

ALEX² Sensitization Profiles and Documented Allergen Immunotherapy Recommendations in Adults with Allergic Rhinitis or Rhinoconjunctivitis

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Abstract

Background/Objectives:

Allergy Explorer 2 (ALEX²) combines allergen extracts and molecular components in a multiplex IgE assay. We described sensitization profiles, clinical and ear, nose and throat (ENT) findings, and documented allergen immunotherapy (AIT) recommendations in adults with allergic rhinitis and/or rhinoconjunctivitis.

Methods

This retrospective observational study included 100 eligible adults evaluated between August 2025 and July 2026. Symptoms, ENT findings, skin prick testing (SPT), total immunoglobulin E (IgE), ALEX² profiles, and AIT-related decisions were extracted from routine records. Polysensitization was defined as sensitization to at least two distinct allergen sources. SPT–ALEX² comparisons were restricted to documented SPT-positive sources with corresponding ALEX² source-group results. Analyses were descriptive and exploratory, without adjustment for multiple comparisons.

Results

Seasonal symptoms were documented in 73% of patients. The most frequently recorded allergen sources were ragweed/Ambrosia (41%), grass pollen (36%), and house dust mites (25%). Polysensitization was present in 57%, and allergologist-interpreted patterns compatible with cross-reactivity in 22%. SPT was performed in 73 patients, with positive results recorded in 71 (97.3%). Across six comparable allergen sources, 64 patients had at least one documented SPT-positive source available for comparison; six had at least one corresponding negative ALEX² result. AIT was recommended in 31 patients (31%) and had been started or was planned in 15 (15%). Among 45 patients with a recorded or considered AIT target, ragweed/Ambrosia was the most frequent target (16/45, 35.6%).

Conclusions

This selected sample showed heterogeneous sensitization profiles and documented AIT recommendations within an integrated ENT–allergy assessment. The study does not establish overall agreement between SPT and ALEX² or the independent contribution of molecular testing to AIT decisions. Exploratory findings require prospective validation.

1. Introduction

Allergic rhinitis and allergic rhinoconjunctivitis are common IgE-mediated airway diseases encountered in both allergology and otorhinolaryngology practice [1,2]. Allergic rhinitis is an IgE-mediated

inflammatory nasal condition that occurs after allergen exposure in a sensitized individual and is clinically associated with symptoms such as sneezing, nasal obstruction, rhinorrhea, and nasal itching [1,2]. In clinical practice, these symptoms may coexist with ocular complaints, cough, sleep disturbance, asthma, rhinosinusitis, or other upper-airway conditions. Consequently, diagnostic interpretation requires the integration of allergy testing with the patient's clinical phenotype [1,2].

The diagnosis of allergic rhinitis requires correlation between symptoms, exposure history, and evidence of allergic sensitization [1–3]. Skin prick testing and extract-based serum-specific IgE assays remain important diagnostic tools; however, a positive sensitization test does not necessarily indicate clinically relevant allergy [1–4]. This limitation is particularly relevant in polysensitized patients, in whom multiple positive results may reflect genuine co-sensitization, cross-reactivity, or clinically irrelevant IgE binding [3–5].

Molecular allergy diagnostics, also referred to as component-resolved diagnostics, evaluate IgE reactivity to individual allergen molecules rather than only to whole allergen extracts [3,4]. This approach may help distinguish primary sensitization to clinically relevant marker allergens from cross-reactive patterns mediated by panallergens or cross-reactive carbohydrate determinants [3–5]. Current consensus documents emphasize that molecular testing may support clinical decision-making, particularly in complex respiratory allergy and allergen immunotherapy selection, but that specific IgE results must still be interpreted in the context of the patient's history and clinical phenotype [3,4].

Polysensitization is a frequent and clinically relevant feature of respiratory allergy [5,7]. At the molecular level, polysensitization may represent co-sensitization to different, unrelated allergen sources, cross-reactivity between structurally related molecules, or a combination of both [5]. This distinction is important because allergen immunotherapy should target clinically relevant allergen sources rather than simply the complete list of positive sensitizations [5,7–10]. Molecular diagnosis may therefore help refine the interpretation of complex sensitization profiles and reduce the risk of inappropriate allergen selection [7–9].

ALEX² is a multiplex allergy assay that combines allergen extracts and molecular components in a single IgE test [6]. Compared with ImmunoCAP ISAC, ALEX² includes a broader allergen panel and incorporates an inhibition step intended to reduce IgE binding to cross-reactive carbohydrate determinants [6]. Comparative data have shown overall agreement between ALEX² and ISAC for shared allergen components, while the inclusion of allergen extracts in ALEX² may identify additional sensitizations in some patients [6]. These characteristics make ALEX² potentially useful in real-life respiratory allergy cohorts, in which polysensitization, cross-reactivity, and uncertain allergen immunotherapy targets are common [3–7].

The clinical relevance of molecular testing is particularly important when allergen immunotherapy is considered [8–12]. Allergen immunotherapy is an allergen-specific, disease-modifying treatment for selected patients with allergic rhinitis or rhinoconjunctivitis [10,11]. Because its efficacy depends on the correct identification of the clinically relevant allergen source, molecular diagnostics may help guide

target selection, particularly in patients with pollen polysensitization or suspected cross-reactivity [8–12]. In seasonal allergic rhinitis, component-resolved diagnosis has been reported to modify the selection of relevant allergens for immunotherapy in a substantial proportion of patients [12].

