

# Clinical relevance of major allergens compared to extract-based IgE diagnosis in pollen-allergic patients in Austria

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## Abstract

**Background:** Pollen-allergy is common in Austria. The present study investigated if the use of major pollen-allergens on top of extract-based diagnosis may improve the diagnostic accuracy and if this additional knowledge helps to identify patients eligible for allergen-specific immunotherapy (AIT). **Methods:** In this retrospective database analysis patients sensitized to a grass, birch, ash-tree, mugwort or ragweed were investigated and results of pollen extracts compared to the respective major allergens (Phl p 1, Bet v 1, Ole e 1, Art v 1, Amb a 1) were assessed. Furthermore, extract- and MD- results of patients double-sensitized to both tree-extracts or both weed-extracts were compared. In patients with discrepant results, their charts were reviewed to define if this difference was clinically relevant in regard to their doctors' AIT-decision. **Results:** 4234 patients (age 1.2-89.7yrs) could be included in the analyses. There was high agreement between extract-based IgE results and major allergens concerning single-sensitization to grass/Phl p 1+5 (94.4%), birch/Bet v 1 (94.4%), and ash-tree/Ole e 1 (82.9%), with lower agreement between mugwort/Art v 1 (64.1%) and ragweed/Amb a 1 (73.8%). Among patients double-sensitized to both tree pollen extracts only 76% were also sensitized to both major allergens (Bet v 1 and Ole e 1) and therefore suitable for AIT. In patients double-sensitized to both weeds, only 25% were also sensitized to both major allergens (Art v 1 and Amb a 1), 46.2% of mugwort extract positive patients tested negative for Art v 1. Of these patients, 41.3% were recommended a ragweed AIT as their Amb a 1 results were positive. **Conclusion:** MD can improve diagnostic accuracy in patients with pollen-allergy and should be implemented in standard clinical care, especially when double-sensitization to allergens of plants with overlapping pollen seasons cannot be clearly discerned by patients' symptoms alone.

## Clinical relevance of major allergens compared to extract-based IgE diagnosis in pollen-allergic patients in Austria

### Short title: Relevance of major allergens in pollen-allergic patients

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FH planned the study, supervised analyses and wrote the first draft of the manuscript

CH was involved in study planning, made analyses and gave input to the final manuscript

BB, EH, MS and ZS were involved in study planning, analyses and adjustment of the manuscript

## ABSTRACT

**Background:** Pollen-allergy is common in Austria. The present study investigated if the use of major pollen-allergens on top of extract-based diagnosis may improve the diagnostic accuracy and if this additional knowledge helps to identify patients eligible for allergen-specific immunotherapy (AIT).

**Methods:** In this retrospective database analysis patients sensitized to a grass, birch, ash-tree, mugwort or ragweed were investigated and results of pollen extracts compared to the respective major allergens (Phl p 1, Bet v 1, Ole e 1, Art v 1, Amb a 1) were assessed. Furthermore, extract- and MD- results of patients double-sensitized to both tree-extracts or both weed-extracts were compared. In patients with discrepant results, their charts were reviewed to define if this difference was clinically relevant in regard to their doctors' AIT-decision.

**Results:** 4234 patients (age 1.2-89.7yrs) could be included in the analyses. There was high agreement between extract-based IgE results and major allergens concerning single-sensitization to grass/Phl p 1+5 (94.4%), birch/Bet v 1 (94.4%), and ash-tree/Ole e 1 (82.9%), with lower agreement between mugwort/Art v 1 (64.1%) and ragweed/Amb a 1 (73.8%). Among patients double-sensitized to both tree pollen extracts only 76% were also sensitized to both major allergens (Bet v 1 and Ole e 1) and therefore suitable for AIT. In patients double-sensitized to both weeds, only 25% were also sensitized to both major allergens (Art v 1 and Amb a 1), 46.2% of mugwort extract positive patients tested negative for Art v 1. Of these patients, 41.3% were recommended a ragweed AIT as their Amb a 1 results were positive.

