

IgE reactivity patterns in patients with allergic rhinoconjunctivitis to ragweed and mugwort pollens

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ABSTRACT

Background: Differential diagnosis between ragweed and mugwort pollen allergy represents a large clinical problem in areas where both plants are present. The aim of this study was to investigate ragweed- and mugwort-sensitized patients to identify specific IgE reactivity profiles. Results were correlated to clinical findings such as medical history and health-related quality of life (HRQL).

Methods: Seventy-four patients with allergic rhinoconjunctivitis between July and October were examined and underwent *in vivo* tests (skin-prick test [SPT] and nasal provocation). Sera were evaluated for IgE reactivity to mugwort and ragweed pollen extracts, major (Art v 1; Amb a 1) and minor (profilin and calcium-binding protein) allergens. HRQL was evaluated using a standardized questionnaire.

Results: Seventy-one patients revealed positive SPT reactivity against mugwort and 60 patients against ragweed extracts. Of these patients, 74 revealed IgE antibodies against mugwort extracts, whereas anti-Art v 1 antibodies were detectable in 50 individuals. Fifty-five patients showed IgE antibodies against natural ragweed extracts; anti-Amb v 1 antibodies were detected in six cases only. Using standardized clinical history and HRQL questionnaires we were not able to detect any differences within different reactivity patterns.

Conclusion: Within the investigated population of 74 weed-allergic patients the prevalence of true mugwort and ragweed sensitization can be calculated as 68 and 8%. High prevalence of ragweed sensitization when testing with full extracts can be explained by cross-reactivity between other weeds, e.g., mugwort rather than cosensitization. Differences in medical history and HRQL between different reactivity patterns were not detectable.

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Pollens from ragweed (*Ambrosia artemisiifolia*) and mugwort (*Artemisia vulgaris*) are abundantly distributed across northern America and Europe. Both plants are taxonomically related to the family of Asteraceae (Compositae), which comprise ~20,000 different species being considered one of the main causes of pollinosis in late summer and autumn.¹

Common ragweed probably has been imported from northern America and first appeared in Europe at the beginning of the 20th century.² Geographical distributions of ragweed are mainly localized in Eastern Europe and parts of France but it is also found in south Germany, eastern Austria, and northern Italy.³ In northern America prevalence of sensitization is ~50% in atopic individuals, hence being one of the most common elicitors of type I allergy.⁴ In central Europe, much lower rates of ragweed sensitization have been reported.⁵ However, prevalence of sensitization are apparently increasing.⁶ Despite a relatively rare distribution of ragweed in south Germany, investigations showed a high prevalence of positive *in vitro* tests under the use of full ragweed extracts.⁷ Approximately 90% of ragweed-sensitized patients reveal IgE antibodies against the major allergen Amb a 1, which consists of a single-chain protein having a molecular weight of ~38 kDa.⁸ Beside the major allergen, several studies reported that 20–35% of the ragweed-sensitized patients displayed IgE antibodies against the panallergens profilin (Amb a 8) and calcium-binding proteins (Amb a 9 and Amb a 10).²

One possible explanation for the high prevalence of ragweed-sensitized patients may be a high sensitization rate against more frequently occurring pollens from taxonomically related anemophilous weeds such as mugwort. Mugwort is widely spread throughout the European temperate climate zone, North America, and parts of Asia and it is one of the main causes of hay fever in late summer and autumn. In Germany, a prevalence of 15–20% for mugwort pollen

allergies has been reported, hence presenting the third most common cause of sensitization to pollen.⁷ Mugwort pollen contains the major allergen Art v 1, which is recognized by 79–95% of mugwort-allergic patients.^{9,10} This 28-kDa glycoprotein has an unusual modular structure, being composed of a cysteine-rich globular domain and a hydroxyproline-rich tail containing uncommon carbohydrate structures.¹¹ In addition to the major allergen, mugwort has been shown to contain the panallergens profilin (Art v 4) and calcium-binding protein (Art v 5).²