Regional exposure patterns are also important when interpreting respiratory molecular profiles [13–18]. Ragweed pollen is a major cause of late-summer and autumn respiratory allergy in parts of Europe, including the Pannonian Plain and neighboring countries such as Romania [13]. *Ambrosia artemisiifolia* has high allergenic potential, and Amb a 1 is a major ragweed allergen relevant to diagnosis and immunotherapy decisions [13,14]. Grass pollen is also one of the leading causes of seasonal respiratory allergy in Europe, and molecular biomarkers may help distinguish genuine grass pollen sensitization from cross-reactivity and support targeted immunotherapy decisions [15]. House dust mites are important perennial allergen sources, with Der p 1, Der p 2, and Der p 23 considered clinically relevant *Dermatophagoides pteronyssinus* components for molecular interpretation [16,17]. *Alternaria alternata* is a relevant mold allergen source, with Alt a 1 described as a major marker of primary sensitization and with sensitization associated with respiratory allergic disease and asthma-related risk [18].

From an ENT perspective, allergic rhinitis should not be interpreted solely on the basis of sensitization testing [1,2,19,20]. Rhinitis-like symptoms may overlap with chronic rhinosinusitis, nasal polyps, septal deviation, postnasal drip, smell impairment, cough, or Eustachian tube dysfunction [1,2,19,20]. EPOS 2020 defines adult rhinosinusitis by symptoms such as nasal obstruction or nasal discharge, with possible facial pressure or smell reduction, together with objective evidence of sinonasal inflammation on endoscopy or computed tomography [19]. Eustachian tube dysfunction is defined by symptoms and signs of middle-ear pressure dysregulation and may require assessment with otoscopy, tympanometry, audiometry, and nasopharyngoscopy [20].

Molecular sensitization results require interpretation within a broader clinical diagnostic pathway [3,4,7]. The primary objective of this study was to describe ALEX² sensitization profiles and clinical and ENT characteristics in adults with allergic rhinitis and/or rhinoconjunctivitis. Secondary objectives were to describe ALEX² positivity among documented SPT-positive allergen sources and to summarize documented AIT recommendations and target allergens. Selected exploratory analyses examined total IgE, sensitization breadth, and AIT recommendation.

2. Materials and Methods

2.1. Study design and setting

This was a retrospective, real-life observational study conducted over a one-year period, between 1 August 2025 and 31 July 2026. The study included adult patients evaluated through an ENT–allergy diagnostic pathway for rhinitis- and/or rhinoconjunctivitis-related symptoms and clinically relevant allergic sensitization.

Allergological assessment was performed in an outpatient allergology setting, while otorhinolaryngological evaluation was performed in an outpatient ENT setting. ALEX² molecular allergy testing was performed in the hospital laboratory of “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania. Clinical, allergological, molecular, and ENT data were retrospectively extracted from medical records, allergology reports, ALEX² laboratory reports, and ENT examination records.

The study was observational, and no diagnostic or therapeutic intervention was performed for research purposes.

The primary objective was to describe ALEX² sensitization profiles and clinical and ENT characteristics. Secondary objectives were to describe ALEX² positivity among documented SPT-positive allergen sources and to summarize documented AIT recommendations and target allergens. Selected exploratory analyses examined total IgE, sensitization breadth, and AIT recommendation.

2.2. Study population

All available records meeting the eligibility criteria within the study period, from 1 August 2025 to 31 July 2026, were included, yielding a final sample of 100 unique adults aged ≥ 18 years. Eligibility required symptoms compatible with allergic rhinitis and/or rhinoconjunctivitis, positive ALEX² results to respiratory allergens, sufficient clinical documentation, and an ENT evaluation. SPT availability was not mandatory for inclusion.

Patients with isolated urticaria, angioedema, food allergy, or venom allergy without a compatible respiratory allergic phenotype were not included in the main analysis. Additional exclusion criteria were age < 18 years, unavailable ALEX² testing, insufficient clinical documentation, acute infectious upper-airway disease as the main presentation, absence of symptoms compatible with allergic rhinitis or rhinoconjunctivitis, and absence of clinically interpretable molecular sensitization in the absence of a compatible respiratory phenotype.

Patients with complex or discordant clinical–molecular profiles were not automatically excluded when the overall clinical and ENT evaluation supported a respiratory allergic phenotype. These cases were retained and flagged for cautious interpretation when appropriate. Because the cohort was selected from patients with available clinically relevant ALEX² testing, it represents an enriched real-life clinical sample and was not designed to estimate diagnostic sensitivity, specificity, or diagnostic accuracy.

No a priori sample-size calculation was performed.

2.3. Clinical data collection

Clinical, allergological, ENT, laboratory, and treatment data were retrospectively extracted from routine medical records using a predefined structured database.

Recorded variables included age, sex, urban or rural background, symptom pattern, seasonality, nasal and ocular symptoms, respiratory comorbidities, relevant allergic comorbidities, previous and current treatments, and allergen immunotherapy-related decisions.

Asthma was coded as present when an asthma diagnosis was explicitly documented in the medical record. Spirometric confirmation, asthma phenotype, and control status were not consistently available and were not analyzed.

