

The Clinical Relevance of Natural Rubber Latex-Specific IgE in Patients Sensitized to Timothy Grass Pollen

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Keywords

Latex allergy · Hev b 8 · Pollen allergy · Cross-reactive carbohydrate determinant · Immunologic tests

Abstract

Background: In pollen-allergic patients, cross-reacting allergens including cross-reactive carbohydrate determinants (CCDs) and profilins may result in positive natural rubber latex (NRL)-specific IgE (slgE) antibody tests but the relationship between this sensitization and clinical NRL type 1 allergy is poorly described. **Objective:** The aims of this study were to determine the frequency and clinical relevance of NRL slgE in grass pollen-sensitized individuals and to investigate which NRL allergen components these individuals were sensitized to. **Methods:** A total of 383 grass-sensitized patients answered questions about NRL allergy symptoms and their stored sera from previous investigations were analyzed for NRL slgE. Patients with NRL slgE ($n = 32$) underwent further investigations comprising medical history, skin prick test with NRL and inhalational allergens, and an additional blood sample. The additional blood samples were analyzed for total IgE and slgE against NRL, timothy grass, birch, rHev b 1, 3, 5, 6.01, 6.02, 8, 9, 11, rPhl p 12, and MUXF3, which was used as a marker of CCD sensitization. **Results:** Overall, 9.4%

of all grass pollen-sensitized individuals showed IgE sensitization to NRL but only 1.6% had a confirmed type I NRL allergy. CCD and Hev b 8 explained the clinically irrelevant NRL IgE sensitization in 65% of the cases. We found a highly significant correlation between NRL profilin (Phl p 12) sensitization and grass profilin (Phl p 12) sensitization ($p < 0.0001$). **Conclusions:** Data from this study support the hypothesis that in patients with grass pollen sensitization, Hev b 8 mono-sensitization has little or no clinical relevance and is caused by cross sensitization from grass profilin (Phl p 12).

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Introduction

Natural rubber latex (NRL) is extracted from the *Hevea brasiliensis* tree and due to its properties, it is widely used in the health care system in the production of medical gloves, catheters, and other products [1].

Immediate type (type 1) NRL allergy is caused by an IgE-mediated reaction to NRL and has been intensively

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studied [2–4]. It is characterized by symptoms which can be mild such as urticaria, rhinitis, conjunctivitis, and mucosal swelling or more severe with generalized urticaria, respiratory symptoms including severe asthma, or anaphylactic shock. Symptoms typically occur within minutes of NRL exposure [4–7]. When suspecting an NRL allergy, skin prick tests (SPTs) and in vitro tests of patient serum are typically used in the diagnosis. However, especially in vitro tests measuring NRL-specific IgE (sIgE) antibodies have raised concerns about the clinical relevance, because a positive test does not differentiate between sensitization and symptomatic allergy [8, 9]. Some clinicians diagnose NRL allergy based on measuring NRL sIgE alone, but it is questioned whether that is a sufficient basis for a diagnosis of NRL allergy.

Localized symptoms of type I allergy can be difficult to distinguish from delayed-type allergy (type IV) and irritant contact dermatitis. Type IV allergy is caused by a T-cell-mediated reaction to the chemicals used in the manufacturing process of NRL and not the NRL itself [6]. Typical type IV symptoms are itchy, eczematous rashes, usually confined to the areas of exposed skin, developing within 24–48 h of exposure [5–7]. Type IV contact dermatitis is diagnosed using patch tests.

Irritant contact dermatitis is caused by a nonimmunological inflammation of the skin [5, 6]. Typical symptoms are dry, erythematous, thickened, scaling and fissuring skin, typically accompanied by an itching or burning sensation [5–7]. The symptoms do not extend beyond the directly exposed skin area. Irritant contact dermatitis is the most frequent NRL glove-associated skin reaction [5], and therefore an important differential diagnosis to NRL allergy.

The Allergen Nomenclature Committee of the International Union of Immunological Societies has identified 15 NRL allergen components denoted Hev b 1–15 [10] given the origin from the *Hevea brasiliensis* tree. The primary allergen components associated with clinical symptoms on NRL exposure are Hev b 1, 3, 5, 6.01, and 6.02 [2, 3, 11–13]. Cross-reacting allergens including cross-reactive carbohydrate determinants (CCDs) and profilins may make the interpretation of a positive NRL sIgE difficult. CCD epitopes are thought to cause clinically irrelevant NRL IgE sensitization [14, 15] and plant allergic patients with a false-positive NRL sIgE have been observed to have a significantly higher prevalence of CCD antibodies than true NRL-allergic patients [9]. Profilins are proteins which are present in all eukaryotic cells. They have a conserved structure across different plant species, resulting in sIgE antibodies reacting to a broad pattern of

cross-reactive profilins [16]. The NRL allergen component Hev b 8 is a profilin, and cross-sensitivity between Hev b 8 and timothy grass has been observed [17, 18], resulting in NRL sIgE-sensitized patients who are asymptomatic on exposure to NRL and mono-sensitized to Hev b 8 [2, 8, 17–20].