**Conclusion:** MD can improve diagnostic accuracy in patients with pollen-allergy and should be implemented in standard clinical care, especially when double-sensitization to allergens of plants with overlapping pollen seasons cannot be clearly discerned by patients' symptoms alone.

## KEY WORDS

allergen-specific immunotherapy, component resolved diagnosis, molecular allergy diagnosis, pollen-allergy

## ABBREVIATIONS

MD molecular allergy diagnosis; AIT allergen-specific immunotherapy; PCC Pearson's correlation coefficients; SPT skin prick test

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## INTRODUCTION

In Europe, the prevalence of allergic asthma (8%) and rhinitis (25%) is high with still increasing trend in the last decades.<sup>1</sup>

The diagnosis of pollen allergy used to be mainly based on medical history, skin prick test (SPT) and the detection of allergen-specific serum IgE by using pollen extracts. Pollen seasons from distinct plants may

overlap, and pan-allergens contained in various pollen may cause IgE-cross-reactions, though. Therefore, molecular allergy diagnosis (MD) assessing IgE reactivity to so-called marker allergens has increasingly gained importance in recent years. However, the question whether MD may improve extract-based diagnosis and help with the decision for allergen-specific immunotherapy (AIT) has not been clarified yet.<sup>2,3</sup>

More than 90% of patients with grass pollen allergy show IgE antibodies against Phl p 1 and Phl p 5,<sup>4</sup> and more than 95% of birch allergic patients are sensitized to the major allergen Bet v 1.<sup>5</sup> Ash tree is another allergen of increasing relevance in Central-Europe.<sup>6</sup> The distinction between birch and ash tree allergy is relevant for the decision which AIT is suitable. However, both pollen seasons overlap in Austria causing concomitant allergic symptoms to either tree species.<sup>8</sup> In MD the structurally homologous Ole e 1 from olive tree is usually used instead of the major ash tree allergen Fra e 1 which is not commercially available.<sup>7</sup>

The defensin-like protein Art v 1 is the single major allergen in mugwort pollen, recognized by IgE antibodies from 90% of patients with mugwort allergy.<sup>9</sup> About 10-14% of patients with rhinoconjunctivitis in Eastern Austria suffer from mugwort allergy.<sup>10</sup> With the mugwort season in eastern Austria overlapping with that of the ragweed season, differentiation based exclusively on patients' history is difficult.<sup>8</sup> Ragweed has gained increasing interest, not only in Eastern, but also in Central Europe. The major allergen available for genuine ragweed allergy is Amb a 1, a pectate lyase with about 50% sensitization rates in ambrosia allergic patients.<sup>11</sup>

Cross-reactivity between mugwort and ragweed extract often leads to inconclusive results. For example, Amb a 1 and Art v 6 as well as Amb a 4 and Art v 1 show structural homologies,<sup>12</sup> however, only Amb a 1 and Art v 1 offer clinical importance for pollen allergic patients. Also, in this case it is important to discriminate between both weed allergies, especially if one has to decide which AIT has to be applied.

The aim of this study was to evaluate whether the use of the major pollen-allergens (Phl p 1+5, Bet v 1, Ole e 1, Art v 1, Amb a 1) can increase the diagnostic accuracy in patients with a single-sensitization against grass, birch, ash, mugwort or ragweed pollen. If there was a discrepancy in the results of extract and major-allergen, we reviewed medical charts to see if this knowledge changed medical recommendations for AIT.

In addition, we intended to analyze how many patients were double-sensitized to birch and ash-tree and/or to mugwort and ragweed, respectively and if this double-sensitization was caused by extracts' cross-reactivity or was based on a relevant sensitization against one or both major allergens.