Because ragweed and mugwort belong to the same botanical family and show nearly identical flowering periods from July until October, a cosensitization against both species results in a relevant problem of diagnosis and therapy. Furthermore, previously reported investigations revealed divergent results concerning a possible cross-reactivity between the major allergens of ragweed and mugwort.^{12,13} To date, diagnosis of type 1 allergies is based on *in vivo* and *in vitro* tests, which are routinely conducted under the use of allergen extracts. Extract-based diagnosis allows the identification of specific allergenic sources but is not suitable to discriminate between specific allergens. Moreover, polysensitization against abundantly distributed panallergens, e.g., profilin and calcium-binding proteins, might be misinterpreted as cosensitization against different allergens.¹⁴

Therefore, the aim of this study was to investigate ragweed- and mugwort-sensitized patients to identify specific IgE reactivity profiles and patterns. Furthermore, patients were regarded in terms of IgE co- and cross-reactivity to ragweed and mugwort major and minor allergens.

Patients suffering from IgE-mediated allergies of the airways mainly show symptoms such as allergic rhinoconjunctivitis, allergic asthma, and oral allergy syndrome (OAS). Because allergic symptoms have negative impact on physical, psychological, and social aspects of daily life, health-related quality of life (HRQL) gained an increasing importance in clinical assessment over the last years.^{15,16} Therefore, results were correlated to clinical findings such as medical history and HRQL.

PATIENTS AND METHODS

Ragweed- and Mugwort-Allergic Patients

Between June 2008 and July 2010 we examined 2367 consecutive patients with complaints of allergic rhinoconjunctivitis. Of these pa-

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Table 1 Sensitization patterns of study patients in SPT with pollen extracts

Characteristics	Amb+/Art- n = 3	Amb-/Art+ n = 14	Amb+/Art+ n = 57
Gender			
Male	0 (0%)	8 (57%)	26 (46%)
Female	3 (100%)	6 (43%)	31 (54%)
Age (yr)	36 ± 18	28 ± 10	40 ± 16
Cosensitization against			
Grass	3 (100%)	6 (43%)	47 (82%)
Trees	3 (100%)	8 (57%)	44 (77%)
Domestic mites	3 (100%)	9 (64%)	41 (71%)
Fungi	2 (76%)	3 (21%)	41 (71%)
Cat	3 (100%)	5 (35%)	31 (54%)
Dog	3 (100%)	6 (43%)	31 (54%)
Horse	2 (76%)	2 (14%)	7 (12%)
Stinging nettle	3 (100%)	6 (43%)	42 (74%)

Values are number of patients total and percent of each evaluated subgroup.
SPT = skin-prick test.

tients, 74 complained about symptoms between July and the beginning of October. All patients underwent routine *in vivo* tests such as skin-prick test (SPT) and/or allergen-specific nasal provocation in accordance with the current guidelines.¹⁷ Serum was evaluated in regard to total IgE and allergen-specific IgE using *in vitro* tests. Patients were included into the study if medical history and SPT/nasal provocation revealed allergy against ragweed and/or mugwort pollen. The study was performed in accordance with the Declaration of Helsinki and the study protocol was approved by the local ethics committee. All patients provided written informed consent.

Allergens

IgE reactivity to purified natural allergen extracts and natural allergen components was investigated using a UniCAP 100 system (Phadia, Freiburg, Germany) according to the instructions of the manufacturer. Ragweed and mugwort pollen extracts and purified natural allergen components were purchased from Phadia. Allergen-specific IgE against the following allergens was measured: (a) natural pollen extracts: ragweed (w1), mugwort (w6), birch (t3), hazel (t4), alder (t2), timothy grass (g6), rye (g12), domestic mites (hx2), fungi (mx1); (b) allergen components: ragweed Amb a 1 (w230), mugwort Art v 1 (w231), profilin (g212), and calcium-binding protein (g210). The results are expressed in kilounits of antibody per liter. Values <0.35 kUa/L were regarded as a negative result. All sera were tested at the allergy laboratory of the Ear, Nose, and Throat Department (University of Munich) using standardized methods.

