

Cross-sensitization to *Artemisia* and *Ambrosia* pollen allergens in an area located outside of the current distribution range of *Ambrosia*

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Abstract

Introduction: The role of long-distance transported (LDT) *Ambrosia* pollen in inducing new sensitization and affecting sensitization rates in *Artemisia*-sensitized patients is unclear.

Aim: The aim of this study was to estimate the degree of cross-sensitization to *Ambrosia/Artemisia* allergens in citizens of Poznan (Western Poland). This area is covered by extensive *Artemisia* populations but does not currently have local *Ambrosia* populations.

Material and methods: Sera of 119 patients were tested by fluoroenzyme immunoassay (CAP-FEIA system) against pollen allergen extracts of *Artemisia vulgaris* and *Ambrosia artemisiifolia*, an allergenic component of *A. vulgaris* (*nArt v 1*), and an allergenic component of *A. artemisiifolia* (*nAmb a 1*). Skin prick tests (SPTs, *n* = 86) were performed with pollen allergen extracts of *A. vulgaris* and *A. artemisiifolia*. *Artemisia* and *Ambrosia* pollen in ambient air was collected (1996–2013) by a Hirst type volumetric trap sited at roof level (33 m).

Results: The SPT showed that the prevalence of sensitization to *Ambrosia* and *Artemisia* pollen exceeded 3.5%, and 10.5%, respectively. The measurements of IgE in blood serum (CAP-FEIA) revealed that among *Ambrosia*-sensitized patients 90.1% (20/22 patients) were concomitantly sensitized to *Artemisia*. 59.1% (13/22) of these patients reacted to *nArt v 1*, suggesting primary sensitization to *Artemisia* pollen. Only 2 (9.1%) patients were mono-sensitized to *Ambrosia* pollen extract, but surprisingly not to *nAmb a 1*.

Conclusions: The LDT *Ambrosia* pollen had a negligible effect on the rate of sensitization to *Ambrosia* allergens in Poznan and did not increase the prevalence of sensitization to *Artemisia* pollen in this region. However, the majority of patients showing hypersensitization to *Artemisia* pollen might also present symptoms during elevated episodes of LDT of *Ambrosia* pollen.

Key words: *Amb a 1*, *Art v 1*, immunoglobulin E (IgE), skin prick test, fluorescence-linked immunosorbent assay.

Introduction

Allergens released from *Artemisia* and *Ambrosia* pollen are an important cause of late-summer allergy worldwide [1–3]. Both genera are phylogenetically related and belong to the *Asteraceae* family [4]. *Artemisia* pollen grains contain several types of allergenic proteins, e.g. the major allergen *Art v 1* (a two-domain glycoprotein) and *Art v 6* (a pectate lyase) [5, 6]. The major allergen of *Ambrosia* pollen (*Amb a 1*) also belongs to the pectate lyase protein family and shares 65% sequence identity with *Art v 6* [5]. Another pro-

tein characterized from *Ambrosia* pollen, *Amb a 4*, is a close homologue of *Art v 1* [7]. A recent study has shown [8] that these proteins play an important role in *Artemisia* and *Ambrosia* pollen allergy and might be responsible for observed cross-sensitization, i.e. the phenomenon of the same IgE binding to several different, but structurally similar allergens, such as *Art v 1* and *Amb a 4* [9]. As a result, patients sensitized to *Artemisia* pollen may also show allergy reactions when in contact with *Ambrosia* pollen (and *vice versa*). However, the cross-sensitization should be clearly distinguished

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from co-sensitization – the simultaneous presence of different IgEs binding to allergens without common structural features, such as Art v 1 and Amb a 1. Although the consequences for patients with co- and cross-sensitization are similar, i.e. allergy symptoms to both pollen types, the etiology (causative factor) is different. This may, in turn, affect the overall cost and efficacy of allergy immunotherapy.

Artemisia pollen grains observed in the air of Poland (Central Europe) are mainly released by local populations of *Artemisia* species, e.g. *A. vulgaris*, *A. campestris* and *A. absinthium* [10]. *Artemisia vulgaris* is the most widely distributed [11] and therefore should be considered as the main pollen contributor. The intensity of *Artemisia* pollen seasons recorded in Poland is one of the highest in Central and Eastern Europe [12]. In addition, *Artemisia* pollen seasons in Poznan (Western Poland) have started earlier and increased in length over the last two decades [13]. *Artemisia* pollen allergens are therefore considered to be one of the most common causes of allergic diseases of the upper respiratory tract in Poland, ranking third behind Poaceae and *Betula* pollen allergens [1].