The study was part of a diploma thesis (C.H.) at the Medical University Vienna, Austria.<sup>13</sup>

## METHODS

### Study design and patients

In this retrospective database-study<sup>13</sup> we included patients from the internal database (ACETOMed 1.6.35) who visited the Allergy Center Vienna West, Austria and were diagnosed as pollen-allergic based on clinical history and allergen-specific IgE levels >0.35 kU/l in ImmunoCAP (Thermo Fisher, Waltham, Massachusetts, USA) between October 2012 (first entry in this database) and the end of July 2016 (when the study was initiated).

For the single pollen analyses, patients with available IgE measurements for pollen extract (grass, birch, ash, mugwort or ragweed) and the respective major allergens (Phl p 1+5, Bet v 1, Ole e 1, Art v 1, Amb a 1) were included (Table 1). For analyses of double-sensitization, we detected both patients with IgE tests available for tree pollen extracts (birch and ash) and their respective major allergens and accordingly patients with IgE tests for both weeds extracts (mugwort, ragweed) and their major allergens.

The study was approved by the ethical committee of the Medical University Vienna (EK Nr.: 1857/2016). As it was a retrospective data-base study no informed consent was demanded.

### Data acquisition and statistical analysis

Data of included patients were exported from ACETomed 1.6.35 to Microsoft Excel (Vers. 15.11) for further analyses.

Extracted data included:

- specific IgE (kUA/l) measured by ImmunoCAP<sup>TM</sup>-system for: timothy-grass, birch, ash, mugwort, ragweed as well as the major allergens Phl p 1+5, Bet v 1, Ole e 1, Art v 1 and Amb a 1. A positive result was determined as an IgE [?] 0.35 kUA/l.
- age (yrs).
- If there was a discrepancy between extract-based diagnosis and major allergens, we reviewed the charts to see if this difference led to a specific recommendation for the patient with regards to AIT.

Microsoft Excel (Vers. 15.11) was used for descriptive analyses and Pearson-correlation.

### Single pollen allergens

In a first step, we compared which patients were extract positive/negative and respective major allergen positive/negative.

Agreement<sup>14</sup> was calculated by adding patients positive to extract and the respective major allergen as well as patients negative to extract and major allergen and describe the result as a percentage of all patients tested with respective extract and major allergen.

Furthermore, we examined medical charts of patients with discrepant extract and major allergen results (=extract positive, major allergen negative or vice versa) to find out if the results of major allergens changed medical recommendations for or against AIT.

Finally, we calculated Pearson's correlation coefficients (PCC) for specific pairs of each allergen extract and its respective major allergen. Values of IgE (kUA/l) were log-transformed as IgE-values following a logarithmic slope. Concentrations below the lower detection limit of 0.35 kUA/l were determined to be 0.1 kUA/l, while values above the upper detection limit of 100 kUA/l were determined to be 200 kUA/l in order to make further calculations and graphical presentation in a scatter plot feasible. A p of [?]0.05 was assumed to be significant.

### Double sensitization

Patients were identified that had IgE tests done for birch and ash extract, as well as their major allergens Bet v 1 and Ole e 1. Extract-double-sensitized patients (=positive for birch and ash extract) were grouped by their major-allergens (Bet v 1 pos. + Ole e 1 pos., Bet v 1 pos. + Ole e 1 neg., Bet v 1 neg. + Ole e 1 pos., Bet v 1 neg. + Ole e 1 neg.) to see if extract-positivity was caused by cross-reaction or if there was a relevant sensitization to both major allergens.

The same analysis was performed for patients with available IgE tests for mugwort and ragweed extract as well as their major allergens Art v 1 and Amb a 1.

## RESULTS

### Single pollen-sensitization

In total, 4234 patients were enrolled (Table 2). The comparison of the sensitization against extracts and the respective major allergens is shown in Table 3.

