

## Sensitization to inhalant allergens in patients with allergic airway disease in Slovenia

Senzibilizacija z inhalatornimi alergeni pri bolnikih s simptomi alergijske bolezni dihalnih poti v Sloveniji

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### Izveček

**Izhodišča:** Kožni vbodni testi so osnovna diagnostična metoda za z IgE posredovane alergijske bolezni. Alergološko testiranje praviloma začnemo s t. i. standardno serijo, ki naj bi vsebovala najbolj pogoste alergene, ki bi bili pri sumu na določeno alergijsko bolezen lahko pomembni. Priporočila za izbiro alergenov za standardno serijo so v literaturi zelo splošna. Poznati moramo pogostost senzibilizacije za različne alergene v lokalnem okolju. Za Slovenijo do sedaj nismo imeli podatkov o pogostosti senzibilizacije za inhalatorne alergene; izbira alergenov je tako temeljila predvsem na osebnih izkušnjah in podatkih iz drugih evropskih držav. Z raziskavo smo želeli opredeliti pogostost senzibilizacije za alergene, ki so t. i. panevropska standardna serija kožnih testov, določiti minimalno serijo alergenov, ki je primerna za epidemiološke študije, in opredeliti pogostost senzibilizacije s temi alergeni v Sloveniji. Želeli smo podati tudi naše mnenje o testiranju z navzkrižnimi alergeni in alergenskimi mešanici.

**Metode:** Pogostost senzibilizacije za panevropsko standardno serijo smo opredelili na terciarnem alergološkem oddelku. Vključili smo bolnike, ki so bili napoteni zaradi suma na alergijsko bolezen dihalnih poti. Do uporabe navzkrižnih alergenov in alergenskih mešanec smo se opredelili tako, da smo hkrati testirali iste bolnike z mešanico alergenov in posameznimi alergeni, ki jih mešanica vsebuje. Na podlagi podatkov smo nato določili minimalno serijo alergenov za epi-

demiološke teste. Pogostost senzibilizacije smo nato opredelili v 13 centrih v Sloveniji. Vključeni so bili bolniki, pri katerih so izbrani zdravniki posumili na alergijsko bolezen dihalnih poti.

**Rezultati:** Pri vseh alergeni iz panevropske standardne serije inhalatornih alergenov za kožne teste je bila pogostost senzibilizacije večja od 2 %. V Sloveniji je torej smiselna uporaba vseh alergenov iz panevropske serije. Glede na rezultate testiranja navzkrižnih alergenov in mešanec alergenov bi v slovensko standardno serijo morali dodati še tri alergene; to so: *Plantago*, *Rumex* in *Urtica*. Glede na te podatke smo določili minimalno serijo alergenov za epidemiološke teste. S serijo osmih alergenov smo diagnosticirali senzibilizacijo pri 95,7 % bolnikov. S to serijo smo testirali osebe v 13 centrih v Sloveniji. Vsaj en kožni test je bil pozitiven pri 63 % oseb.

**Zaključek:** Slovenska standardna serija inhalacijskih alergenov za kožne teste naj vsebuje 19 alergenov. Ti so: *Alternaria*, *Ambrosia*, *Artemisia*, *Aspergillus*, *Betulacea*, ščurek, mačka, cipresa, *Cladosporium*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, pes, oljka, *Parietaria*, *Penicillium*, platana, *Plantago*, *Rumex*, mešanica trav in *Urtica*. Potrebne so nadaljnje študije za potrditev pomembnosti dodanih alergenov, ki jih panevropska serija ne vsebuje, in za opredelitev drugih alergenov, ki so lahko lokalno pomembni. Za epidemiološke raziskave senzibilizacije z inhalatornimi alergeni v Sloveniji naj minimalna serija vsebuje 8 alergenov. Ti so: *Alternaria*, *Artemisia*, breza, *Dermatophagoides*

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*farinae*, *Dermatophagoides pteronyssinus*, mačka, oljka in mešanica trav.

