, had measurable airflow (Table 3). Based on the burden polyp patients experience, we can only call patients sensitized and not allergic. Sensitized polyp patients have a positive skin prick test, but there is no observation of increase of symptoms during the grass pollen season.

Second, we believe that the underlying pathomechanisms might explain the difference between CRSwNP and AR in the response to allergen exposure. Allergic rhinitis and CRSwNP are characterized by Th2 cell-type inflammation, eosinophilia and high concentrations of IgE in the diseased tissues. However, the IgE repertoires in these tissues exhibit exactly opposite trends in diversity. A large fraction of the total IgE in the nasal mucosa in AR, compared with the circulation, is allergen-specific, which results in sensitization of the resident mast cells for immediate hypersensitivity on exposure to allergen. Mast cell activation initiates the

cascade of events leading to the symptoms of AR. In contrast, IgE in the CRSwNP is polyclonal, and although allergen specificities can sometimes be detected and they are able to sensitize mast cells for allergen activation *in vitro*, they constitute a small fraction of the total IgE. However, this polyclonal IgE might induce a permanent mast cell degranulation in CRSwNP, masking the effect of a single allergen provocation.

In this study, we found local total IgE, measured in nasal secretions, in 10 of 12 grass pollen-sensitized CRSwNP patients. Importantly, we succeeded in measuring grass pollen-specific IgE in nasal secretions derived from filter discs in 6 of 12 CRSwNP patients. This local IgE measured in nasal secretion is a reflection of the local IgE present in tissue, due to the close interplay between T and B cells in lymphoid aggregates [3, 17, 18]. We could not identify a correlation between the amount of local IgE (total or specific) measured in nasal secretions and the development of a positive NAPT. However, these subgroups (NAPT positive versus negative) are too small to draw definite conclusions.

The presence of nasal polyps makes the performance of NAPT more difficult because nasal polyp tissue in the nasal cavity obstructs the application of the allergen with a spray and only certain parts of the nose are reached by the allergen. In allergic rhinitis, it is presumed that most of the allergen is captured by the inferior turbinate; this may not be the case in CRSwNP, specifically in those patients with severe polyposis obstructing the nasal cavity. In our study, the mean nasal polyp score in the allergic CRSwNP group was comparable between a positive NAPT and a negative NAPT (respectively, 4.5 and 4.8). Perhaps, by performing NAPT before and after surgery, we could differentiate the response of the nasal polyps versus inferior turbinates in this mechanism.

Our *in vivo* observation of a clinical response after NAPT in sensitized CRSwNP patients was in line with the *in vitro* results published by Zhang et al. [10]. These authors showed that nasal polyp tissue with specific local grass pollen IgE reacted to grass pollen allergens by mast cell degranulation (measured by the release of prostaglandins); however, the maximal release triggered by stimulation with grass pollen allergen was only one-third of the release triggered by stimulation with anti-IgE. In both CRSwNP and AR patients, they found a Gaussian distribution of the magnitude of prostaglandin release in relation to the concentration of allergen, which was administered. Also the prostaglandin release induced by allergen stimulation did correspond with the release after anti-IgE stimulation in AR but not in CRSwNP. Thus, local IgE in CRSwNP is functional when triggered with a specific allergen, but this reaction is