Symptom and ENT finding variables were assessed in all 100 included patients. In these fields, 1 indicated presence, while 0 and blank entries indicated verified absence. Blank entries were therefore coded as 0 for analysis. For AIT recommendation, blank entries indicated that AIT had not been recommended and were also coded as 0. These coding rules were variable-specific and were not applied to source-specific SPT fields. Procedures not performed and genuinely undocumented information were retained as missing or not applicable. No statistical imputation was performed.

2.4. Allergological assessment and skin prick testing

All patients underwent allergological assessment based on clinical history, skin prick testing and/or serum IgE evaluation, according to clinical indication. Allergic rhinitis/rhinoconjunctivitis was defined by the presence of compatible nasal and/or ocular symptoms associated with documented allergic sensitization, identified through skin prick testing and/or allergen-specific IgE testing, interpreted in correlation with clinical history.

Skin prick testing was performed according to clinical indication using Alyostal Prick extracts (Stallergenes, Antony, France); the selection of tested allergen sources varied between patients. The available extracts included standardized extracts of grass, weed, and tree pollens; house-dust and storage mites, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*; cat and dog dander; and latex. The pollen panel included individual and mixed extracts covering clinically relevant grass species, *Ambrosia elatior*, *Artemisia vulgaris*, *Parietaria* spp., *Salsola kali*, *Alnus glutinosa*, *Betula pendula*, *Carpinus betulus*, *Corylus avellana*, *Olea europaea*, *Fraxinus excelsior*, *Platanus acerifolia*, and *Juniperus ashei*. Histamine dihydrochloride 10 mg/mL was used as the positive control, and the negative control contained saline, glycerol, and phenol. A skin prick test was considered positive when the wheal diameter was at least 3 mm larger than the negative control, in the presence of an appropriate positive histamine response.

SPT results were extracted by allergen source where available. Comparisons with ALEX² were restricted to explicitly documented SPT-positive sources with corresponding ALEX² source-group results. In source-specific SPT fields, a value of 0 indicated that the source had not been tested; these entries and blank cells were excluded from the comparison. The analysis described ALEX² positivity among SPT-positive sources and did not estimate overall agreement, sensitivity, or specificity.

2.5. ALEX² molecular allergy testing

ALEX² multiplex molecular allergy testing (MADx, Macro Array Diagnostics) was performed as part of routine diagnostic assessment. The assay report specifies 165 allergen sources and includes both allergen extracts and molecular components, covering pollen, mite, fungal, animal-derived, food, insect/venom, latex, and other allergen categories. Allergen-specific IgE results were reported in kUA/L for allergen extracts and molecular components. Sensitization to an allergen extract or molecular component was defined by a specific IgE concentration ≥ 0.3 kUA/L, in accordance with the laboratory report threshold. Values < 0.3 kUA/L were classified as negative or uncertain and were not counted as positive sensitizations. Results were interpreted according to the laboratory-defined categories provided in the reports: <0.3 kUA/L negative or uncertain, $0.3-1$ kUA/L low, $1-5$ kUA/L moderate, $5-15$ kUA/L high, and > 15 kUA/L very high.

An ALEX² allergen-source group was classified as positive when at least one corresponding allergen extract or molecular component had a specific IgE concentration ≥ 0.3 kUA/L. Multiple positive extracts or components within the same group were counted once for the group-level analysis. PR-10 and profilin sensitization were recorded separately as molecular-family variables and were not counted as additional allergen-source groups.

Total IgE was reported in kU/L. Adult reference values were defined as < 100 kU/L according to the laboratory reports. Values reported as > 2500 kU/L were coded as 2500 kU/L and flagged as right-censored for statistical analysis.

For analysis, sensitizations were grouped into clinically relevant categories: grass pollen, ragweed/Ambrosia, mugwort/Artemisia, Betulaceae/tree pollen, house dust mites, molds, Alternaria, animal-derived allergens, cat sensitization, and cross-reactive molecular families. Particular attention was given to major allergen components relevant for clinical interpretation and immunotherapy targeting, including Amb a 1, Art v 1, Der p/Der f components, grass group 1 and group 5 allergens, Betulaceae/PR-10-related components, Fel d 1, profilins, lipocalins, serum albumins, and tropomyosins.

2.6. ENT examination

All patients underwent routine ENT examination, including anterior rhinoscopy and nasal endoscopic assessment using a 0° rigid Karl Storz endoscope.

Recorded ENT variables included septal deviation, inferior turbinate hypertrophy, mucosal appearance, nasal secretions, nasal polyps or nasal polyposis, narrowed nasal fossae, postnasal drip or pharyngeal secretions, and Eustachian tube-related findings.

Nasal polyps or nasal polyposis were coded as present when explicitly documented during the completed ENT assessment and as absent when no polyps were documented. This variable was not automatically equated with chronic rhinosinusitis with nasal polyps unless a compatible chronic rhinosinusitis context was explicitly documented.

Flexible fiberoptic nasopharyngoscopy was not performed routinely. It was performed selectively, particularly in younger patients when symptoms or previous medical documentation raised suspicion of gastroesophageal reflux disease and/or adenoid hypertrophy.

Tympanometry was not performed routinely. It was performed only when symptoms or examination findings raised suspicion of Eustachian tube dysfunction or otitis media with effusion, using an Interacoustics Titan tympanometer. When otitis media with effusion was identified, patients received routine clinical treatment and were recalled for reassessment. Because otitis media with effusion was not a predefined study endpoint, it was not included as a primary outcome.