## Abstract

**Background:** Skin prick testing is the basic diagnostic method for IgE-mediated allergies. To define a standard battery of allergens, data about local sensitization pattern are needed. The aims of the study were to define the prevalence of sensitization to allergens recommended as Pan European standard prick test panel in Slovenija, to define a minimum battery of allergens for epidemiological studies and to assess sensitization to those allergens across the country. Another aim was to define the usefulness of testing with extensively cross-reactive allergens and the use of allergen mixtures.

**Methods:** The prevalence of sensitization for Pan-European standard prick test panel was assessed at single referral allergy centre, where the same patients were also tested with cross reactive allergens and allergen mixtures. A minimum battery for epidemiological study was defined

from this data. The prevalence of sensitization to those allergens was then assessed in 13 centers in Slovenija.

**Results:** All allergens suggested as Pan-European standard prick test panel showed a prevalence of more than 2 %, so all of them should be used in clinical setting. Additional three allergens are needed for Slovene standard skin prick test panel. Eight allergens are needed for a minimum battery of allergens needed in epidemiological studies.

**Conclusion:** Slovene standard panel for skin prick tests should include: *Alternaria*, *Ambrosia*, *Artemisia*, *Aspergillus*, *Betulacea*, *Blatella*, *Cat*, *Cladosporium*, *Cypres*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Dog*, *Grass*, *Olive*, *Parietaria*, *Penicillium*, *Plane*, *Plantago*, *Rumex*, *Urtica*.. Further studies are needed to confirm the importance of added allergens and to define other allergens that might be locally important.

## Introduction

Skin prick tests are most commonly used method to detect sensitization in patients with allergic airway disease.<sup>1</sup> Confirmed sensitization is crucial for correct diagnosis and treatment of allergic patients. Guidelines on skin prick testing offers only general recommendations about allergen selection.<sup>2</sup> Data on local sensitization pattern are needed to make a reliable conclusion about standard battery of tests used in most patients in clinical setting and for epidemiological studies. For individual patient care all allergens that show a frequency of sensitization of more than 2 % are regarded as eligible for standard batteries.<sup>3</sup> By using only the relevant allergens it is possible to reduce the costs. Many allergens show extensive cross-reactivity. The use of one locally prevalent allergen is considered adequate for a standard battery.<sup>4</sup> Mixtures of allergens from similar sources, like Moulds or Weeds mix, which are not extensively cross-reactive, are also commercially available and used in some centers.<sup>5</sup> Epidemiological population-based and patient-based data about sensitization pattern exists for many European

countries, but Slovenia has so far not been included in those studies.<sup>6-9</sup>

The aim of the study was to define the prevalence of sensitization to inhalant allergens proposed as Pan-European standard prick test panel.<sup>5</sup> Next aim was to assess whether the use of marker allergen form cross reacting allergens and the use of allergen mixtures are appropriate. Another aim was to define a minimum battery of allergens for Slovenia for epidemiologic studies and to obtain data about the prevalence of allergic sensitization to those allergens in patients suspected to have allergic airway disease across the country.

## Methods

### Patients

The first part of the study was done at a single allergy referral center. All patients referred for allergy testing were screened for symptoms of allergic airway disease. In this part of the study the aim was to determine which allergens should be tested in Slovenia in clinical setting for patients with suspected allergy to inhalant allergens and to define a minimum battery of inhalant allergens for

epidemiological studies. Patients referred for allergy tests at the University Hospital for Pulmonary and Allergic diseases Golnik were screened for 6 months. All patients completed a questionnaire about signs and symptoms compatible with allergic rhinoconjunctivitis and/or asthma. The patient questionnaire was anonymous and was distributed to patients during the clinical consultation. Patients were asked about nasal symptoms: sneezing, itching, watery rhinorrhea and nasal obstruction. Questions about eye symptoms included: itching and eye redness. Patients were also asked about symptoms of lower airways: dyspnea, prolonged cough (more than 6–8 weeks), wheezing and limitation at exercise. Only patients with symptoms of rhinoconjunctivitis and/or asthma were included in the analysis.