In Denmark, grass pollens are a frequent cause of pollen allergy [21–23]. Studies from Austria and Italy have shown that 25–30% of grass-allergic patients are sensitized to grass profilin (Phl p 12) [16, 24]. Since grass profilin (Phl p 12) sensitization is associated with sensitization to NRL profilin (Hev b 8) [17, 18], NRL sIgE sensitization in grass pollen-sensitized patients may not always be clinically relevant.

The aims of this study were (1) to determine the frequency and clinical relevance of NRL sIgE in grass pollen-sensitized individuals and (2) to investigate to which NRL allergen components these individuals were sensitized. Our hypothesis is that in patients with sIgE to timothy grass, Hev b 8 mono-sensitization is caused by cross-sensitization from Phl p 12 and has little or no clinical relevance.

Materials and Methods

Definitions

NRL allergy was defined as clinically relevant type 1 allergic symptoms in combination with one or more positive tests. Symptoms were considered as type I allergic symptoms if the patient reported one or more of the following symptoms occurring within minutes of exposure to NRL: local or generalized urticaria, mucosal swelling, conjunctivitis, rhinitis, and/or respiratory symptoms. Patients experiencing type I allergic symptoms were classified as having “immediate symptoms”.

Symptoms upon NRL exposure were considered to be caused by either irritant contact dermatitis or type IV allergy when symptom onset was delayed (hours to days after exposure), when local itching was the only symptom, when the patient had already been diagnosed with a type IV allergy and only reported hand symptoms, or when the patient only experienced local itching and rash and reported no allergic reactions at the dentist, general practitioner, hair dresser, gynecologist, or the hospital. If the patient did not avoid using NRL products or did not react on exposure to NRL-containing products, symptoms were considered to be caused by irritant contact dermatitis. Patients not experiencing symptoms compatible with NRL allergy or experiencing no symptoms at all were classified as having no immediate symptoms.

Study Design

A flowchart of inclusion is shown in Figure 1. All patients investigated for allergy in the period from October 1, 2013 to July 1, 2015 at the Allergy Clinic, Department of Dermatology and