2.7. Molecular phenotype classification and source-specific comparisons

A clinically interpreted dominant molecular profile was assigned for each patient using the molecular components, allergen families, IgE pattern, clinical history, seasonality, exposure pattern, and potential relevance for allergen-specific immunotherapy. Dominant profiles were not assigned solely according to the highest numerical IgE value.

Polysensitization was defined as sensitization to at least two distinct allergen sources based on ALEX² results. Reactivity to multiple molecular components from the same allergen source was not counted as polysensitization. This classification did not require demonstrated clinical relevance of each sensitization.

For the separate correlation analysis, the number of positive ALEX² source groups was calculated using seven non-overlapping groups: grass pollen, ragweed/Ambrosia, mugwort/Artemisia, house dust mites, molds, animal-derived allergens, and Betulaceae/tree pollen. Alternaria and cat were treated as descriptive subcategories and were not counted separately in this analysis.

Patterns compatible with cross-reactivity were identified by the allergologist through interpretation of the overall ALEX² profile, taking into account sensitization to PR-10 proteins and profilins. These patterns were considered suggestive of cross-reactivity rather than experimental confirmation of IgE cross-reactivity and were not interpreted as evidence of clinical allergy to all implicated sources.

Interpretation caution was an allergologist-assigned flag based on the overall clinical and molecular assessment, including the sensitization profile and total IgE. It was summarized descriptively. Associations with features contributing to this assessment were not subjected to inferential analysis to avoid circularity.

Clinical–ALEX² concordance was defined as agreement between the molecular profile and the patient's symptoms, seasonality, or exposure history. Clinical–ALEX² discordance was defined as molecular sensitization without clear clinical relevance or as a clinically suggested allergen exposure that was not confirmed by ALEX².

Source-specific comparisons included grass pollen, ragweed, mugwort, house dust mites, *Alternaria*, and cat. Patients could contribute to more than one comparison. Generic tree and mold categories were excluded because the specific SPT extracts could not be established from the structured dataset.

2.8. Allergen immunotherapy decision-making and treatment data

AIT-related variables included recommendation, discussion, deferral or pending status, initiation or planning, previous administration, target allergen group, and documented product. These categories were not mutually exclusive. Recommendations reflected the decision recorded at the assessment analyzed and were based on the integrated clinical and allergological evaluation rather than ALEX² positivity alone. Absence of a recommendation was not interpreted as a contraindication or definitive ineligibility for AIT. Initiation and planned initiation were recorded as a combined category and were not interpreted as an actual treatment initiation rate.

AIT targets were standardized into ragweed/*Ambrosia*, house dust mites, grass pollen, *Betulaceae*/tree pollen, mugwort/*Artemisia*, cat/animal dander, and multiple candidate or unspecified targets. The last category included records listing alternative potential targets or lacking a clearly established target and was not interpreted as a recommendation for multi-allergen immunotherapy.

Product-specific analyses were restricted to patients with a documented AIT product. Acarizax was classified as house dust mite-directed immunotherapy, Ragwizax as ragweed/*Ambrosia*-directed immunotherapy, and Itulazax as *Betulaceae*/tree-pollen-directed immunotherapy.

Treatment data were extracted retrospectively from the available clinical records. Treatment classes were not mutually exclusive, and no comparative treatment-efficacy analysis was planned.

2.9. Statistical analysis

Statistical analyses were performed using jamovi version 2.7.38 and IBM SPSS Statistics version 32.0.0.0. Categorical variables were summarized as absolute numbers and percentages. Continuous variables were summarized as medians, interquartile ranges, and minimum–maximum values because non-normal distributions were expected, particularly for total IgE.

The association between house dust mite sensitization and AIT recommendation was assessed using Pearson's chi-square test. Total IgE concentrations were compared between polysensitized and non-polysensitized patients, and age was compared between patients with and without an AIT recommendation, using two-sided Mann–Whitney U tests. Spearman's rank correlation was used to explore the association between top-coded total IgE and the number of positive ALEX² source groups.

An unadjusted odds ratio with a 95% confidence interval was calculated for the association between house dust mite sensitization and AIT recommendation. Interpretation caution was summarized descriptively. Comparisons between this flag and features contributing to its assignment, and between

polysensitization and patterns compatible with cross-reactivity, were omitted from inferential reporting because their classifications could share defining information. All inferential analyses were exploratory and unadjusted for multiple comparisons. Nominal p-values were not interpreted as confirmatory evidence, and no causal inference was made.

2.10. Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. The analysis was retrospective and observational and used anonymized clinical and laboratory data collected during routine allergological, ENT, and laboratory evaluation. No additional diagnostic or therapeutic intervention was performed for research purposes.

The study received a favorable opinion from the Local Ethics Committee of “Dr. Carol Davila” Central Military Emergency University Hospital, Bucharest, Romania (meeting minutes No. 908, dated 18 August 2026). Patient confidentiality was maintained throughout data extraction, coding, and analysis.

3. Results

3.1. Study population

A total of 100 adult patients with clinically relevant allergic rhinitis or rhinoconjunctivitis and positive ALEX² multiplex testing were included in the main analysis. Demographic data were available for all patients. The median age was 33 years [interquartile range (IQR), 26–42; range, 18–66]. The cohort included 54 female patients and 46 male patients. Most patients were from urban environments (69/100), while 31/100 were from rural areas.