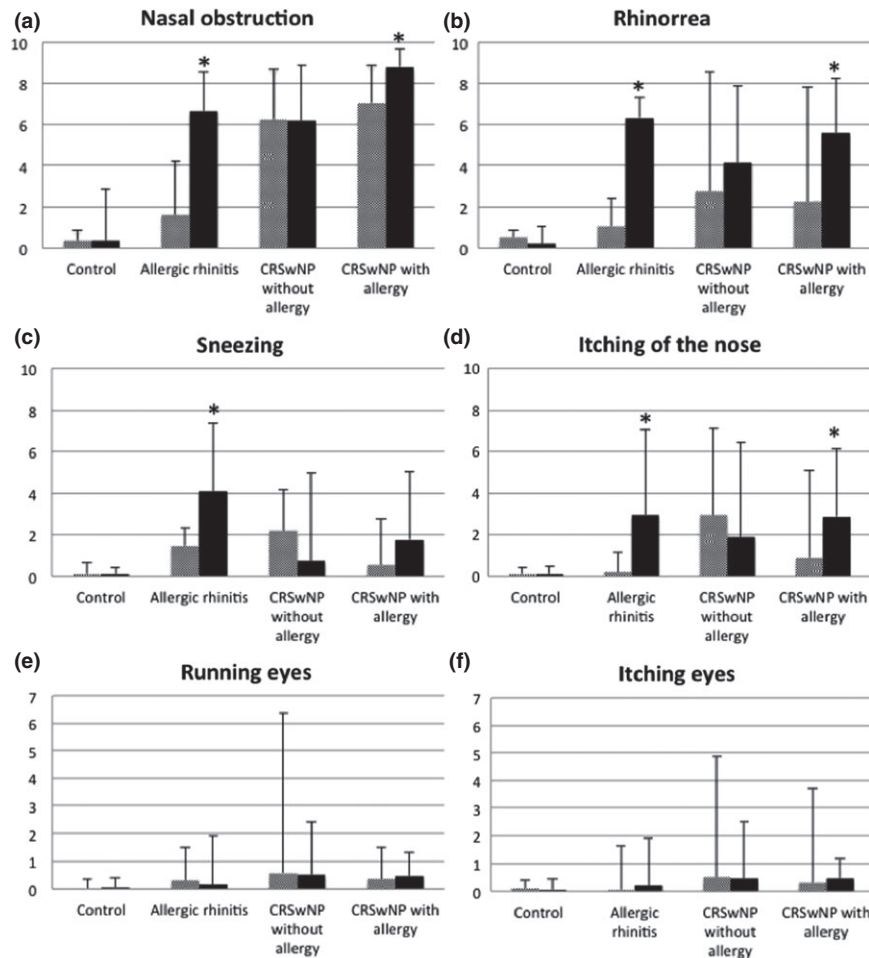


Fig. 2. Symptoms measured via VAS scores before (grey) and after (black) NAPT are shown. The three disease groups are shown for each symptom as median (bar) and upper IQR. Chi-square test with a Bonferroni-correction was used. The significant increases are marked with an asterisk.

reduced in comparison with non-specific anti-IgE stimulation.

Other factors in the underlying inflammation in CRSwNP could be a reason for the partial clinical response after NAPT. We could presume that there are agents that block the IgE-activity in CRSwNP, like IgA, IgG₄ and sCD23. IgA acts as a shield against intruding allergens at mucosal sites [19, 20], and perhaps, IgA has an inhibitory effect on IgE [21]. IgG₄ can be produced as a side effect of IgE production in the lymphoid follicles in CRSwNP, and IgG₄ decreases IgE receptor-facilitated allergen binding to B cells and diminishes activation of mast cells and basophils [8]. However, IgG and IgG₄ are measurable in the nasal secretions without significant difference between the groups. Further, in CRSwNP, the concentration of soluble low affinity IgE receptor (sCD23) has been reported higher than in control tissue, which could contribute to a reduction of magnitude of response after NAPT [2]. This soluble IgE receptor could interfere by capturing IgE and preventing binding to the receptor. To

completely understand these results, further research into the pathways of B cell switching to IgE and mechanism of IgE memory in patients with CRSwNP is essential.

Finally, CRSwNP is also characterized by continuous mast cell degranulation, so the activation status of mast cells could also impact on the release of mediators [22–24]. Tryptase was measured as marker to monitoring mast cell activity, but at baseline, there was no significant increase of tryptase in nasal secretion in the CRSwNP group versus control. In contrast, in AR there was an increased amount of tryptase in nasal secretions, which is probably due to exposure to other allergens in the polysensitized patients [25]. After NAPT, neither increase in tryptase in AR nor CRSwNP was significant.

In summary, the exposure of patients with grass pollen sensitized CRSwNP to grass pollen induced a clinical response. However, compared with allergic rhinitis, this response was attenuated. Different underlying pathomechanisms could possibly induce this phenomenon in CRSwNP with grass pollen sensitization.