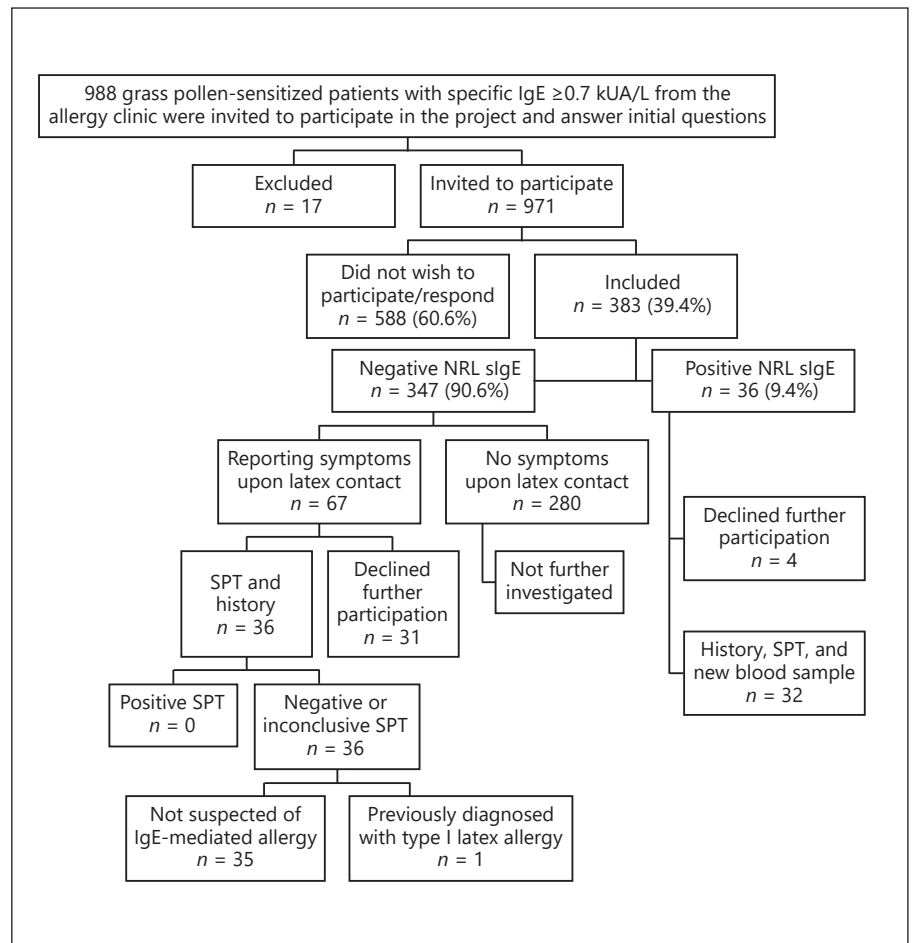


Fig. 1. Flowchart of inclusion.

Allergy, Copenhagen University Hospital at Gentofte, Denmark, who were sensitized to timothy grass with sIgE level ≥ 0.7 kUA/L were identified from the database at the Laboratory of Medical Allergology in the Allergy Clinic. The laboratory biobank contains serum from patients investigated for allergy at the Allergy Clinic. Here 1,050 blood samples with sIgE antibodies against timothy grass were identified in the given time period. If a patient had more than a single serum sample, the most recent was included. After correcting for duplicates, 988 patients remained. Seventeen patients were excluded due to death ($n = 1$), emigration from Denmark ($n = 12$), unknown address ($n = 2$), or investigation in the Danish Anaesthesia Allergy Center ($n = 2$). In total, 971 patients were invited, by letter, to participate in the study.

Initial Questioning

The initial medical history taking was standardized and comprised questions on types of symptoms occurring on contact with NRL, the period from exposure to reaction(s), any previous diagnosis of either a type I or type IV NRL allergy, any allergic reactions at the dentist, general practitioner, hair dresser, gynecologist and/or during surgery, use of products containing NRL, and any pro-

fessional and/or occupational exposure to NRL. Symptoms upon NRL contact were defined as patient-reported symptoms based on the medical history recorded at the allergy clinic.

Skin Prick Test

SPTs were carried out using a standard inhalation panel with birch (*Betula verrucosa*), timothy grass (*Phleum pratense*), mugwort (*Artemisia vulgaris*), animal dander (horse, dog, cat), house dust mites (*Dermaphagoides pteronyssinus*, *Dermaphagoides farina*), moulds (*Alternaria alternata* and *Cladosporium herbarum*), and in addition an NRL extract (Soluprick® SQ; ALK-Abelló Nordic). Histamine was used as a positive control and saline as a negative control. SPT reactions were considered positive when the wheal reaction was ≥ 3 mm after 15 min according to guidelines [25].

sIgE Antibody Analysis

Serological testing was performed using the ImmunoCAP® 1000 system (Thermo Fisher, Uppsala, Sweden) in accordance with the manufacturer's instructions. All samples drawn at the time of the investigation (termed "new blood samples") were analyzed for total IgE and sIgE against NRL (k82), timothy grass, birch, all available NRL allergen components from Thermo Fisher