To analyze the usefulness of allergen mixtures, patients were tested at the same time with allergen mixture and individual allergens. Mixture of allergens in the commercial extract and the allergens contained in that mixture were tested on the same day.

**Table 1:** Prevalence of sensitization in patients with allergic airway disease at a single center in Central Slovenia

	Prevalence n (%)
<i>Dermatophagoides pteronyssinus</i>	75 (32.6 %)
<i>Dermatophagoides farinae</i>	73 (31.7 %)
Grass pollen	56 (24.3 %)
<i>Betulacea</i>	48 (20.9 %)
Olive pollen	36 (15.6 %)
Cat	35 (15.2 %)
<i>Fagacea</i>	28 (12.2 %)
Weeds mix	20 (8.7 %)
<i>Blatella</i>	19 (8.3 %)
Dog	14 (6.1 %)
<i>Ambrosia</i>	10 (4.3 %)
Moulds mix	9 (3.9 %)
<i>Parietaria</i>	8 (3.5 %)
Cypress	7 (3.0 %)
Plane pollen	6 (2.6 %)

The second part of the study was multicenter, 13 study centres were included. Patients were tested in the period of two months. Patients in whom general practitioners suspected rhinoconjunctivitis were invited in the study. They were asked about their nasal/eye symptom and symptoms of lower airways disease. Only patients with symptoms compatible with allergic airway disease were included in the analysis. Sensitization prevalence was calculated.

Patients gave their informed consent; the study was approved by the national ethic committee.

### Skin tests

Inhalant allergens were tested. The first step of the study was done at a single referral center. The aim was to define the prevalence of sensitization for all allergens recommended as European battery of inhalant allergens and to define a minimum battery of allergens that identify most sensitized patients. The following commercial extracts were used: *Ambrosia*, *Betulacea*, *Blatella*, Cat, Cypress pollen, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, Dog, *Fagacea*, Grass mix pollen, Moulds Mix, Olive pollen, *Parietaria*, Plane pollen and Weeds mix. *Betulacea* contained: hazel, birch and alder pollen. *Fagacea* contained: beech, chestnut and oak pollen. Moulds mix contained: *Aspergillus fumigatus*, *Penicillium notatum*, *Alternaria alternata*, *Mucor mucedo*, *Cladosporium cladosporioides*. Weeds mix contained: *Urtica dioica*, *Plantago lanceolata*, *Artemisia vulgaris*, *Rumex actosella*.

The second part of the study was done in 13 centers. The aim was to assess the prevalence of sensitization to a minimum battery of inhalant allergens in patients with allergic airway disease in Slovenia. The following commercial extracts were chosen on the basis of results from the first step of the study: *Alternaria alternata*, *Artemisia vulgaris*, Birch pollen, Cat, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, Grass pollen mix, Olive pollen.

Positive control was performed with histamine and negative control with saline. Results of skin prick tests were recorded

after 15 minutes. The diameter of the wheal was measured. Subjects with positive control of less than 3 mm and/or negative control of more than 2 mm were excluded from further analysis. The test was considered positive if the diameter of allergen induced wheal was more than half of histamine-induced wheal. Subjects with at least one positive skin prick test were considered as sensitized. Allergen extracts were purchased from companies Stallergens (Anthony, France) or HAL (Leiden, The Netherlands).

### Statistical analysis

In the first step, we wanted to define the number and the type of skin prick test allergens required to identify a subject as sensitized for clinical setting. The prevalence of sensitization for all allergens recommended as the European battery of inhalant allergens was calculated. To define the number of allergens needed for an epidemiological step-by-step conditional approach was used as previously described.<sup>3</sup> Results of testing from a single referral allergy center were

used. Allergens were classified from the one that gives the highest increase in prevalence of sensitization to the one that gives the lowest. The procedure started by defining the most prevalent allergen. Then, to identify the next allergen that gives the highest increase in sensitization prevalence, the allergen that gives the highest prevalence of sensitization was looked for in the subgroup of subjects not sensitized for the previous allergen. The procedure was repeated until none of the resulting allergens induced a change in prevalence. To define the usefulness of mixtures of cross-reactive allergens, results of tests with a mixture of allergens were compared with results of tests for different allergens contained in the mixture.