In contrast, *Ambrosia artemisiifolia* (the most common ragweed species) is an invasive weed from North America, which has currently infested many regions in Asia, particularly Eastern China and South Korea [14, 15], Australia [16], and Europe, especially the Pannonian Plain (mainly Hungary and Northern Serbia) as well as parts of France, Northern Italy (Po Valley) and Ukraine [17]. An expansion of the plant's distribution into Central and Northern Europe (e.g. Germany) has also been observed in recent years [18]. In Poznan, however, *Ambrosia* has only occurred ephemerally and the majority of recorded *Ambrosia* pollen grains are transported from distant sources, particularly from the Pannonian Plain and Ukraine [19–21]. As a result, exposure to *Ambrosia* pollen allergens in Poznan depends highly on meteorological conditions facilitating long-distance transport [22].

It is interesting to note that in areas where both plant species are present, e.g. Northern Italy, an unexpectedly high prevalence of concomitant sensitization to these airborne allergens has been recorded [8, 23]. In other words, the expansion of *Ambrosia* not only resulted in the increase of *Ambrosia* pollen allergy but also a parallel increase in *Artemisia* pollen sensitization. This phenomenon might be especially important in such areas as Poland where: (1) a high rate of *Artemisia* pollen allergy is observed; (2) *Ambrosia* invasion is still in its initial phase (mainly limited to the south of the country), and the majority of ragweed pollen arrived from distant sources. With overlapping pollination periods and cross-reacting pollen allergens these species may concomitantly affect sensitized patients, hindering appropriate allergy treatment.

Aim

Therefore, the aim of this study is to determine the prevalence of *Artemisia* and *Ambrosia* pollen allergy and

to identify the degree of cross-sensitization among citizens of Poznan. In addition, we examined airborne *Artemisia* and *Ambrosia* pollen concentrations recorded over the last 18 years, as exposure is an important factor that plays a role in the induction of allergy sensitization.

Material and methods

Clinical survey

Clinical studies were performed at the Department of Dermatology, University of Medical Sciences in Poznan. Poznań is the main city of Western Poland (and the fifth largest city in Poland) with a population of ~550 000 [24]. The examined group included 119 consecutive patients (48 males and 71 females), aged 3–66 years. Mean age was 22.8 years. Patients were hospitalized, or attended our outpatient clinic. They presented various manifestations of atopy: mostly atopic dermatitis (83 patients – 70% of examined group), allergic rhinitis and conjunctivitis (29 patients – 24% of examined group) and asthma (7 patients – 6% of entire group). All patients were residents of Poznan and the surrounding area. In all recruited patients, total and antigen-specific IgE (as IgE) levels were measured in serum using fluoroenzyme immunoassay (FEIA) (CAP System 100, Phadia) against:

- allergen extract of *Artemisia vulgaris* pollen,
- allergen extract of *Ambrosia artemisiifolia* pollen,
- allergenic component *Artemisia vulgaris* (*nArt v 1*),
- allergenic component of *Ambrosia artemisiifolia* (*nAmb a 1*).

Fluoroenzyme immunoassay (CAP System FEIA – Phadia) was used according to the producer's instructions. Results were presented as kU/l and interpreted according to the age standard (total IgE), or interpreted as classes (asIgE) – an antibody level lower than 0.35 kU/l was interpreted as “serum antibodies undetectable”.

On the basis of obtained results, the patients were divided into 3 subgroups:

- Subgroup I. Detectable serum antibodies against *Artemisia* and *Ambrosia* pollen extracts (Art+/Amb+), cross-sensitized patients.
- Subgroup II. No detectable serum antibodies against *Artemisia* pollen extract, but detectable against *Ambrosia* (Art–/Amb+), patients mono-sensitized to *Ambrosia* pollen.
- Subgroup III. Detectable serum antibodies against *Artemisia*, but not against *Ambrosia* pollen extract (Art+/Amb–), patients mono-sensitized to *Artemisia* pollen.