Table 1. Demographic, serological, and clinical data of the study population

Patient No.	Age, years	Gender (M/F)	NRL SPT, mm	NRL sIgE (initial), kU _A /L	NRL sIgE (new), kU _A /L	rHev b, kU _A /L					Timothy Grass SPT, mm	Timothy Grass sIgE (new), kU _A /L	Phl p 12 sIgE, kU _A /L	CCD, kU _A /L	Total IgE, kU _A /L			
						1	3	5	6.01	6.02						8	9	11
1	52	F	15	42.5	17.2	0	0.43	0	27.7	34.9	1.13	0.46	10.0	9	32.5	0.68	0	>5,000
2	68	F	5	0.75	1.57	0	0	0	2.52	2.28	0	0	0	2	1.04	0	0	170
3	70	F	5	20.3	24.4	0	0	0	0	0	0	0	0	4	21.5	0	19.8	45.1
4	38	F	4	11.9	8.63	0	0	6.54	4.78	5.32	0	0	0.79	4	19.5	0	0	768
5	44	F	5	1.37	0.76	0	0	0	1.24	1.40	0	0	0	7	52.6	0	0	1,994
6	64	F	5	5.72	6.5	0.42	0.46	0.39	0.38	0.81	0.54	0.66	0.77	2	6.17	0.63	4.21	336
7	79	F	0	3.49	0.76	0	0	0	0	0	0	0	0	0	0.99	0	0.74	111
8	51	F	0	0.46	0	0	0	0	0	0	0	0	0	0	0	0	0	20.4
9	38	F	0	1.22	0.48	0	0	0	0	0.36	0	0	0	6	6.05	0	0	75.4
10	46	M	0	0.67	1.75	0	0	0	0	0	0	0	0	11	23.0	0	0.54	485
11	33	M	0	0.38	1.12	0	0	0	0	0	3.42	0	0	6	72.1	1.86	0	167
12	36	F	0	0	0.39	0	0	0	0	0	1.23	0	0	8	43.5	0.52	0	341
13	66	M	0	0.71	0.43	0	0	0	0	0	0	0	0	0	0.91	0	0	575
14	62	M	0	0.73	0.39	0	0	0	0	0	0	0	0	4	3.45	0	0	201
15	43	M	0	0.35	0.58	0	0	0	0	0	0	0	0	5	18.0	0	0	130
21	28	F	0	1.93	3.01	0	0	0	0	0	3.16	0	0	7	4.52	1.64	0	53.2
17	54	F	0	0.43	0	0	0	0	0	0	0	0	0	4	2.27	0	0	88.4
18	20	F	0	0.45	0	0	0	0	0	0	0	0	0	3	48.4	0	0	1,526
19	40	F	0	0.89	0.50	0	0	0	0	0	0	0	0	7	6.24	0	0.35	429
20	64	M	0	5.11	2.04	0	0	0	0	0	0	0	0	0	2.09	0	1.61	451
16	31	M	0	0.41	1.07	0	0	0	0	0	0	0	0	8	1.86	0	0	288
22	64	F	0	1.23	0	0	0	0	0	0	0	0	0	2	4.65	0	0	72.8
23	28	F	0	3.35	1.39	0	0	0	0	0	0	0	0	4	2.37	0	0.86	703
24	73	F	0	1.29	1.77	0	0	0	0	0	0	0	0	2	1.63	0	1.36	60.3
25	28	F	0	0.54	0.66	0	0	0	0	0	0	0	0	9	9.87	0	0.46	2,573
26	28	F	0	12.7	8.24	0	0	0	0	0	33.8	4.50	0	5	26.7	9.12	0.93	628
27	50	M	0	0.52	0.83	0	0	0	0	0	0	0	0	0	0.76	0	0.57	758
28	68	M	0	0.55	0	0	0	0	0	0	0	0	0	6	10.5	0	0	452
29	72	F	0	495	183	4.81	5.28	5.21	4.85	6.91	7.08	6.75	7.36	0	163	6.47	143	869
30	43	F	0	0.64	0.36	0	0	0	0	0	0.74	0	0	4	12.4	0	0	654
31	41	F	0	1.23	0	0	0	0	0	0	0	0	0	0	0	0	0	74.9
32	36	F	0	0.75	0	0	0	0	0	0	0	0	0	3	2.98	0	0	275
33	37	M	0	1.07	1.35	0	0	0	0	0	8.13	0	0	4	3.35	3.09	0.37	823

Only patients with a "new blood sample" taken at the time of investigation are shown. Patients marked in grey are patients with immediate symptoms. NRL, natural rubber latex; SPT, skin prick test.

Table 2. The quantitative levels of NRL sIgE in the patients with and without immediate symptoms

	Immediate symptoms	No immediate symptoms	<i>p</i> value
Median NRL sIgE, kU _A /L	8.81 (IQR: 2.46–18.2)	0.73 (IQR: 0.49–1.26)	0.009*
NRL sIgE levels from the initial blood samples. IQR, interquartile range. * Mann-Whitney U test.			

(rHev b 1, 3, 5, 6.01, 6.02, 8, 9, 11), grass profilin: rPhl p 12, and MUXF3, which was used as a marker of CCD sensitization. A value of sIgE ≥ 0.35 kU_A/L was considered positive.

Patient Inclusion

In total, 383 patients with timothy grass pollen sIgE sensitization agreed to participate and answered the initial questions at inclusion. Serum samples from their original allergy investigations (termed “initial blood samples”) were analyzed for NRL sIgE (k82 ImmunoCAP).

Patients with a positive NRL sIgE ($n = 36$) were scheduled for an appointment at the Allergy Clinic offering investigations as described above comprising medical history, SPT with inhalational allergens and NRL, and an additional blood sample (“new blood sample”). Four patients with positive NRL sIgE declined further investigations (2 due to illness and 2 did not respond to repeated contacts). A total of 32 patients with NRL sIgE underwent further diagnostic workup.