**Grass:** We found 1984 patients with IgE-measurements to timothy grass extract and Phl p 1+5. Median age was 26.9 years (1.2-89.7 years). While 1745 displayed IgE for timothy grass extract, 1658 were positive for Phl p 1+5. 94.3% of extract positive patients tested positive for the major allergen, 11.4% of all patients were negative for both measurements. Twelve patients (=5% of all extract-negative patients) with a negative extract result were positive for Phl p 1,5. This observation was clinically relevant for 6 patients (2.5%) in which AIT was recommended despite negative extract results. Ninety-nine patients (=5.7% of all extract

positive patients) were extract-positive, but Phl p 1+5-negative. This observation was clinically relevant for 18 (1%) of these patients, as the treating physicians advised against AIT.

There was a strong correlation between timothy-grass extract and Phl p 1+5 (PCC 0.87,  $p < 0.01$ ; Fig. 1A) and a very high agreement (94.4%) between extract and major-allergen results (Table 3).

**Birch:** Parallel analyses of sensitization to birch extract and Bet v 1 were available for 1555 patients. Median age was 31.3 years (2.7-89.7 yrs). While 1350 patients had a positive IgE result for birch extract, 1279 were positive for Bet v 1. In summary, 94.1% of extract positive patients were also positive for the major allergen, while 12.7% of all patients were negative for both measurements. Eight patients (3.9%) with a negative result for the extract tested positive to Bet v 1, of whom 5 had an IgE  $< 0.69$  kUA/l. In the other 3 patients (1.5%), AIT was recommended based on a positive major allergen value. Seventy-nine (5.9%) of extract positive patients showed negative results in Bet v 1. In only one patient AIT was performed and this showed no clinical benefit. In 14 patients (1% of all birch extract positive tests) the physicians advised against AIT because of the negative Bet v 1 result.

There was a strong correlation between birch extract and Bet v 1 (PCC 0.82,  $p < 0.01$ ; Fig. 1B) and a very good agreement (94.4%) between extract and major-allergen results (Table 3).

**Ash-tree:** One hundred and eighty-one patients could be included with parallel measurements of ash-tree extract and Ole e 1. The patients' median age was 27 years (3.8-65.5 yrs). One hundred and fifty-seven patients tested positive for the extract, 128 for the major allergen. While 80.9% of extract positive patients were also positive for the major allergen, 12.7% of all patients were negative for both measurements. Only 1 patient was positive for Ole e 1 (with a value of 1.09 kUA/l) while testing negative for the extract. On the other hand, 19.1% of patients were extract positive and Ole e 1 negative. AIT wasn't recommended for any of these patients, and in 2 (1.3%) patients AIT was specifically advised against due to the results. Half of these 30 Ole e 1 negative, extract positive patients suffered from birch pollen allergy, and AIT against birch was recommended or had already begun.

There was a strong correlation between ash extract and Ole e 1 (PCC 0.94,  $p < 0.01$ ; Fig. 1C) and a high agreement (82.9%) between extract and major-allergen results (Table 3).

**Mugwort :** Over the study period, 262 patients were included with results for both mugwort extract and Art v 1. The patients' median age was 31.2 (5-73 yrs). One hundred and ninety-nine patients had a positive result for the extract, while 109 were positive for the major allergen. 53.8% of extract positive patients were also positive for Art v 1, while 23.3% of all patients were negative for both measurements. Two patients (3.2%) with a negative result for the extract tested positive for Art v 1. Ninety-two (46.2%) extract positive patients showed negative results for Art v 1. Only one of these patients received AIT against mugwort, in 6 patients AIT was definitely advised against in the patient chart, while in 38 (41.3%) a ragweed-allergy was detected and AIT against ragweed recommended.

There was a strong correlation between mugwort extract and Art v 1 (PCC 0.87,  $p < 0.01$ ; Fig. 1D) and a moderate agreement (64.1%) between extract and major-allergen results (Table 3).