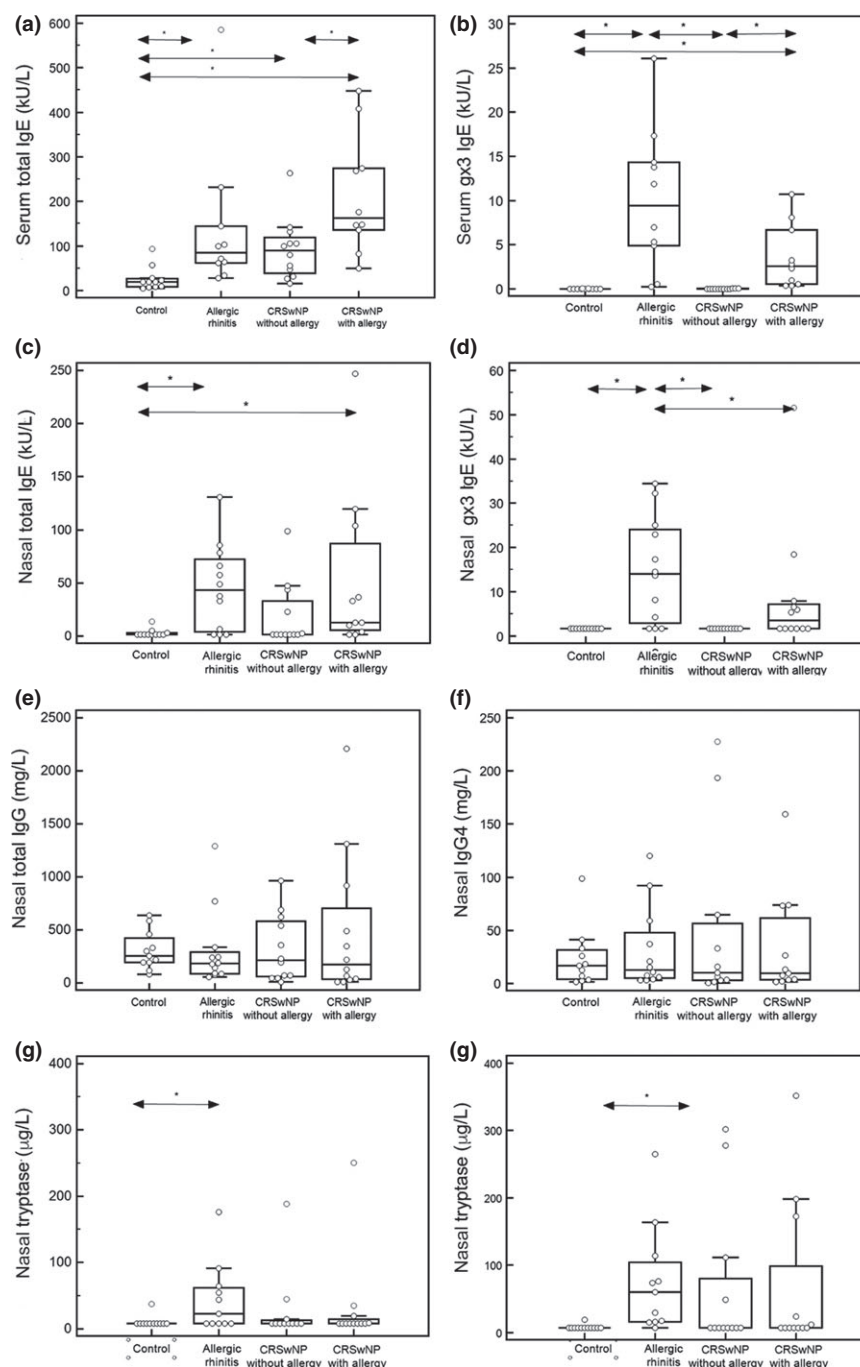


Fig. 3. Measurements of total IgE (a) and specific grass pollen IgE (b) in serum before NAPT in our four different groups: control, allergic rhinitis, CRSwNP without allergy and CRSwNP with allergy. In nasal secretion, total IgE (c), specific grass pollen IgE (d), total IgG (e) and IgG₄ (f) are measured before NAPT. Tryptase measurements before and after NAPT are shown in panels (g) and (h). All measurements are marked with a black open circle. Mann–Whitney *U*-test was used. The significant differences are marked with an asterisk.

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the concept and contributed to sample collection, interpretation of the data and development of the manuscript. All authors critically revised the article and approved the final version. The opinions, results and conclusions reported in this article are those of the authors and are independent of the funding sources. The corresponding author had full access to all data in

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Conflict of interest

The authors declare no conflict of interest.

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