Total IgE values were available for all included patients. The median was 179 kU/L [IQR, 94–665.5]. The minimum was 24 kU/L; ten values were reported as > 2500 kU/L and top-coded at 2500 for analysis. Total IgE ≥ 100 kU/L was recorded in 73 patients.

Demographic, clinical, and ENT characteristics of the cohort are summarized in Table 1.

Table 1
Demographic, clinical and ENT characteristics of the cohort

Characteristic	Value
Main cohort, n	100
Age, years, median [IQR]; range	33 [26–42]; range 18–66
Female sex	54/100 (54.0%)
Male sex	46/100 (46.0%)
Urban environment	69/100 (69.0%)
Rural environment	31/100 (31.0%)
Total IgE, kU/L, median [IQR]; range	179 [94–665.5]; minimum 24; 10 values > 2500, top-coded at 2500
Total IgE > = 100 kU/L	73/100 (73.0%)
Total IgE censored > 2500 kU/L	10/100 (10.0%)
Seasonal symptoms	73/100 (73.0%)
Perennial symptoms	30/100 (30.0%)
Indoor symptoms	24/100 (24.0%)
Nocturnal symptoms	13/100 (13.0%)
Nasal obstruction	58/100 (58.0%)
Watery rhinorrhea	37/100 (37.0%)
Paroxysmal sneezing	33/100 (33.0%)
Nasal itching	20/100 (20.0%)
Ocular symptoms	32/100 (32.0%)
Asthma	29/100 (29.0%)
Cough	12/100 (12.0%)
Postnasal drip	11/100 (11.0%)
Nasal polyps / nasal polyposis	8/100 (8.0%)
Inferior turbinate hypertrophy	60/100 (60.0%)
Pale nasal mucosa	51/100 (51.0%)
Nasal secretions	54/100 (54.0%)
Watery secretions	29/100 (29.0%)

Characteristic	Value
Septal deviation	16/100 (16.0%)

3.2. Clinical and ENT characteristics

Seasonal symptoms were documented in 73 patients, while perennial symptoms were recorded in 30 patients. Indoor symptoms were present in 24 patients, and nocturnal symptoms in 13 patients.

The most frequently documented nasal symptom was nasal obstruction, reported in 58 patients, followed by watery rhinorrhea in 37 patients, sneezing in 33 patients, ocular symptoms in 32 patients, and nasal itching in 20 patients. Asthma was documented in 29 patients, cough in 12 patients, and postnasal drip in 11 patients. Nasal polyps or nasal polyposis were documented in 8 patients.

Common ENT findings included inferior turbinate hypertrophy in 60 patients, pale nasal mucosa in 51, nasal secretions in 54, and watery secretions in 29. Septal deviation was recorded in 16 patients.

3.3. Molecular sensitization profiles identified by ALEX²

ALEX² multiplex testing identified heterogeneous molecular sensitization profiles across the cohort. The most frequent allergen group was ragweed/Ambrosia, identified in 41 patients, followed by grass pollen sensitization in 36 patients. House dust mite sensitization was documented in 25 patients, tree pollen or Betulaceae-related sensitization in 24 patients, and animal-derived sensitization in 23 patients. Cat sensitization was present in 18 patients.

Mold-related sensitization was identified in 20 patients, including Alternaria sensitization in 16 patients. Mugwort/Artemisia sensitization was present in 8 patients.

PR-10 sensitization was recorded in 13 patients and profilin sensitization in ten. The allergologist identified patterns compatible with cross-reactivity in 22 patients. Polysensitization, defined as sensitization to at least two distinct allergen sources, was present in 57 patients. ALEX² sensitization profiles are summarized in Table 2.

Table 2
ALEX² molecular sensitization profiles

ALEX ² variable	n/N (%)
Ragweed/Ambrosia	41/100 (41.0%)
Grass pollen	36/100 (36.0%)
House dust mite	25/100 (25.0%)
Tree/Betulaceae-related	24/100 (24.0%)
Animal dander	23/100 (23.0%)
Mold	20/100 (20.0%)
Cat	18/100 (18.0%)
Alternaria	16/100 (16.0%)
Mugwort/Artemisia	8/100 (8.0%)
PR-10	13/100 (13.0%)
Profilin	10/100 (10.0%)
Pattern compatible with cross-reactivity	22/100 (22.0%)
Polysensitization involving ≥ 2 allergen sources	57/100 (57.0%)

Allergen categories were not mutually exclusive. Alternaria was treated as a mold subcategory, and cat sensitization as an animal-derived allergen subcategory.

3.4. ALEX² positivity among SPT-positive allergen sources

SPT was performed in 73 patients (73%), with positive results recorded in 71/73 (97.3%). Across six comparable allergen sources, 64 patients had at least one documented SPT-positive source with an available corresponding ALEX² source-group result. Six of these 64 patients had at least one SPT-positive source with a negative corresponding ALEX² result. Source-specific findings are presented in Table 3. Separately, clinical–ALEX² discordance was recorded in four patients (4%).

