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The prevalence of sensitization to lupin flour in France and Belgium: a prospective study in 5,366 patients, by the Allergy Vigilance Network

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KEY WORDS

Lupin flour, sensitization, prevalence, atopy, peanut, cross-reactivity

SUMMARY

Objectives: To determine the prevalence of sensitization to lupin flour in patients consulting allergists, in order to evaluate the risk of primary and secondary allergies to lupin. **Methods:** A prospective study carried out by members of the Allergy Vigilance Network, using prick-tests with a commercial lupin flour extract in patients with various allergic symptoms. The study design classified patients into four groups: peanut allergy, current atopic disease, latent atopy, no atopy. Data were collected and analysed by Network coordinators. **Results:** Over a two-month period, 88 French and Belgian allergists tested 5,366 patients: 2,680 children and 2,686 adults aged over 16 years. Of the 2,680 children, 11.15% presented with peanut allergy. The frequency of cross-reactivity with lupin was 17.1% for patients with peanut allergy, 2.5% for children with current atopic disease and 1.7% for healthy children with latent atopy. In the 2,686 adults, peanut allergy was diagnosed in 1.86% of patients with cross-reactivity to lupin in 14.6%. Sensitization to lupin was detected in 3.7% of patients with current atopic disease and in 1.8% of those with latent atopy. **Conclusion:** The relative frequency of latent sensitisation to lupin in patients of all ages presenting with atopic disease is a new factor indicating the likelihood of an increase in primary food allergies to lupin flour. This justifies the recent decision requiring mandatory labelling of lupin, and shows the need to inform consumers who may be unaware that this ingredient is being used increasingly. Sensitization to lupin should be searched by prick-tests in any case of peanut allergy. Prick-test to lupin may be valuable whenever a food allergy is suspected when no current food allergens have been identified.

Introduction

Food allergy is constantly increasing (1, 2). Changes in life styles, new foods, interaction with environmental agents are revealing genetic predispositions that remained without clinical expression until very recently.

Lupin is a legume. Of 450 different species, three, known as sweet lupin, are regularly used in the food in-

dustry (*Lupinus albus*, *Lupinus Luteus* and *Lupinus angustifolius*). Lupin is rich in proteins (39 to 45%) and essential amino acids (lysine, leucine, threonine) (3). Though salted lupin seeds have been introduced in human foods in Mediterranean countries, the use of lupin flour as an ingredient is more recent: 1996-1997 in France and the United Kingdom. It is an ingredient found in many biscuits, snacks, bread, pizzas, industrial pastry, croissants,

and may represent up to 10% of wheat flour (4). Large consumers of bread and other bakery products consume lupin flour at least once a week. The allergic risk, first identified amongst patients allergic to peanut, is well documented (5-10). Cases of primary food allergy have recently been observed (11, 12). In December 2006, due to the constant increase in the prevalence of lupin allergy, lupin was added to the list of allergens for which mandatory labelling is required in the European Union (13). The aim of this Allergy Vigilance Network study, carried out by 88 allergists in France and Belgium, was to evaluate the prevalence of sensitisation to lupin flour (using the same extract) in different categories of patients presenting with allergic symptoms. The study highlights the relative frequency of sensitization to lupin, particularly in those with current atopic diseases, such as respiratory disease or atopic eczema/dermatitis syndrome (AEDES), and indicates that increased prevalence of allergies to lupin may be detected if they were routinely screened for.

Methods

Study design

Members of the Allergy Vigilance Network were invited to participate in the study (14). They were asked to include all patients who were to undergo skin tests with routine airborne allergens for diagnostic purposes. The patient's informed consent to add two extra prick-tests (lupin flour and peanut) was obtained.

The patient population was divided into four groups, according to the interview and the results of prick-tests with routine airborne allergens.

- *Group I: non-atopic subjects*, with no history of atopic disease (atopic dermatitis, allergic rhinitis, asthma) and whose prick-tests for routine airborne allergens were negative. These non-atopic subjects consulted for adverse reaction to medication, non-allergic rhinitis, nasal and sinus polyposis, non-specified skin reactions or allergy to hymenoptera.
- *Group II: latent atopy*: patients with the same symptoms as above who did not have the atopic diseases mentioned above, but whose skin tests revealed one or more positive prick-tests to airborne allergens.
- *Group III: current atopic disease*: patients with atopic dermatitis, and/or seasonal or perennial allergic rhinitis, and/or allergic asthma, or food allergy other than peanut

allergy, and one or more positive prick-tests to airborne allergens.

- *Group IV: certain IgE-mediated food allergy to peanut*: the diagnosis was established on a clear history of immediate allergic symptoms to peanut, confirmed by positive prick-tests to peanut and /or presence of specific IgEs. In some cases, the diagnosis was established on sensitization to peanut and a positive Oral Challenge.