**Ragweed:** Two hundred and fifty-two patients with measured IgE against extract of ragweed and Amb a 1 as the major allergen were included. Median age was 32.0 years (6.1-74.3yrs). Of these patients 217 were positive for the extract and 161 for the major allergen Amb a 1. While 71.3% of extract positive patients were also positive to the major allergen, 11.9% of all patients were negative for both. Five patients had a negative result for the extract while testing positive for Amb a 1 (1 of these patients were prescribed AIT for ragweed). On the other hand, 61 had a positive extract and a negative Amb a 1 result. Of these patients only 1 was prescribed AIT for ragweed, 5 were advised against AIT and 6 were prescribed AIT for mugwort (as they were positive for Art v 1).

There was a moderate correlation between ragweed extract and Amb a 1 (PCC 0.71,  $p < 0.01$ ; Fig. 1E) and moderate agreement (73.8%) between extract and major-allergen results (Table 3).

## Double pollen-sensitization

**Tree pollen:** In 131 patients birch extract, ash-tree-extract, Bet v 1 and Ole e 1 IgE were determined. 117 patients showed a positive result to ash-tree extract, and 104 to birch. Twenty patients were mono-sensitized to ash-tree extract, while 7 were mono-sensitized to birch. Seven of 131 were negative for both birch and ash-tree extract. The 97 patients who were positive for birch and ash-tree extract (=double-sensitized) were further evaluated using the major allergens (Figure 2A). While 74/97 patients had a double-sensitization to Bet v 1 and Ole e 1, 15 were positive only for Bet v 1, 4 only for Ole e 1, and 4 were negative for Bet v 1 and Ole e 1.

**Weeds-Pollen:** We obtained results for mugwort extract, ragweed extract and the major allergens Art v 1 and Amb a 1 in 158 patients. Looking at the extracts, 137 were positive for ragweed and 121 for mugwort. Six were mono-sensitized to mugwort, 22 to ragweed. Further evaluation was done for 115 patients with double-sensitization to both extracts (Figure 2B). Twenty-nine of 115 patients were double-sensitized to both major allergens Art v 1 and Amb a 1. Seventeen patients were exclusively positive to Art v 1, whereas 48 patients were exclusively positive to Amb a 1. 21 patients were double-negative for Art v 1 and Amb a 1.

## DISCUSSION:

In this retrospective, data-base study we found that the use of major-allergen analysis improves the diagnosis of pollen allergy and helps recommending for or against AIT. The added value of MD was especially important in patients allergic to tree pollen or weed pollen as the differentiation between birch/ash-tree or mugwort/ragweed was not easily discernable without MD due to overlapping pollen seasons of these plants in Austria. Interestingly, in patients only sensitized to grass, birch or ash-tree, the agreement of IgE-reactivity to extract and major allergen was very high (83-94%), whereas it was lower for mugwort or ragweed (64%, 74%). Hence, it can be assumed that in single-sensitized grass- or tree pollen patients either the extract or the major allergens can be used interchangeably. This does not hold true for weed-sensitized patients or patients double-sensitized to birch and ash-tree or mugwort and ragweed.

Sastre et al<sup>14</sup> investigated if MD (using a micro-array-based panel including pollen-allergens) rather than results of SPT had an influence on the AIT decision in 141 patients with allergic rhinoconjunctivitis and/or asthma living in Spain. They found that including MD changed the AIT decision in 46% of the patients. As the pollen season is different in Spain compared to Austria with mainly cypress, plane, olive and grass-pollen being prevalent in the Spanish study region, the data cannot be directly compared to our observation. However, we found in our study similar results as 50% of patients with positive ash-tree extract, and negative Ole e 1 was prescribed birch-AIT (as they were Bet v 1 positive), and 41.3% of mugwort/ragweed double-sensitized patients were prescribed ragweed-AIT because of MD-results. An Italian study in 651 children found comparable results.<sup>15</sup> In 47% of pollen-allergic patients MD led to a change of AIT-recommendations. The pollen season in Italy is more comparable to Spain than to Austria but the principle is the same.