Patients with a negative NRL sIgE, but who reported symptoms on contact with NRL at the initial questioning ($n = 67$) were offered an appointment at the Allergy Clinic (36 agreed to participate and 31 declined) to obtain a medical history and perform SPT with inhalational allergens and NRL.

Patients with a negative NRL sIgE reporting no symptoms on NRL contact ($n = 280$) did not undergo further investigations. Patients ($n = 16/280$) reporting to be uncertain about having symptoms on NRL contact or who did not respond to the questioning at all were included in the group of patients reporting “no symptoms” upon NRL contact.

Statistical Analysis

The χ^2 test was used to assess gender bias in the participating group, Fisher’s exact test was used to compare correlation between variables, and the Mann-Whitney U test was used for sIgE median comparison and assessment of age bias in the participating group. Statistical calculations were performed using RStudio (RStudio Inc., Boston, MA, USA). *p* values < 0.05 were considered significant.

Results

Characteristics of the Included Subjects

In total, 383 out of 971 invited grass-sensitized patients (39.4%) agreed to participate. Of these, 257 (67%) were women and 126 (33%) were men. This was significantly

different from the group of patients who chose not to participate where 335 (57%) were women and 253 (43%) were men ($p = 0.002$). The median age was 41 years (range: 19–87 years), which was 5 years older (95% CI: 3.0; 6.0) than the median age in the group of patients who chose not to participate ($p < 0.0001$).

In total, 36 of the 383 included patients (9.4%) were NRL IgE-sensitized. In all, 32/36 NRL-sensitized patients (initial blood sample) and 1 patient not NRL-sensitized (initial blood sample) but previously diagnosed with NRL allergy (patient No. 12; Table 1) were investigated. Demographic, clinical, and serological data can be found in Table 1. Six out of 32 patients with a positive NRL sIgE (18.8%) reported immediate symptoms (marked in grey in Table 1), resulting in a 1.6% prevalence of NRL allergy in the investigated population. NRL SPTs were helpful in differentiating between sensitized and allergic patients; all 6 patients with immediate symptoms had positive SPTs and all 26 patients without immediate symptoms had negative SPTs. All but one NRL-sensitized patient reported pollen allergy symptoms and 22/32 were timothy grass SPT positive. Comparing patients with and without immediate symptoms, there was a significantly higher median NRL sIgE level (initial blood sample) in patients experiencing immediate symptoms ($p = 0.009$) (see Table 2).

There were 347 patients (90.6%) with a negative NRL sIgE and among these, 67 patients (19.3%) reported symptoms when exposed to NRL on initial questioning; 36/67 patients (53.7%) were interested in undergoing further investigation. None of these patients reported immediate symptoms consistent with NRL allergy; 35/36 patients had a negative NRL SPT. One SPT was inconclusive due to dermatographism.

sIgE Analysis

In total, the sera of 33 patients were analyzed; 32 NRL sIgE positive in the initial blood sample and patient No. 12 who was NRL sIgE negative but previously diagnosed with NRL allergy.

The median level of NRL sIgE in the new blood samples was 0.76 kU_A/L (interquartile range, IQR: 0.39–1.77 kU_A/L). The median level of total IgE was 339 kU_A/L (IQR: 105–666 kU_A/L).

In total, 6 NRL-sensitized patients experienced immediate symptoms and of these 5 were sensitized to one or more of the primary allergen components: rHev b 1, 3, 5, 6.01, and/or 6.02 (Fig. 2, number shown in parentheses). One patient was exclusively sensitized to CCD with sIgE of 19.8 KU_A/L (patient No. 3; Table 1). One patient was sensitized to all tested NRL allergen components and CCD (patient No. 6; Table 1). As shown in Figure 3, rHev b 3, 5, 6.01, and 6.02 were predominantly recognized by patients experiencing immediate symptoms upon NRL contact. We found a significant correlation between experiencing immediate symptoms and sensitization to one or more of the primary allergen components: rHev b 1, 3, 5, 6.01, and 6.02 ($p = 0.0005$). rHev b 6.01 and 6.02 were the allergen components most frequently identified in patients with immediate symptoms (5/6). No patients with immediate symptoms were mono-sensitized to rHev b 8, as shown in Figure 3.