Medical History and HRQL Questionnaire

The objective of the present study was to compare medical history and HRQL in patients allergic to mugwort and ragweed pollen with different IgE reactivity profiles in SPT, radioallergosorbent test (RAST), and major (Art v 1/Amb a 1) or minor allergens (profilin and calcium-binding protein). A standardized clinical history questionnaire included seasonal complaints, demographic data, living environment, OAS, and medical history. For the evaluation of patient's HRQL we used a slightly shortened and translated questionnaire as developed by Juniper.¹⁸ The self-translated questionnaire was not validated in the converted form. A symptom score of 0 (not troubled) to 3 (extremely troubled) or 0 (never) to 3 (always) was used. The HRQL questionnaire was answered by the patients during the allergy season and without any influence of antiallergic medication. We performed subgroup analysis and compared polysensitized versus monosensitized.

Statistical Analysis

OriginPro 7.5 software (OriginLab Corp., Northampton, MA) was used for the statistical evaluation and graphical presentation of the data. The paired *t*-test was used for the comparison in the individual group and ANOVA for the comparison of the different groups. A value of $p \leq 0.05$ was judged significant.

RESULTS

SPT and Nasal Provocation

Of 2367 investigated patients, 74 fulfilled the criteria and were included into the study. Clinical characteristics and sensitizations patterns of the patients are depicted in Table 1. We investigated three sensitivity patterns regarding SPT and nasal provocation: Amb+/Art-, Amb-/Art+, and Amb+/Art+. Within these subgroups, ragweed and mugwort sensitivity were strongly associated because only three patients (4%) showed reactivity against ragweed but not against mugwort. In contrast, 14 patients (19%) were sensitized against mugwort without showing reactivity toward ragweed pollen extracts. In 57 patients (77%) reactivity against both ragweed and mugwort pollens were found.

In Vitro Tests

Amb+/Art- patients showed IgE antibodies against natural ragweed and mugwort pollen extract in all cases (Fig. 1). The mean value obtained for ragweed was 7.27 kUa/L and for mugwort was 2.96 kUa/L. However, testing with natural ragweed and mugwort major antigens, no patient revealed anti-Amb a 1 IgE (mean value, 0.13 kUa/L), whereas two patients showed anti-Art v 1 antibodies (mean value, 0.59 kUa/L). Anti-profilin and anti-calcium-binding protein IgE was found in two cases and thus can explain positive reactions against ragweed extracts.

In Amb-/Art+ individuals IgE antibodies directed toward natural mugwort extracts were found in all cases showing a mean value of 2.25 kUa/L. Nine patients (64%) also revealed anti-Art v 1 antibodies (mean value, 2.14 kUa/L). Despite negative SPT, six patients (43%) showed antibodies against natural ragweed extract (mean value, 0.58 kUa/L). However, anti-Amb v 1 antibodies were not found in any of these patients, whereas antibodies against the minor allergen calcium-binding protein were found in two cases (14%).

Patients with a positive SPT against mugwort and ragweed (Amb+/Art+) showed IgE antibodies against natural mugwort extracts in all and against Art v 1 in 39 cases (68%), showing mean