Table 3
ALEX² positivity among documented SPT-positive allergen sources

Allergen source	ALEX ² -positive / SPT-positive, n/N (%)
Grass pollen	25/26 (96.2%)
Ragweed	25/28 (89.3%)
Mugwort	3/4 (75.0%)
House dust mites	16/19 (84.2%)
Alternaria	13/14 (92.9%)
Cat	13/13 (100%)

Patients could contribute to more than one allergen-source comparison. Untested sources and blank SPT entries were excluded. Generic tree and mold categories were not compared because the specific SPT extracts could not be established from the structured dataset. These percentages describe ALEX² positivity among documented SPT-positive sources, not overall assay agreement or diagnostic accuracy.

3.5. Allergen immunotherapy decision-making

AIT-related decision-making was evaluated in relation to molecular sensitization profiles, clinical relevance of exposure, symptom seasonality, SPT findings, and ENT evaluation.

AIT was recommended in 31 patients (31%) and had been started or was planned in 15 (15%). AIT remained under discussion in 15 patients, while a pending or deferred decision was recorded in 13. Previous AIT was documented in six patients. These categories were not mutually exclusive, and the combined initiation/planning category did not distinguish actual treatment initiation from intended treatment.

Among the 45 patients in whom an AIT target was recorded or considered, the most frequent standardized target was ragweed/Ambrosia, identified in 16 patients, followed by house dust mite in 8 patients, grass pollen in 5 patients, and Betulaceae/tree pollen in 4 patients. Mugwort/Artemisia and cat/animal dander were each recorded in 1 patient. In ten patients, multiple candidate targets were listed or the target remained unspecified.

Among the 15 patients with a documented AIT product, Acarizax was recorded in 8 patients, Ragwizax in 3, Staloral in 3, and Itulazax in 1 patient. AIT decision-making, standardized AIT targets, and recorded AIT products are summarized in Table 4.

Table 4
AIT decision-making, standardized AIT targets and recorded AIT products

AIT-related variable	n/N (%)
AIT decision status	
AIT recommended	31/100 (31.0%)
AIT under discussion	15/100 (15.0%)
AIT pending/deferred	13/100 (13.0%)
AIT started or planned	15/100 (15.0%)
Previous AIT	6/100 (6.0%)
Standardized AIT target	
Ragweed/Ambrosia	16/45 (35.6%)
House dust mite	8/45 (17.8%)
Grass pollen	5/45 (11.1%)
Betulaceae/tree pollen	4/45 (8.9%)
Mugwort/Artemisia	1/45 (2.2%)
Cat/animal dander	1/45 (2.2%)
Multiple candidate or unspecified targets	10/45 (22.2%)
Recorded AIT product	
Acarizax	8/15 (53.3%)
Ragwizax	3/15 (20.0%)
Staloral	3/15 (20.0%)
Itulazax	1/15 (6.7%)

Percentages for AIT decision status were calculated for the entire cohort (N = 100). Percentages for standardized AIT targets were calculated among patients with a recorded or considered AIT target (N = 45). Percentages for AIT products were calculated among patients with a documented product (N = 15). AIT-related decision categories were not mutually exclusive. The combined initiation/planning category does not represent an actual treatment initiation rate. Multiple candidate or unspecified targets do not indicate a recommendation for multi-allergen immunotherapy.

3.6. Exploratory analyses

Interpretation caution was recorded in 37 patients (37%) and was summarized descriptively. Retained exploratory analyses examined total IgE, polysensitization, sensitization breadth, and AIT recommendation.

Total IgE was higher in polysensitized than in non-polysensitized patients (median, 300 versus 143 kU/L; n = 57 versus 43; Mann–Whitney p = 0.0024). Total IgE correlated weakly with the number of positive ALEX² source groups (Spearman’s rho = 0.267; n = 100; p = 0.0073).

A nominal association was observed between house dust mite sensitization and AIT recommendation (OR, 2.72; 95% CI, 1.06–6.98; chi-square p = 0.0338). Patients with an AIT recommendation were younger than those without a recommendation (median age, 28 versus 34 years; n = 31 versus 69; Mann–Whitney p = 0.0481).

All retained analyses were exploratory and unadjusted for multiple comparisons. The estimates are presented in Table 5 and were not interpreted as independent predictors or confirmatory evidence.

Table 5
Exploratory associations **A. Categorical associations**

Comparison	Effect estimate	Test	p-value
House dust mite sensitization vs AIT recommendation	OR 2.72 (95% CI 1.06–6.98)	Chi-square	0.0338
B. Continuous and correlation analyses			

Comparison	Test	Effect / contrast	p-value
Total IgE: polysensitized vs non-polysensitized	Mann-Whitney U	median 300.0 vs 143.0 kU/L; n = 57 vs 43	0.0024
Total IgE vs number of positive ALEX ² groups	Spearman	rho = 0.267; n = 100	0.0073
Age: AIT recommended vs not recommended	Mann-Whitney U	median 28.0 vs 34.0 years; n = 31 vs 69	0.0481

OR, odds ratio; CI, confidence interval; AIT, allergen immunotherapy. P-values are exploratory and unadjusted for multiple comparisons.

4. Discussion

4.1. Principal Findings

This real-life ENT–allergology cohort of 100 adults with allergic rhinitis and/or rhinoconjunctivitis showed heterogeneous molecular sensitization profiles by ALEX² testing. Seasonal sensitization

patterns were frequent, especially ragweed/Ambrosia and grass pollen, while perennial and mixed patterns were represented by house dust mites, animal dander, molds, and other allergen groups. This distribution is consistent with the clinical heterogeneity of allergic rhinitis, which may be seasonal, perennial, mixed, and associated with asthma, conjunctivitis, rhinosinusitis, or other upper-airway conditions [1,2,19].