Of the 27 NRL-sensitized patients without immediate symptoms, 20/27 (74%) were NRL-sensitized in the new blood sample, as shown in Figure 2. In total, 13/20 patients (65%) had sIgE antibodies directed exclusively against rHev b 8 and/or CCD: 8 CCD mono-sensitized, 4 rHev b 8 mono-sensitized, and 1 sensitized to CCD and rHev b 8. Of the remaining 7/20 patients, 1 patient (patient No. 29; Table 1) was sensitized to all tested NRL components and CCD, 1 patient (patient No. 9; Table 1) was sensitized exclusively to rHev b 6.02, and 1 patient (patient No. 26; Table 1) was predominantly sensitized to rHev b 8 with a co-sensitization to rHev b 9 and CCD. In 4 patients (patients No. 13–16; Table 1) we could not find any explanation for the positive NRL sIgE. In 8/9 cases of rHev b 8 sensitization, the sensitization was associated with Phl p 12 sensitization. The correlation between rHev b 8 (NRL profilin) sensitization and Phl p 12 (grass profilin) sensitization was highly significant ($p < 0.0001$). In the 7 patients without NRL sensitization in the new blood sample, the median sIgE level was 0.55 kU_A/L (range: 0.43–1.23 kU_A/L) in the initial blood sample.

Discussion

The general prevalence of NRL sIgE antibodies in the population of grass-sensitized patients was 9.4% (36/383 patients), which is lower than that reported from other

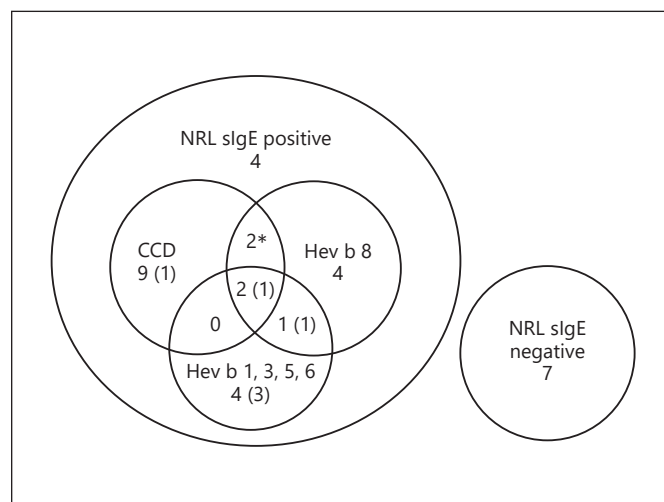


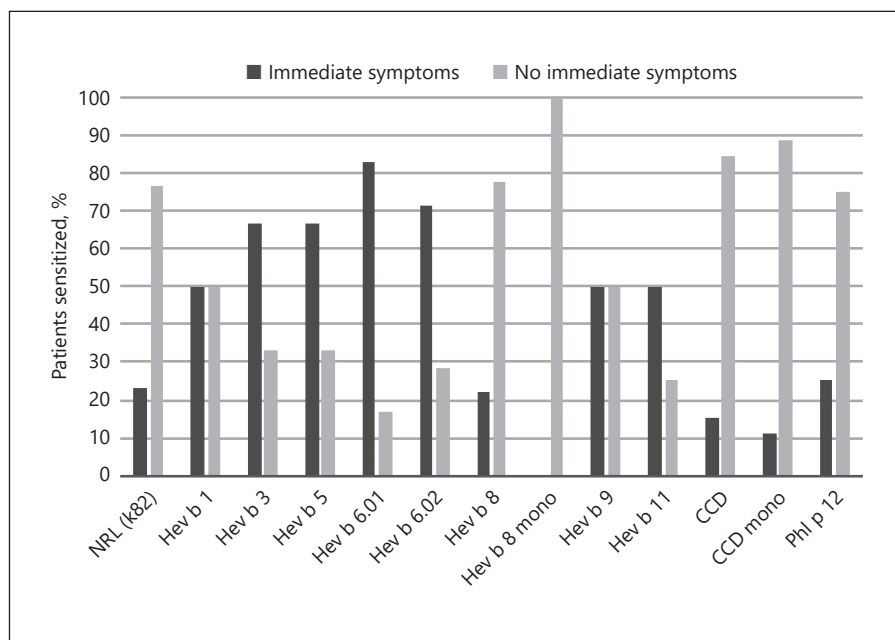
Fig. 2. Sensitization patterns in all patients who had NRL-specific IgE measured in the “new blood sample” from the time of investigation ($n = 33$). Patients are distributed according to results of NRL sIgE, specific allergen components, and CCD. Numbers in parentheses indicate the number of patients with immediate symptoms. Hev b 1, 3, 5, 6 denotes patients sensitized to Hev b 1 and/or 3 and/or 5 and/or 6.01 and/or 6.02. * One of the 2 patients with Hev b 8 and CCD co-sensitization was also sensitized to Hev b 9.

countries [16, 24]. Only 6 out of the 32 NRL sIgE-positive patients (18.8%) had a clinically relevant history of immediate symptoms on NRL exposure fulfilling the criteria for NRL allergy. Interestingly, all 6 were positive in SPT and 5/6 were sensitized to the primary allergen components. We found a highly significant correlation between having immediate symptoms and sensitization to the primary allergen components (rHev b 1, 3, 5, 6.01, and 6.02). This has also been shown in other studies [2, 3, 11–13].