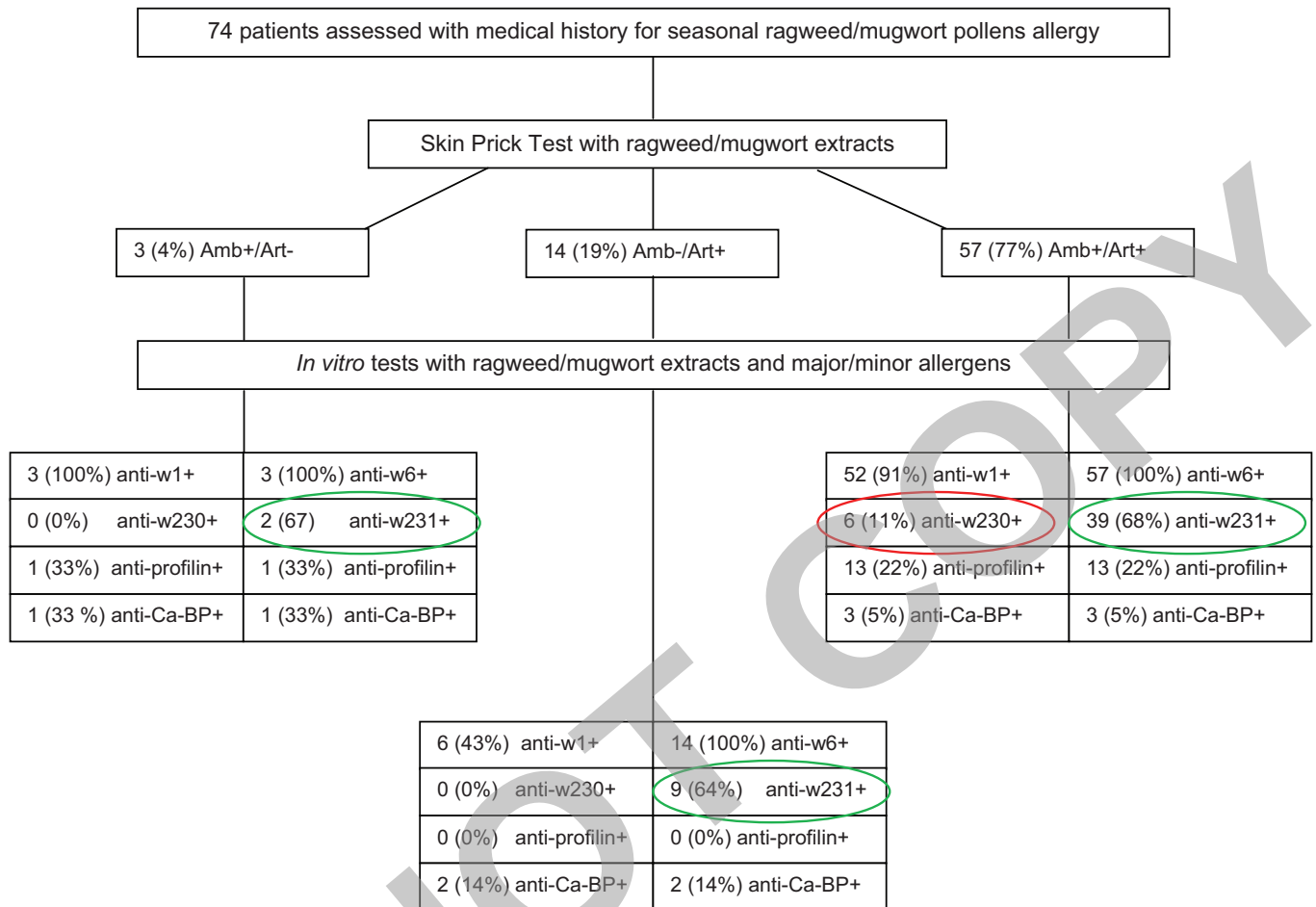


Figure 1. This figure shows reactivity profiles of ragweed- and mugwort-allergic patients using skin-prick testing and *in vitro* tests. w230 = nAmb a 1 ragweed, w231 = nArt v 1 mugwort, w1 = natural allergen extract ragweed, w6 = natural allergen extracts mugwort, Ca-BP = calcium-binding protein, red circled = genuine ragweed allergic patients, green circled = genuine mugwort allergic patients.

values of 7.92 and 2.42 kUa/L. Using natural ragweed extracts, 52 patients (91%) tested positive for IgE antibodies (mean value, 2.52 kUa/L). Of these patients, only 6 (11%) showed antibodies against the ragweed major allergen Amb a 1 (mean value, 2.00 kUa/L). Of these patients, 3 (50%) revealed antibodies against minor allergens. Overall, 13 patients (22%) showed antibodies against the minor allergen profilin and 3 patients (5%) against calcium-binding protein.