In the second step, the prevalence of sensitization for the minimum battery of allergens needed in epidemiological study was calculated from the results of test from 13 centers in Slovenia.

The analysis was performed using SPSS 16 for Windows®.

**Table 2:** Prevalence of sensitization, allergen ranked from the most prevalent to the allergen with the least increase in identifying additional sensitized subjects

	Prevalence n (%)	Increase in identifying sensitized patients by adding allergen in prick test	
		n	%
<i>Dermatophagoides pteronyssinus</i>	75 (27.9 %)		
Grass pollen	98(36.4 %)	23	8.6 %
<i>Betulacea</i>	111 (41.3 %)	13	4.8 %
Olive pollen	119 (44.2 %)	7	2.6 %
Cat	125 (46.5 %)	6	2.2 %
<i>Dermatophagoides farinae</i>	130 (48.3 %)	5	1.9 %
Weeds mix	133( 49.4 %)	3	1.1 %
Moulds mix	135 (50.2 %)	2	0.7 %
Blatella	137 (50.9 %)	2	0.7 %
Cypress	139 (51.7 %)	2	0.7 %
Parietaria	140 (52.0 %)	1	0.4 %
Plane pollen	141 (52.4 %)	1	0.4 %

*Ambrosia, Fagacea, Dog did not increase the number of identified sensitized subjects. Cells in gray: More than 95 % of sensitized subjects are identified.*

## Results

### Slovene battery of inhalant allergens for clinical setting

In the first part of the study, which was done at a single referral allergy center, a total of 370 subjects were included. Out of these, 48 (13 %) subjects were excluded because tests were not adequate or the answers to the questionnaire were incomplete. 322 set of tests were further analyzed. 230 (71.4 %) patients had symptoms compatible with rhinoconjunctivitis. 131 (57 %) patients had at least one positive test and were considered as subjects with allergic rhinoconjunctivitis. Another 39 subjects who did not have symptoms of rhinoconjunctivitis but claimed symptoms of lower airways were analyzed. 10 of them were positive to at least one allergy test and were considered as patients with allergic asthma. There were altogether 269 patients with symptoms compatible with allergic airway disease; 141 (52.4 %) of these patients had at least one positive allergy test with inhalant allergen, comprising the group of patients with confirmed allergic airway disease, involving 57 (40.4 %) men. Their average age was 31.5 (22–43) years. The most prevalent allergens in patients with allergic airway disease were *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass pollen, *Betulacea*, olive pollen and cat (Table 1).

**Table 3:** Prevalence of sensitization to Moulds in 129 tested patients

Moulds	Prevalence n (%)
<i>Alternaria</i>	6 (4.7)
<i>Cladosporium</i>	4 (3.1)
<i>Aspergillus</i>	3 (2.3)
<i>Penicillium</i>	3 (2.3)
<i>Mucor</i>	2 (1.6)

### Minimum battery of skin tests needed in epidemiological studies for Slovenia

Eight out of 15 allergens were needed to identify 95.7 % of allergic patients (Table 2). The prevalence of sensitization ranged from 27.9 %, when only most prevalent allergen *Dermatophagoides pteronyssinus* was tested, to 52.4 %, when all 15 allergens were tested. None of the patients allergic to *Ambrosia*, *Fagacea* or Dog were monosensitized.