A central finding was the high frequency of molecular polysensitization, documented in 57% of the cohort. Polysensitization is common in respiratory allergic disease and is clinically relevant because it may complicate diagnosis and treatment selection [5,7]. However, multiple positive molecular results should not automatically be interpreted as multiple clinically relevant allergies, because molecular positivity may reflect co-sensitization, cross-reactivity, or clinically irrelevant IgE binding [3–5]. This distinction is essential in real-life practice, where patients often have overlapping seasonal and perennial exposures [1,5,7].

4.2. Molecular Polysensitization and Cross-Reactivity

Polysensitization and allergologist-interpreted patterns compatible with cross-reactivity were recorded in 57% and 22% of patients, respectively. Polysensitization described reactivity to at least two distinct allergen sources and did not establish clinical allergy to each source. Specific IgE results must be interpreted in relation to clinical history, as emphasized by WAO–ARIA–GA²LEN and the EAACI Molecular Allergology User’s Guide [3,4].

Reactivity to PR-10 proteins and profilins informed the interpretation of patterns compatible with cross-reactivity. However, this classification did not experimentally demonstrate cross-reactivity or establish its clinical relevance in individual patients. Such patterns were therefore distinguished from multiple independent clinically relevant allergies [3–5].

Total IgE was higher in polysensitized patients and correlated weakly with the number of positive ALEX² source groups. These exploratory findings describe sensitization breadth within the selected sample but do not establish total IgE as an independent measure of clinical relevance or interpretive complexity [3,5].

4.3. Clinically Relevant Molecular Sensitization Profiles

Ragweed/Ambrosia was the most frequent molecular sensitization group and the most frequent standardized allergen immunotherapy target in this cohort. This is clinically plausible in Romania and nearby regions, because *Ambrosia artemisiifolia* has expanded in Europe and important sources of ragweed pollen are described in the Pannonian Plain and neighboring countries, including Romania [13]. Ragweed pollen is associated with late-summer and autumn allergic rhinitis and asthma, and Amb a 1 is a major allergen relevant for diagnosis and immunotherapy selection [13,14]. The predominance of ragweed among immunotherapy targets in our cohort therefore likely reflects regional exposure and clinical relevance rather than isolated molecular positivity.

Grass pollen sensitization was also frequent and contributed to the seasonal sensitization burden. Grass pollen is among the leading aeroallergens in Europe and is associated mainly with seasonal rhinoconjunctivitis and, in some patients, asthma [15]. Molecular biomarkers may help distinguish genuine grass pollen sensitization from cross-reactivity and may support targeted allergen immunotherapy decisions [15]. Douladiris et al. also emphasize the value of component-resolved diagnosis in pollen-allergic patients when immunotherapy is considered, particularly where overlapping pollen seasons and multiple sensitizations complicate clinical interpretation [9].

House dust mite sensitization represented an important perennial profile. *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are major mite allergen sources worldwide, and Der p 1, Der p 2, and Der p 23 are considered clinically relevant components for HDM diagnosis and molecular interpretation [16,17]. Posa et al. describe progression from early mite sensitization to broader molecular recognition in some patients, with broader sensitization patterns associated with clinical symptoms and disease progression [17]. In the present cohort, HDM sensitization was clinically relevant for immunotherapy-related decisions, particularly in patients with perennial symptoms or Acarizax-related treatment planning.

Mold sensitization, including *Alternaria*, was less frequent than pollen sensitization but remains clinically important. *Alternaria alternata* is a potent aeroallergen source, and Alt a 1 is considered its main elicitor and a marker of primary sensitization [18]. *Alternaria* sensitization has been associated with respiratory allergic disease and asthma-related risk, although diagnostic and immunotherapy standardization is less developed than for major pollen or mite allergens [18]. In this study, *Alternaria*-positive profiles were therefore interpreted cautiously and in relation to clinical phenotype, asthma history, and exposure context.

4.4. Source-specific SPT–ALEX² comparisons

ALEX² positivity was frequent among documented SPT-positive sources, although some SPT-positive sources had negative corresponding ALEX² results. These findings describe source-specific positivity overlap rather than overall agreement between the tests. The analysis excluded untested sources and did not systematically include SPT-negative results; it therefore cannot establish sensitivity, specificity, or superiority of either method. Interpretation is also limited by selection of patients with positive respiratory ALEX² findings and by differences between SPT extracts and ALEX² source-group representation. Conventional and molecular allergy testing should be integrated according to the clinical question and the patient's history [2–7].

4.5. Implications for Allergen Immunotherapy

Allergen immunotherapy was recommended in 31% of patients and had been started or planned in 15%. These findings should be interpreted descriptively, because the study did not assess immunotherapy efficacy, adherence, or long-term outcomes. AIT-related categories were not mutually exclusive and represented documented clinical decisions rather than standardized treatment allocation. Among the 45 patients with a recorded or considered AIT target, ragweed/Ambrosia was the most frequent target,

followed by house dust mite, grass pollen, and Betulaceae/tree pollen. ALEX² results were considered alongside clinical history, exposure patterns, SPT findings, total IgE, and ENT examination when AIT decisions were documented; the present design cannot determine the independent contribution of ALEX² to those decisions [3,4,9–12]. The combined initiation/planning category does not measure actual treatment uptake. Absence of an AIT recommendation at the assessment analyzed does not establish contraindication or definitive ineligibility. Because decisions before and after ALEX² testing were not recorded, changes in recommendations attributable to molecular testing could not be assessed.