A more recent German study found no improvement by including MD in weed-allergic patients.<sup>16</sup> However they included only mugwort and English plantain in their IgE-profiles without looking at ragweed which is more relevant in Eastern-Austria than in the described German region.<sup>10, 17</sup>

The relevance of the present study is that it included a large number of patients (>4000) from the database of an allergy-center with a wide age group from toddlers to the elderly in eastern Austria. The number of double-sensitized patients with parallel extract and MD measurements provides a good basis for the analyses performed. We could also review the patients' charts if additional clinical information (such as the doctor's decision for or against AIT) was necessary.

## CONCLUSION

Molecular diagnostics (MD) has become an important tool in the hand of the allergologist. In patients with single sensitization to one pollen-species, extract-diagnosis would be sufficient. However, we definitely need MD in patients with sensitization to allergens of plants with overlapping pollen seasons when symptoms cannot be accounted to a single plant species.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest in relation to this work

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**Table 1. Pollen extracts and respective major allergens used in the study**

POLLEN EXTRACT	MAJOR ALLERGEN
Timothy-grass	Phl p 1+5
Birch	Bet v 1
Ash-tree	Ole e 1
Mugwort	Art v 1
Ragweed	Amb a 1

**Table 2. Patients' characteristics**

<b>N</b>	4234
<b>Age; mean (SD; range), years</b>	30.01 (15.40; 2.59-89.41)
<b>Children (0-12 years); n (%)</b>	673 (15.90)
<b>Adolescents (13-17 years); n (%)</b>	460 (10.86)
<b>Adults ([?] 18 years); n (%)</b>	3101 (73.24)

**Table 3. Comparison of sensitization to extracts and major allergens**

Pollen-extract/Major allergen; n	Major allergen positive; n	Major allergen negative; n	Agreement; n (%)
<b>Grass/Phl p 1+5</b> 1745 pos. grass extract 239 neg. grass extract	1646 12	99 227	1873 (94.4)
<b>Birch/Bet v 1</b> 1350 pos. birch extract 205 neg. birch extract	1271 8	79 197	1468 (94.4)
<b>Ash/Ole e 1</b> 157 pos. ash extract 24 neg. ash extract	127 1	30 23	150 (82.9)
<b>Mugwort/Art v 1</b> 199 pos. mugwort extract 63 neg. mugwort extract	107 2	92 61	168 (64.1)
<b>Ragweed/Amb a 1</b> 217 pos. ragweed extract 35 neg. ragweed extract	156 5	61 30	186 (73.8)

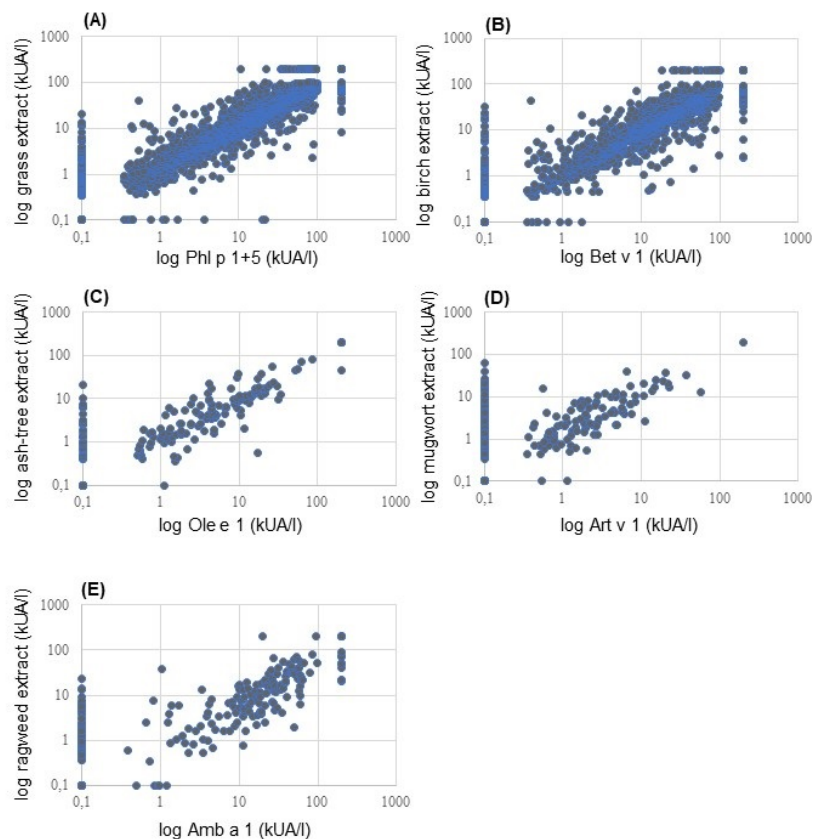
**Figure 1. Correlation between pollen extracts and respective major allergen +**