### Cross-reacting allergens and allergen mixtures

Separately we analyzed allergy to cross reactive allergens and the usefulness of allergen mixtures. Most patients positive to *Dermatophagoides farinae* were also positive to *Dermatophagoides pteronyssinus*, but 9 (3.3 %) patients were only sensitized to *Dermatophagoides farinae* and not to *Dermatophagoides pteronyssinus*. On the other hand, 11 (4.1 %) patients were only sensitized to *Dermatophagoides pteronyssinus* and not to *Dermatophagoides farinae*. Most patients positive to *Fagacea* were also positive to *Betulacea*, except for 3 (1.1 %) patients who were exclusively sensitive to *Fagacea*. 129 patients were tested with moulds mix and moulds separately; 11 (8.5 %) were only positive with separate allergen extracts and not with the allergen mixture. Among the tested Moulds *Alternaria* was the most prevalent (Table 3). 72 patients were tested with Weeds mix and weeds separately; 6 (8.3 %) were positive only with separate allergen extracts and not with allergen mixture. *Artemisia* was the most prevalent (Table 4). 19 patients were tested with *Betulacea* compared with

**Table 4:** Prevalence of sensitization to Weeds in 72 tested patients

Weeds	Prevalence n (%)
<i>Artemisia</i>	8 (11.1)
<i>Plantago</i>	6 (8.3 %)
<i>Urtica</i>	3 (4.2 %)
<i>Rumex</i>	2 (2.8 %)

**Table 5:** Prevalence of sensitization in Slovenia

	Prevalence n (%)
Allergen	All (326)
Birch	175 (54 %)
Grass	168 (51 %)
<i>Dermatophagoides pteronyssinus</i>	141(43 %)
<i>Dermatophagoides farinae</i>	138 (42 %)
Olive	86 (26 %)
Cat	75 (23 %)
<i>Artemisia</i>	51 (16 %)
<i>Alternaria</i>	47 (14 %)

Birch, Hazel and Adler. None were positive to separate allergen extracts only.

### Sensitization in Slovenia

In the multicenter part of the study a total of 571 subjects were included. There were 239 (42 %) men. Their average age was 33 (2–89) years. 37 data sets were excluded because allergy tests were not adequate or the answers to the questionnaire were incomplete. 18 subjects did not have symptoms of rhinoconjunctivitis and/or asthma and were also excluded from further analysis.

516 subjects claimed symptoms of rhinoconjunctivitis and/or asthma, 326 (63 %) of them were sensitized to at least one allergen and those patients were characterized as patients with allergic airway disease and were

**Table 6**

Number of positive tests	All (516) n (%)
0	190 (37 %)
1	90 (17 %)
2	73 (14 %)
3	68 (13 %)
4	42 (8 %)
5	34 (7 %)
6	13 (3 %)
7	5 (1 %)
8	1 (0 %)

further analyzed. The prevalence of sensitization is shown in Table 5.

Most tested subjects 90 (17 %) were sensitized for one allergen, but one subject was sensitized for all 8 tested allergens (Table 6).

### Discussion

With this study we first defined allergens necessary for testing patients with suspected allergy to inhalant allergens in clinical setting in Slovenia. The allergen list was composed in accordance with GA<sup>2</sup>LEN Pan-European skin prick test study.<sup>5</sup> To decrease the number of extracts needed for skin testing, mixtures or only one marker allergen from cross reacting allergens were used. All tested allergens had prevalence of sensitization higher than 2 %, so all of them should be used in clinical setting in Slovenia. We also defined a minimum battery of inhalant allergens needed in epidemiological studies for Slovenia. Eight allergens were necessary to identify 95.7 % of all sensitized patients. This is comparable with overall results from the Pan-European multicenter study where eight allergens also allowed identification of 95 % of sensitized subjects, but differences between countries were considerable, ranging from 2 up to 13 allergens. Those eight allergens for Slovenia were *Dermatophagoides pteronyssinus*, Grass pollen, *Betulacea*, Olive pollen, Cat, *Dermatophagoides farinae*, Weeds mix and Moulds mix. Similar allergens as in the Pan European study were identified, except for *Blatella*, which is not a part of minimum battery for Slovenia. Both *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are needed to correctly diagnose allergic patients. The usefulness of mixtures was evaluated by separate testing to compare the efficacy of mixtures with single allergen extracts. We used mixtures of extensively cross-reactive allergens, such as *Betulacea*, and compared the results in the same patients with allergens contained in the mixture. All subjects tested with *Betulacea* were also positive to birch and none of the subjects were positive exclusively to hazel or alder, so birch was chosen for the minimum battery for epidemiological studies. The use of *Fagacea* did not seem necessary