Within these three subgroups, the number of patients with positive reactions to particular allergenic components *nArt v 1/nAmb a 1* was calculated. Asero *et al.* [8] stated that the major *Artemisia* pollen allergen (Art v 1) can be considered as the best marker of primary *Artemisia* sensitization. On the other hand, due to the high cross-reactivity between Amb a 1 and Art v 6, it is not possible to distinguish between co- and cross-sensi-

tization in patients reacting to both major *Artemisia* and *Ambrosia* pollen allergens (*nArt v 1+/nAmb a 1+*). As we did not include Art v 6 in the FEIA test, all patients showing reactions to *Artemisia* and *Ambrosia* pollen extracts/allergens will be considered, in this study, as cross-sensitized. If sensitization to *nArt v 1* was observed (*Art+/Amb+* and *nArt v 1+*), patients were further classified as primary sensitized to *Artemisia*.

In addition, skin prick tests (SPTs) were performed on a total of 86 patients (72% of the 119 patients examined). It was not possible to carry out SPTs on the remaining 33 patients because of significant exacerbations of skin inflammatory lesions and constant administration of anti-inflammatory and anti-allergic treatment. The SPTs were performed using airborne allergen extracts (Allergopharma GmbH & Co. KG, Reinbek, Germany): *Artemisia vulgaris* and *Ambrosia artemisiifolia*. Each allergen concentration in a tested substance was expressed as biological units (BE/ml or SBE/ml) and/or PNU.

The SPTs were applied to the skin on the inner part of the forearm, approximately 5 cm from the wrist and 3–5 cm from popliteal fossa. The SPTs were examined 20 min after application and the results were presented as the mean diameter of the wheal and erythema, but also on a “plus” scale by comparing the diameters of allergen wheal and control wheal (both positive and negative control). In addition, a doubtful result was defined as an allergen wheal with mean diameter equal to or larger than the half of the mean diameter of a histamine wheal [25].

Pollen monitoring

Artemisia and *Ambrosia* pollen grains in ambient air were collected (1996–2013) by a volumetric trap of the Hirst design [26] sited at roof level (33 m) approximately 8 km from Poznan city centre (52°24'N 16°53'E). The pollen counting methods used in Poznan during the years studied have previously been described in detail [27]. Daily averages of airborne *Artemisia* and *Ambrosia* pollen counts were converted to concentrations and expressed as particles per cubic metre of air (P/m³) [28].

The number of days with daily mean *Artemisia* and *Ambrosia* pollen concentrations ≥ 30 P/m³ was extracted from the pollen database. The threshold value (≥ 30 P/m³) was chosen based on the atmospheric concentrations of *Artemisia* and *Ambrosia* pollen often reported to evoke allergic symptoms (De Weger *et al.* [29] and references therein). Next, the probability risk (*p*) of recording at least 1, 3, 5, 7 and 10 days with an elevated daily mean *Artemisia* and/or *Ambrosia* pollen level (≥ 30 P/m³) during every decade from the beginning of July to the end of September was calculated. The following descriptors of probability were applied (adapted from [30]: very low ($0.0 \leq p \leq 0.25$), low ($0.25 < p \leq 0.50$), medium ($0.5 < p \leq 0.75$), high ($0.75 < p \leq 0.90$), very high ($0.90 < p \leq 1.00$).

Results

Clinical survey

FEIA: Out of all recruited patients (*n* = 119), positive results for *Artemisia* and/or *Ambrosia* allergen extracts were obtained in 29 patients (29/119 – 24.4%) (Table 1).