None of the remaining 27 NRL sIgE-positive patients had a clinically relevant history of symptoms on NRL exposure and all were negative on SPT, therefore none of them fulfilled the criteria for NRL allergy. Thus, in this study of grass pollen-sensitized patients, just over 80% of the NRL sIgE-sensitized patients had a false-positive NRL sIgE.

Two of the 27 nonallergic but NRL sIgE-positive patients were sensitized to primary NRL allergen components: one was mono-sensitized to Hev b 6.02 (sIgE 0.36 kU_A/L) and the other was positive for all allergen components available. Neither of them had a positive SPT nor had they clinically relevant symptoms. The remaining 25 were not sensitized to any of the primary NRL allergen components.

Fig. 3. Comparison of prevalence of specific IgE against NRL, recombinant NRL allergen components, and CCD in patients with and without immediate symptoms. “Immediate symptoms” refers to patients reporting symptoms consistent with type I NRL allergy. “No immediate symptoms” refers to patients either without symptoms upon NRL contact or symptoms consistent with irritant/type IV contact dermatitis. “mono” refers to patients being mono-sensitized to the given allergen. Patients sensitized, %: indicates the percentage of patients either with or without immediate symptoms out of the total number of patients sensitized to the allergen in question. The sIgE levels are measured on the new blood samples.



In general, potentially false-positive NRL sIgE appears to be due to borderline positive NRL sIgE levels or mono-sensitization to Hev b 8 or CCD. The potential implication of this is that clinicians not familiar with the difference in clinical relevance of the different NRL allergen components and the presence of CCD might wrongfully diagnose a pollen-allergic individual with NRL allergy on the basis of unspecific symptoms on NRL exposure and a (false) positive NRL sIgE test.

The Level of NRL sIgE

Whether NRL sIgE-sensitized patients have clinically relevant symptoms or not appears to be mirrored in the concentration of sIgE, a correlation also found in other studies [11, 20]. We found significantly higher levels of NRL sIgE in patients with immediate symptoms compared to patients without (see Table 2). This suggests that a higher cutoff level might improve the ability of the sIgE to differentiate between sensitized, but asymptomatic, patients and allergic patients, thereby improving the specificity.

In 4 nonallergic but NRL IgE-sensitized patients (new blood sample), we could not find an explanation for the positive test (see Fig. 2): all NRL allergen components tested negative and the NRL sIgE values were low (range: 0.39–1.07 kU_A/L). The total IgE ranged from 130 to 575 kU_A/L.

This lack of explanation has also been observed in other studies [20]. It could be explained by sensitization to

some pollen-related NRL determinant(s) not available for testing or perhaps not yet discovered.

Seven patients appeared to have lost their NRL sensitization from the initial blood sample to the new. The sIgE levels in the initial blood sample were all low with a median of 0.55 kU_A/L. None of these patients reported immediate symptoms upon NRL exposure and all were SPT negative. If the patients were still NRL IgE-sensitized, we would expect them to fall into the same categories as the rest of the patients without immediate symptoms who remained NRL-sensitized, primarily with a sensitization to Hev b 8 and/or CCD.

CCD and Hev b 8

CCD and Hev b 8 mono-sensitization primarily occurred in patients without immediate symptoms (see Fig. 3) and the two allergens explained the NRL sIgE sensitization in the nonallergic patients in 65% of the cases. There was a highly significant correlation between rHev b 8 and rPhl p 12 sensitization, confirming the highly cross-reactive nature of profilins also shown by Casquete-Román et al. [17]. Our hypothesis that Hev b 8 mono-sensitization would have little or no clinical relevance in patients with grass sIgE antibodies appears to be supported by our data. However, numbers are small and further studies with more Hev b 8 mono-sensitized patients are needed. Other studies have suggested that Hev b 8 is clinically irrelevant [2, 8, 17–20] and Quercia et al. [26] have

shown that asymptomatic, Hev b 8 mono-sensitized patients could undergo surgery in a normal NRL-containing setting without any allergic reactions. NRL allergy in patients with Hev b 8 mono-sensitization has, however, been described [20, 27].