- (A) Grass - Phl p 1+5
- (B) Birch - Bet v 1
- (C) Ash-tree - Ole e 1
- (D) Mugwort - Art v 1
- (E) Ragweed - Amb a 1



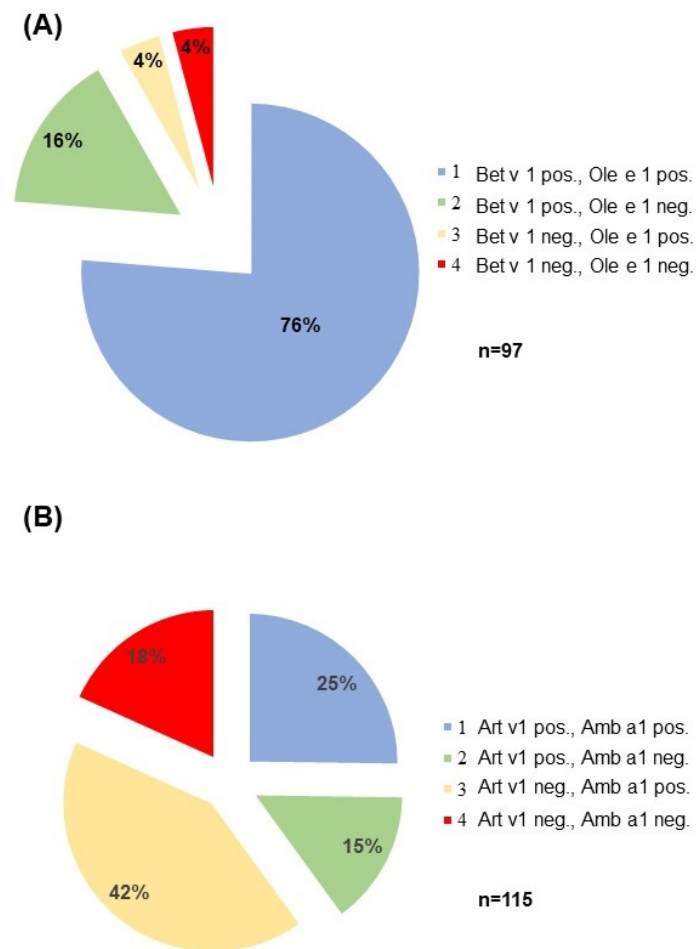
+ Figure adapted from Ref. 13 with permission of the author C.H.

**Figure 2.** IgE results for major allergens of patients double sensitized to: (A) birch and ash-tree extract (n=97), (B) mugwort and ragweed extract (n=115)



Correlation between pollen extracts and respective major allergen.  
(A) Grass - Phl p 1+5; (B) Birch - Bet v 1; (C) Ash-tree - Ole e 1; (D) Mugwort - Art v 1;  
(E) Ragweed - Amb a

Figure 1



IgE results for major allergens of patients double sensitized to: (A) birch and ash-tree extract, (B) mugwort and ragweed extract

Figure 2