The allergists received eight tables corresponding to the four categories; children (<15 years) and adults (>15 years) were classified separately. Data recorded included sex, age, size in mm of prick-test wheals for negative and positive controls and for lupin and airborne allergens. Results were centralised and analysed by the Allergy Vigilance Network coordinator.

Material and methods for prick-tests

Allergists who agreed to participate in the study received a commercial extract of lupin flour prepared by the Allerbio laboratory (Varennes en Argonne, France).

The positive control was 9% codeine or 1 mg histamine and a negative control was saline solution. Prick-tests included an acarion, dog and cat epithelia, *Alternaria*, grass pollens, artemisia pollen, birch pollen (Northern France), olive and cypress pollen (Southern France). The extracts tested, chosen by the allergists, were provided by the laboratory Allerbio (Varennes en Argonne, France) or by Stallergènes (Antony, France). Two prick-tests to lupin flour (extract from Allerbio) and peanut (extract from Allerbio or Stallergènes) were added. For peanut a prick-in-prick test to natural roasted peanut was accepted.

The positive criterion for prick-tests to lupin was defined as a wheal diameter 2/3 that of the codeine or histamine control, where the control was equal to or more than 2.5 mm. For infants (< 1 year), the positive value was a wheal diameter equal to that of the positive control.

Results

The study was performed over a two-month period by 88 allergists located throughout France and Belgium (May-June 2006). A total of 5,366 patients were included, 2,680 children aged less than 16 years and 2,686 adults.

Of the 2,680 children, 315 (11.1%) presented with peanut allergy. 434 were sensitized meaning that 72% of sensitized children (positive prick-tests to peanut) were really allergic. The frequency of cross-reactivity with lupin was

Table 1 - Prevalence of sensitization to peanut and lupin, studied by prick-tests in 2,680 children

Groups	n 2680	Positive peanut PT					Positive lupin PT						
		Boys		Girls		Total %	p	Boys		Girls		Total %	p
		n	%	n	%			n	%	n	%		
Non atopic	797	2/408	0.5	5/389	1.3	0.87	} ≤ 0.001	2/408	0.5	0/389	0	0.2	} ≤ 0.05
Latent atopy	173	4/95	4.2	4/78	5.1	4.6		1/95	1	2/78	2.5	1.7	
Atopic disease	1395	84/863	9.7	47/532	8.8	9.4		22/863	2.5	13/532	2.4	2.5	
Peanut allergy	315	193/211	91.5	95/104	91.3	91.4		33/209	15.8	21/101	20.8	17.1	

Table 2 - Prevalence of sensitization to peanut and lupin, studied by prick-tests in 2,686 adults

Groups	n 2686	Positive peanut PT					Positive lupin PT						
		Males		Females		Total %	p	Males		Females		Total %	p
		n	%	n	%			n	%	n	%		
Non atopic	990	2/315	0.6	4/675	0.6	0.6	} ns	2/315	0.6	4/675	0.6	0.6	} ns
Latent atopy	226	1/85	1.2	3/7141	2.1	1.8		0/85	0	4/141	2.8	1.8	
Atopic disease	1422	45/584	7.7	53/838	6.3	6.9		18/584	3.1	35/829	4.2	3.7	
Peanut allergy	48	22/23	95.6	22/25	88	91.6		2/23	8.7	5/25	20	14.6	

17.1%. For 173 patients who had atopy revealed by positive prick-test to inhalants, sensitization to lupin was observed in 1.7% vs 2.5% in 1,395 patients with current atopic disease (ns). The difference of both groups with non-atopic patients, 0.2% of them being sensitized is significant ($p < 0.05$) (table 1).

Amongst the 2,686 adults, 48 patients (1.86%) were diagnosed with peanut allergy, representing 25% of patients with positive prick-tests to peanut. 14.6% were cross-reactive to lupin. Primary sensitization to lupin characterised 3.7% of the 1,422 patients with current atopic disease and 1.8% of the 226 patients with latent atopy ($p < 0.05$) (table 2). However, there are no significant difference between non-atopic patients (0.6%) and patients with latent atopy. The ratio of peanut allergy to simple sensitization, documented by prick-tests, was 0.72 for children and 0.25 for adults.

Discussion

The prevalence of food allergy to lupin in France is estimated from data collected by the CICBAA¹ who record serious, documented food allergy accidents (5). From the 983 reports concerning food allergy, 3.7% concerned lupin. Lupin allergy affects 5.2% of children aged from 1 to 15 years and 1.7% of young adults between 15 to 30 years. It does not affect infants. Clinical signs are severe (15). Between 2002 and 2004, the Allergy Vigilance Network reported 15 cases of severe anaphylaxis with lupin, out of 294 cases of food-related anaphylaxis, i.e. 5.1%. In 2005-2006, nine cases were found, i.e. 4.7% of all notifications. Lupin is ninth in the rank order of "risk" allergens (14, 16). The reactivity threshold is low: 265 to 965 mg for objective symptoms (4, 7, 17). These doses correspond to amounts found in routinely consumed products such as snacks or biscuits containing lupin.