as most allergic subjects were already diagnosed with *Betulacea*. We also compared mixtures of related allergens and allergens contained in that mixture. Moulds mixture failed to diagnose correctly 8.5 % of mould positive patients. Also Weeds mix missed 8.3 % of Weeds positive subjects. On the basis of the prevalence of sensitization to allergens contained in the mixture, we chose *Artemisia* from the Weeds mix and *Alternaria* from the Moulds mix for the minimum battery needed for epidemiological studies. In general, we can conclude that mixtures of cross-reacting allergens, such as *Betulacea*, can be used in clinical setting and epidemiological studies, but the use of mixtures of related but not extensively cross-reactive allergens, such as Moulds or Weeds mix, is not appropriate. Considering that allergens that have prevalence of sensitization higher than 2 % should be used in clinical setting, allergens from Weed mix and Mould Mix should be added to the standard battery of inhalant allergens in Slovenia. The prevalence of sensitization was higher than 2 % for *Plantago*, *Urtica*, *Rumex*, *Cladosporium*, *Aspergillus* and *Penicillium*.

With the minimum battery of inhalant allergens for epidemiological studies in Slovenia, defined in the first part of the study, sensitization prevalence in 13 centers was assessed in subjects with allergic airway disease. 63 % of tested subjects were positive to at least one allergen which is comparable to a similar patient-based European study where 68.2 % of patients involved were sensitized to at least one allergen.<sup>3</sup> Most prevalent allergen was Birch pollen followed by *Dermatophagoides pteronyssinus*, grass pollen and cat. Sensitization to Olive pollen most probably reflects important sensitization to Ash pollen, since Olive pollen is not normally present in most of Slovenia.<sup>10</sup>

Until now, allergens for skin prick testing in Slovenia could only be chosen on the basis of personal experience and data from other European countries. Sensitization to inhalant allergens in Europe has been investigated in several studies that included neighbouring countries such as Italy, Austria and Hungary.<sup>10</sup> This is the first study to identify the sensitization pattern in Slove-

nia. The study was patient based. To identify a minimum battery of skin tests for Slovenia, a high enough number of tests was performed to allow reliable conclusions.

To correctly diagnose all allergic patients, it is very important to include all locally prevalent allergens in the usual panel for clinical work. It is also important to include only important allergens to minimize the costs. Sensitization and exposure to inhalant allergens causes predominantly allergic rhinoconjunctivitis. Only allergens that are small enough to penetrate into lungs also cause allergic asthma. Standardized local inhalant allergen panel should help generate more relevant data for clinical practice and clinical research. A minimum battery of inhalant allergens for epidemiological studies in Slovenia should include *Dermatophagoides pteronyssinus*, Grass pollen, *Betulacea*, Olive pollen, Cat, *Dermatophagoides farinae*, *Artemisia* and *Alternaria*. For clinical setting, other allergens should be used to also detect sensitizations that are not prevalent but are important for individual patient. It is appropriate to add *Ambrosia*, *Aspergillus*, *Blattella*, *Cladosporium*, Cypress, Dog, *Parietaria*, *Penicillium*, Plane pollen, *Plantago*, *Rumex*, *Urtica*. Additional suspected candidate allergens, depending on the patient's history, should be added.<sup>11</sup> For example, in patients with other pets, besides cats and dogs, those allergens should be tested. The list of allergens tested should be updated regularly with new data from literature. Recently, Permaul et al. showed that there is a substantial exposure to mouse allergens in schools and homes.<sup>12</sup> Further larger studies should be done to confirm importance of these allergens and to define other allergens that might be locally important. More data about regional differences in sensitization pattern are also needed to properly address influences related to geographical and vegetation differences in Slovenia.

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