Twenty-two (18.5%) patients reacted to *Ambrosia* pollen extract (Subgroups I and II):

- The majority of these patients (20/22 – 90.1%) were also sensitized to *Artemisia* pollen extract, and 59.1% (13/22) of them reacted to *nArt v 1*, suggesting primary sensitization to *Artemisia* pollen.
- Eight patients (8/22 – 36.4%) did not react to *nAmb a 1* and *nArt v 1*; they could therefore be sensitized to other (minor) *Ambrosia/Artemisia* pollen allergens. Six of them (Subgroup I; *Art+/Amb+*) were potentially cross-sensitized, and the remaining 2 (Subgroup II, *Art-/Amb+*) were mono-sensitized to *Ambrosia* pollen, but surprisingly not to the major *Ambrosia* pollen allergen – *nAmb a 1*.
- Four patients (4/22 – 18.2%) reacted to the major *Ambrosia* pollen allergen – *nAmb a 1*, but only 1 patient did not show concomitant reaction to *nArt v 1*. As this patient also reacted to *Artemisia* pollen extract (*Art+*),

Table 1. Results of FEIA grouped according to hypersensitivity to *Ambrosia* and/or *Artemisia* pollen extract detected in 119 examined patients

Clinical test		<i>Ambrosia</i> and <i>Artemisia</i> allergen extracts (subgroups)			Sum
		Subgroup I <i>Art+/Amb+</i> (number of patients; %)	Subgroup II <i>Art-/Amb+</i> (number of patients; %)	Subgroup III <i>Art+/Amb-</i> (number of patients; %)	
		20/119 (16.8%)	2/119 (1.7%)	7/119 (5.9%)	29/119 (24.0%)
Allergenic components	<i>nAmb a 1 (+)/nArt v 1 (+)</i>	3 (15%)	0	0	3 (10.4%)
	<i>nAmb a 1 (+)/nArt v 1 (-)</i>	1 (5%)	0	0	1 (3.4%)
	<i>nAmb a 1 (-)/nArt v 1 (+)</i>	10 (50%)	0	4 (57%)	14 (48.3%)
	<i>nAmb a 1 (-)/nArt v 1 (-)</i>	6 (30%)	2 (100%)	3 (43%)	11 (37.9%)

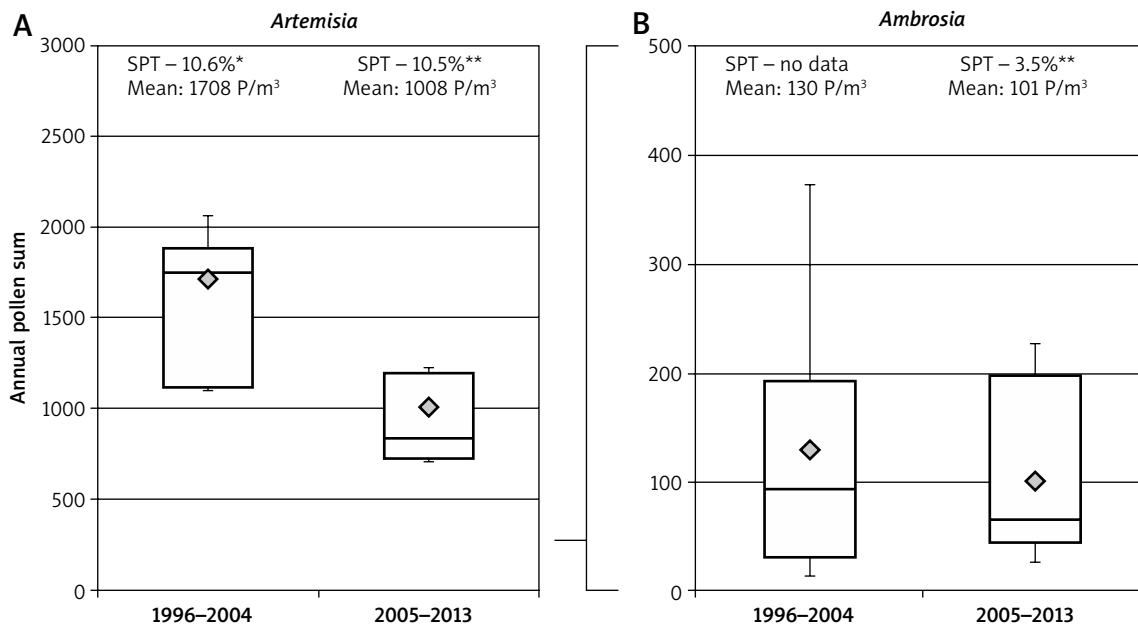


Figure 1. Comparison of the annual sum of airborne *Artemisia* (A) and *Ambrosia* (B) pollen between 1996–2004 and 2005–2013, and SPT values against *Ambrosia* and *Artemisia* pollen in Poznan. Data collection: *2002–2004 from Stach *et al.* (2005); **2010–2012 from this study

there is a high chance that the reaction to *nAmb a 1* resulted from the cross-sensitivity to Art v 6.