In total, 60 patients revealed positive SPT reactivity against ragweed. Of these patients, 55 (92%) showed IgE antibodies against natural ragweed extracts, but only in 6 cases were we able to detect anti-Amb v 1 antibodies. Within the investigated population of 74 weed-allergic patients the prevalence of true ragweed sensitization can be calculated as 8.1%. Moreover, 71 patients showed sensitivity against mugwort in SPT; IgE antibodies against mugwort extracts were found in 74 patients, whereas anti-Art v 1 antibodies were detectable in 50 patients, signifying a prevalence of 68% within the cohort of weed-allergic patients. In 61 patients we found antibodies against natural ragweed and mugwort extracts. A cosensitization of ragweed and mugwort was found only in 6 of 61 patients (10%), whereas cross-reactivity was seen in 55 of 61 cases (90%).

Medical History and HRQL Questionnaire

To describe the disease-related impact on patient's overall daily life, results from *in vitro* investigations were compared with medical history and HRQL scales. Using standardized clinical history and

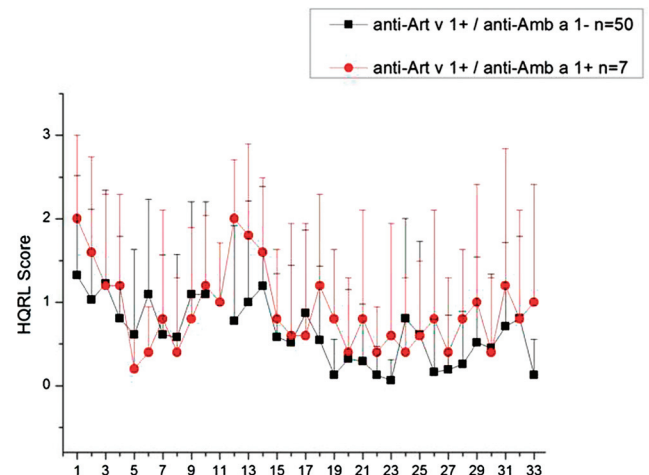


Figure 2. This figure shows health-related quality of life (HRQL) of patients with a sole sensitization against Art v 1 versus a sensitization against Art v 1 and Amb a 1. No significant differences in HRQL were observable.

HRQL questionnaires we were not able to detect any differences within the SPT and RAST reactivity patterns. Regarding patients with only sensitization against Art v 1 versus a sensitization against Art v 1 and Amb a 1, we observed no significant differences (Fig. 2).

A comparison between solely anti-Art v 1- and anti-Amb a 1-positive patients was not possible because no patients with a sensitization only against the ragweed major allergen Amb a 1 were found. In addition, when comparing monosensitized versus polysensitized and anti-profilin/calcium-binding protein-positive versus anti-profilin/calcium-binding protein-negative patients no significant differences were observed. Furthermore, patients were asked for symptoms of OAS; however, no typical cross-reactivity of ragweed with, e.g., bananas and mugwort, with, e.g., kiwi, was found among the investigated patients.

DISCUSSION

Weed pollens such as mugwort and ragweed are widely distributed in northern America and are among the most common causes for respiratory allergies.⁴ In Europe, the occurrence of weed, especially of ragweed, is rare. Despite the rare distribution of ragweed in Germany a high prevalence of positive *in vitro* tests is reported.⁷ A recently published European epidemiological study revealed a pan-European frequency of ragweed sensitization of 14.1%. In Germany a comparable prevalence of weed pollen allergies was reported.⁵ However, large variations between different European countries from 2 to 54% have been found. Increasing frequencies of ragweed sensitization may result from an augmented allergen occurrence over the last decades with a three times increase in ragweed pollen load from the late 1980s to the end of the 1990s. Recent studies from Austria revealed that the frequency of ragweed-specific IgE antibodies among allergic patients had risen from 20 to ~30% within the same period of time.¹⁹ However, differential diagnosis between ragweed and mugwort pollen allergy represents a large clinical problem in areas where both plants are present. Besides clinical history, diagnosis of ragweed and mugwort allergy is based on skin tests, provocation tests, and *in vitro* investigations with natural allergen extracts. Because pollination of both plants in central Europe starts at the end of July and lasts until the end of October, a distinction on a solely clinical basis is hardly feasible. In this situation *in vitro* investigations may provide additional information but also are limited when using whole pollen extracts because conventional extracts are suited to identify allergy sources but are not eligible for the identification of one specific major allergen.¹⁸