There are three possible forms of clinical allergy to lupin: 1) the most frequent is primary allergy to peanut with cross-reactivity to lupin, b) primary allergy to lupin (10, 17), c) primary allergy by inhalation of lupin pollen or flour (often occupational) (18-20).

Numerous allergens have been identified: glycosylated globulins, alpha (33% protein), beta (45%), gamma (5%) and delta (12%) conglutins, a PR-10 protein, gamma conglutins, a 2S albumin (21-26). There is marked thermo-resistance: it persists after one hour of boiling (27). Moreover, the inter-patient variability in allergenicity of boiled and cooked lupin suggests possible interaction between lupin and matrix factors or food processing techniques. Processing that involves an instantaneous controlled pressure drop at several pressure and time points could reduce lupin allergenicity (28). Cross-reactivity between peanut and lupin has been described both *in vitro* and *ex vivo*: strong homology between PR-10 and Ara h 8, the precursor of beta conglutin and Ara h 1, homology between other lupin proteins and Ara h 3 explain, cross-allergenicity between lupin pollen and peanut (4, 17, 24, 26). *In vivo*, cross-allergenicity between legumes and peanut is rare, (29) but peanut-lupin cross reactivity, if screened for routinely, concerns 11% of patients with peanut allergy (CICBAA data).

In this large population (5,366 patients), there is a predominance of young males with current atopic disease (61.8%) and with peanut allergy (67%). This fact is well-known (30). However, for adults in the current atopic disease group, there was a marked female predominance (62.4%), whilst for peanut allergy there was only slight predominance (52%). The frequency of peanut allergy was 11.2 % for children and 1.8% for adults. In the current atopic disease and latent atopy groups, the frequency of peanut sensitisation in both children (9.4% and 4.6% respectively) and adults (6.9% and 1.8% respectively) was as expected from an earlier study using the same methods (31). The comparison of peanut allergy with simple non-allergic sensitization, as detected by prick-tests, indicated a very different ratio between children and adults. 72% of sensitized children were allergic, but only 25% of sensitised adults. It may be postulated that tolerance mechanisms are more effective in adults.

17% of children and 14.5% of adults allergic to peanut showed cross sensitisation to lupin. This cross sensitization could be 34% in another study (32). It was rare in patients with latent atopy but no current atopic disease: 1.7% and 1.8% respectively. It nearly doubled in patients presenting with current atopic disease (allergic asthma, al-

lergic rhinitis, atopic dermatitis): 2.5% and 3.7% respectively. The origin of sensitization remains unknown: either routine ingestion, or primary inhalation of lupin pollen followed by cross sensitisation to lupin seeds. Cross reactivity between pollen and seed has been demonstrated *in vitro* (4). A third possibility is occupational sensitisation due to inhalation of lupin flour (18-20).

This study documents the relative frequency of latent sensitisation to lupin flour, and confirms the findings of an earlier French study in 323 atopic children, that revealed a sensitisation rate of 8%, 75% of whom presented cross sensitization to peanut and 25% isolated, primary sensitisation (33). Such data support the notion that there will probably be more primary allergy to lupin in the near future in Europe, and that lupin should be included in the airborne allergens causing asthma in children (34). This is all the more worrying that lupin flour is used increasingly in packet soups, pasta and even as a clarifying agent for wine (35). It is a common replacement for wheat flour for patients with celiac disease (36). Lupin is often present as a hidden allergen in processed foods (5, 8, 9). Screening for lupin sensitization can be carried out by CapSystem Phadia and by skin-test with the commercial extract. These diagnostic tools are not extensively used as yet. This screening could be offered routinely to patients with peanut allergy and should be performed where food allergy is suspected, but not detected to usual foods. In this sample of more than 5,000 patients, only one had known allergy to lupin. The ratio sensitization/allergy will have to be evaluated in further studies including DBPCFC (4, 32, 37). This risk may be elevated since it was evaluated in 2/9 English children or 1/10 Norwegian children challenged with lupin flour (32, 37). Information on lupin allergy and on the fact that it is an ingredient masked in other foods should be circulated more widely to the general public. Mandatory labelling, based on the analysis of published cases of allergy and anaphylaxis, is all the more justified by the finding that up to 3.7% of patients with atopic disease may also be latently sensitized to lupin.

Allergists who participated in the study:

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