Twenty-seven patients were sensitized to *Artemisia* pollen extract (20 from Subgroup I and 7 from Subgroup III). 74.1% (20/27) of them reacted to *Ambrosia* pollen extract. A total of 63% (17/27) showed reactions to the major *Artemisia* pollen allergen *nArt v 1*.

SPT: Of the 86 atopic patients examined, a total of 16 patients presented either positive (9/86 – 10.5%) or doubtful (7/86 – 8%) results for *Artemisia* allergen extract (Figure 1). With respect to the *Ambrosia* allergen extract, 3 patients exhibited positive (3/86 – 3.5%) and 9 patients doubtful (9/86 – 10.5%) SPTs. Simultaneously positive and/or doubtful SPT results for both allergens were obtained in 9 out of 86 patients (10.5%).

Aerobiological data

During the examined period (July–September) the highest probability of recording at least 1 day with an elevated *Artemisia* pollen level (≥ 30 *Artemisia* P/m³ daily average) was observed in the 1st and 2nd 10-day period in August ($p = 0.89$) (Figure 2 A). During the 1st 10-day period the probability was also high for ≥ 3 days ($p = 0.83$) and medium for ≥ 5 and ≥ 7 days with ≥ 30 *Artemisia* P/m³ ($p = 0.72$ and $p = 0.61$, respectively). In relation to *Ambrosia* pollen, the probability of exceeding the threshold value (≥ 30 *Ambrosia* P/m³ daily average) at least once during a 10-day period was the highest in the 3rd 10-day period in August and the 1st 10-day period in September ($p = 0.28$ and $p = 0.22$, respectively) (Figure 2 B). In general, the probability of recording elevated daily

mean *Ambrosia* pollen levels during selected 10-day periods was very low or low (never exceeding ≥ 0.30). When both taxa were considered together (Figure 2 C) the most distinct differences were observed in the 3rd 10-day period in August, when the probability of recording at least one day with mean daily *Artemisia* or *Ambrosia* pollen levels ≥ 30 P/m³ increased to 0.39. However, the probability of simultaneously observing elevated daily average concentrations of pollen (≥ 30 P/m³) from both pollen taxa in the same 10-day period was very low (e.g. $p = 0.19$ in the 3rd 10-day period of August) (Figure 2 D).

Discussion

The prevalence of pollen allergy to particular plant species is closely related to the geographical location, and more specifically to the intensity of pollen exposure in that area. In Poland, where *Artemisia* pollen is considered to be an important aeroallergen, the prevalence to *Artemisia* pollen allergens is over 20% and one of the highest in Europe [1]. In our study, depending on tests used, the prevalence varies from 10.5% (positive reaction in SPT), to 15.1% (positive reaction to Art v 1), and 22.7% (positive reaction to *Artemisia* pollen extract). It is worth noting that among patients sensitized to *Artemisia* pollen extract (FEIA), 63% reacted positively to the allergenic component *nArt v 1* (the major *Artemisia* pollen allergen). The remaining *Artemisia*-hypersensitive patients presumably reacted to other *Artemisia* pollen allergens. For instance, according to Gadermaier *et al.* [31] Art v 3 can be considered as a new major *Artemisia* pollen allergen, with the