One explanation for the relatively high prevalence of IgE antibodies against common ragweed may be the fact that patients produce cross-reacting IgE antibodies against antigens shared with abundantly distributed weed species such as mugwort or minor allergens, e.g., profilin or calcium-binding proteins. Previously reported investigations revealed divergent results in regard to cross-reacting IgE antibodies of ragweed and mugwort. Hirschehr *et al.*¹² concluded that in addition to profilin, mugwort and ragweed pollen contain numerous cross-reactive allergens including the major mugwort allergen Art v 1. Data of the present study are in line with these results. Here, we used common extracts in combination with major and minor allergens and performed subgroup analyses of reactivity profiles in patients with seasonal symptoms of ragweed and mugwort allergy. Using mugwort and ragweed extracts, nearly all patients seemed to be cosensitized against both allergens showing a high prevalence of >70% among the weed-allergic patients. However, when testing major and minor allergens, only a small proportion with a calculated prevalence of 8% was truly sensitized against the ragweed major allergen Amb a 1. *Vice versa*, the vast majority of positive ragweed RAST results may be explained by cross-reacting IgE antibodies against both allergens. Furthermore, a high percentage of patients were polysensitized against minor allergens that occur in many biologically unrelated allergen sources. Recognized by the same IgE antibody, these patients appear to be polysensitized against both mugwort and ragweed.²⁰ In contradiction, other studies found no or only little cross-reactivity between ragweed and mugwort^{13,21} and concluded that patients showing both ragweed- and mugwort-positive SPT and/or RAST results are cosensitized.²²

We found a high prevalence (68%) of Art v 1 sensitization among the investigated weed-allergic patients. These data are in line with results from former studies and reflect the latitude of south Germany.

The clinical relevance of a cosensitization against mugwort and/or ragweed is still not clear. However, previous histamine release experiments and nasal provocations revealed that mugwort and ragweed extracts induced comparable histamine releases and nasal obstructions.¹² To date, investigations concerning HRQL and clinical relevance of a sensitization against the major allergens Art v 1 and Amb a 1 have not been performed. Therefore, the objective of the present study was to compare HRQL and clinical relevance in patients allergic to mugwort and ragweed pollen that showed different IgE reactivity profiles toward major (Art v 1/Amb a 1) and minor allergens (profilin and calcium-binding protein). We performed subgroup analysis and compared polysensitized versus monosensitized. Here, we could not detect any significant differences in patients suffering from mugwort versus mugwort plus ragweed sensitization. A comparison between a sole sensitization against Art v 1 or Amb a 1 was not possible, because all anti-Amb a 1-positive patients also showed IgE against Art v 1. In addition, we were not able to detect any differences in monosensitized versus polysensitized (anti-profilin and/or anti-calcium-binding protein positive) patients. However, HRQL depends on the patient's perception and severity of symptoms is subjective and influences HRQL directly.¹⁵ Numbers of patients in the subgroups were very small and thus the present study deserves replication with larger groups of patients.

CONCLUSION

Within the investigated population of 74 weed-allergic patients the prevalence of true mugwort and ragweed sensitization can be calculated as 68 and 8%. High prevalence of ragweed sensitization when testing with full extracts can be explained by cross-reacting antibodies from other weeds, e.g., mugwort. Differences in medical history and HRQL between different reactivity patterns were not detectable.

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