In 1 NRL-allergic patient (patient No. 3; Table 1) we could not identify sensitization to any of the tested NRL allergen components but solely to CCD with quite high titers. Whether the CCD sensitization is causing the patient's NRL immediate symptoms or whether it is just an expression of her concomitant pollen allergy is unknown. One could hypothesize from this case that the clinical relevance of CCDs is a question of concentration and that they can become clinically relevant if the CCD sIgE level is high enough. However, it seems more likely that this patient is sensitized to a clinically relevant NRL allergen component (Hev b 2, 12, or 13) which was not available for testing. Raulf-Heimsoth et al. [3] and Kurup et al. [12] have both shown that Hev b 2 and 13 are part of the primary allergen components involved in sensitizing health care workers. Patient No. 3 was a retired nurse, and it therefore seems plausible that sensitization to either of these two allergen components are the real cause of her allergic symptoms, rather than CCD. Hev b 12 is another plausible explanation for her symptoms. Faber et al. [28] have reported on 2 symptomatic patients who were not sensitized to any of the NRL allergen components used in our study: rHev b 1, 3, 5, 6.01, 6.02, 8, 9, and 11, but sensitization to Hev b 12 was diagnosed by flow cytometric bead array, thereby implicating a clinical relevance of Hev b 12.

In this study, it appears that patients with false-positive NRL sIgE can be separated from the true-positive NRL sIgE by supplementing the investigations with SPT, given that none of the patients without immediate symptoms had a positive SPT. This supports the statement from Griffiths et al. [29] concluding that SPT should be the preferred test when diagnosing NRL allergy. It is therefore important to have standardized NRL SPT extracts available for testing. Unfortunately, the NRL SPT extracts are currently being taken off the market in some countries in Europe.

Limitations

Other studies have included provocation tests to distinguish between sensitized and true allergic patients [19, 27, 30, 31]. We chose not to do provocations in this study due to the lack of consensus on the ideal provocation method. Different provocation models have been investigated for their diagnostic accuracy which differs a lot

from study to study [32–34] due to differences in definitions of allergic and nonallergic patients, differences in provocation protocols and NRL materials/extracts used, and lastly different diagnostic criteria. We included all patients reporting NRL-induced symptoms regardless of the type of symptom, and several provocation models would have been needed to cover the different organs. A glove test might for instance have been insufficient in patients exclusively experiencing mucosal symptoms.

A clear limitation of this study is the relatively few NRL-sensitized patients included. We had a low response rate of just under 40% which might be partly explained by the large number of surveys sent out electronically nowadays. From the literature, we expected the NRL sIgE sensitization to be around 25–30% of included patients as profilin sensitization among grass-allergic individuals is described to be at this level in studies from Austria and Italy [16, 24]. In our study from Denmark, it was only around 9%, indicating a difference in sensitization patterns between countries.

There could be a selection bias, given that patients experiencing symptoms might be more prone to participate in the study compared to asymptomatic patients. This, however, only lowers the risk of missing the relevant patients.

Another limitation is the selection of the study population, a selection based on the grass sIgE levels instead of information of a genuine grass pollen hypersensitivity. Thus, some of the invited patients might have been grass-sensitized but not allergic. Questioning on pollen allergy symptoms as well as SPT with standard inhalation panel were performed to compensate for this limitation.

Conclusion

In conclusion, Hev b 8 mono-sensitization appears to have little or no clinical relevance in pollen-sensitized patients and is instead caused by cross-sensitization from grass profilin (Phl p 12). Several patients were sensitized to CCDs which, unexpectedly, caused quite a few false-positive NRL sIgE tests as well. NRL sIgE measurements should only be done when there is a convincing medical history of immediate symptoms on NRL exposure, and it should always be supplemented by SPT and/or allergen component analysis. The apparent lack of clinical relevance of Hev b 8 suggests that the NRL sIgE test (k82) might benefit from removal of Hev b 8 to avoid false-positive testing. Further studies on larger populations are needed to determine the exact clinical relevance of the cross-reacting molecules Hev b 8 and CCDs.

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Statement of Ethics

The Ethics Committee of the Capital region of Denmark approved the study (Ref. No. H-15015871, approval granted December 23, 2015). Approval was obtained from the Danish Data Protection Agency (journal No. 2012-58-0015). Verbal and written informed consent was obtained from all participants.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The three authors S.L. Brandi, L.K. Poulsen and L.H. Garvey developed the hypothesis and study design in collaboration. S.L. Brandi, under the supervision of L.H. Garvey, wrote the protocol and conducted the clinical trial. All three authors contributed to data analysis and all approved the final paper.

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