In contrast, broad cross-reactive patterns, multiple low-level positives, or uncertain symptom correlation may justify discussion, reassessment, or deferral rather than automatic immunotherapy prescription. These considerations are consistent with current guidance emphasizing clinical relevance and exposure correlation when selecting allergen immunotherapy targets [3–5,7–12].

4.6. ENT Findings and Clinical Integration

The ENT examination added an important clinical layer to molecular interpretation. Inferior turbinate hypertrophy, pale mucosa, nasal secretions, septal deviation, postnasal drip, nasal polyps or nasal polyposis, and Eustachian tube-related findings may all contribute to symptoms attributed to allergy. Rhinitis practice parameters emphasize that allergic rhinitis should be assessed alongside differential diagnoses, comorbidities, and overlapping upper-airway conditions [1,2]. Therefore, molecular sensitization was interpreted as part of an integrated ENT–allergology assessment rather than as an isolated diagnostic endpoint.

Nasal polyps or nasal polyposis were documented in eight patients. This variable was based on routine clinical documentation and was not uniformly verified as chronic rhinosinusitis with nasal polyps using a standardized diagnostic protocol. Its frequency should not be interpreted as evidence that allergic sensitization causes nasal polyposis. In these patients, allergic rhinitis symptoms, chronic rhinosinusitis-related symptoms, and molecular sensitization findings require separate clinical assessment [1,2,19].

Eustachian tube dysfunction was evaluated as part of the broader ENT phenotype rather than as a primary endpoint. The ETD consensus statement defines Eustachian tube dysfunction as a syndrome involving symptoms and signs of middle-ear pressure dysregulation and recommends assessment with otoscopy or otomicroscopy, tympanometry, audiometry, and nasopharyngoscopy when appropriate [20]. In patients with allergic rhinitis, ETD-like symptoms may coexist with nasal inflammation, but this relationship cannot be inferred from molecular sensitization alone [20].

4.7. Strengths and Limitations

This study has several strengths. It reflects real-life clinical practice and includes adult patients evaluated through both allergology and ENT assessment. It integrates symptoms, seasonality, SPT data, ALEX² molecular profiles, total IgE, ENT findings, and immunotherapy-related decisions. It also retains complex cases rather than excluding them, which reflects the practical reality of molecular allergy interpretation in routine care.

Several limitations should be acknowledged. The study was retrospective and observational, and no causal inference can be made. The sample was selected for compatible respiratory symptoms, positive ALEX² sensitization to respiratory allergens, sufficient documentation, and ENT evaluation; it was not an unselected population of adults with rhinitis. SPT was not performed in all patients, and source-specific comparisons were restricted to documented SPT-positive results. Consequently, overall assay agreement and diagnostic accuracy could not be estimated. Some variables depended on record completeness. Patterns compatible with cross-reactivity and interpretation caution were allergologist-interpreted variables, and inter-rater reliability was not assessed. Interpretation caution was summarized descriptively because its assignment incorporated clinical and molecular information. AIT-related categories could overlap, and combined initiation/planning did not measure actual treatment initiation, efficacy, adherence, or long-term outcomes. The absence of recorded decisions before and after ALEX² prevented assessment of its independent effect on AIT recommendations. Exploratory analyses were unadjusted for multiple comparisons, and some estimates were based on small subgroups. The findings require prospective validation.

This study describes ALEX² sensitization profiles and documented AIT recommendations within an integrated ENT–allergy pathway. Its selected sample and retrospective design do not establish incremental diagnostic benefit, changes in AIT recommendations attributable to molecular testing, or improved clinical outcomes. These questions require prospective assessment with reproducible classification criteria and recorded decisions before and after molecular testing.

5. Conclusions

In this retrospective observational study of 100 adults with allergic rhinitis and/or rhinoconjunctivitis and positive respiratory ALEX² findings, ragweed/Ambrosia, grass pollen, and house dust mites were the most frequently recorded allergen sources. Polysensitization and patterns compatible with cross-reactivity were common. Source-specific comparisons described ALEX² positivity among documented SPT-positive sources, while AIT recommendations and recorded or considered targets were summarized within an integrated clinical assessment. The study does not establish overall agreement between SPT and ALEX² or the independent contribution of molecular testing to AIT decisions. Exploratory associations require prospective validation.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
AIT	Allergen immunotherapy
ALEX ²	Allergy Explorer 2 multiplex molecular allergy assay
CI	Confidence interval
ENT	Ear, nose and throat
ETD	Eustachian tube dysfunction
HDM	House dust mite
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
IQR	Interquartile range
kUA/L	Kilo allergen-specific IgE units per liter
kU/L	Kilo units per liter
LABA	Long-acting beta ₂ -agonist
OR	Odds ratio
PR-10	Pathogenesis-related protein family 10
SPT	Skin prick testing

Declarations

Supplementary Materials: Not applicable.

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