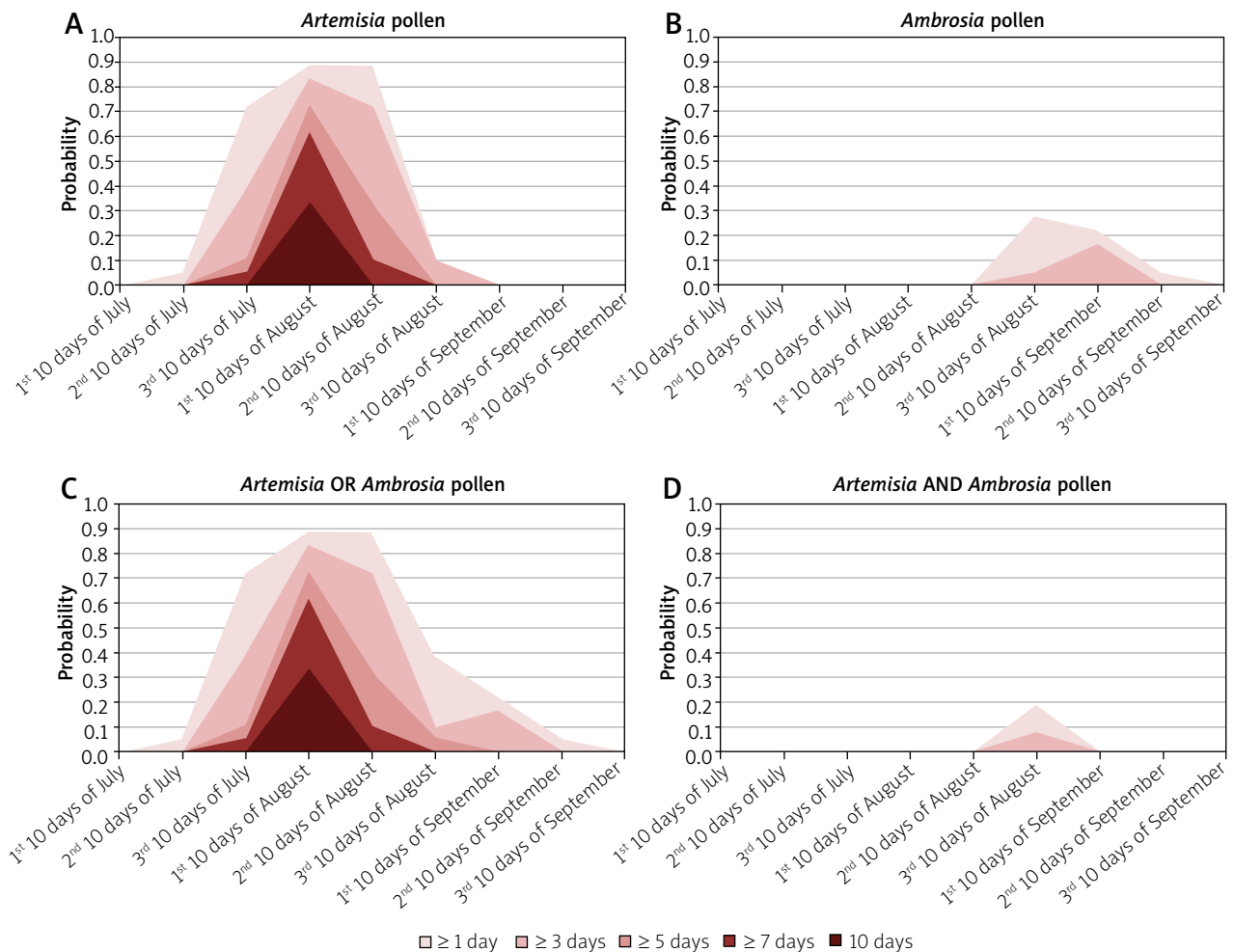


Figure 2. Probability of recording a certain number of days with elevated mean daily *Artemisia* (A), *Ambrosia* (B), *Artemisia* OR *Ambrosia* (C), and *Artemisia* AND *Ambrosia* (D) pollen concentrations (≥ 30 P/m³) in the air of Poznan during every 10-day period from the beginning of July to the end of September

prevalence of reactivity among *Artemisia* pollen-sensitized individuals reaching 85%. The comparison of SPT results obtained in this study with results obtained in a previous analysis conducted during 2002–2004 [32] shows that the rate of sensitization to *Artemisia* pollen has not markedly changed (from 10.6% to 10.5%). Although the pollen level of *Artemisia* is gradually decreasing in Poznan (likely due to drier summers and reduction of suitable habitats) [13], *Artemisia* still represents a major source of allergenic sensitization in atopic patients, with stable prevalence over the last decade. These results are in contrast with Northern Italy, where the prevalence of *Artemisia* sensitivity has increased dramatically during recent decades in parallel with the spread of *Ambrosia* [33].

For *Ambrosia*, the highest rate of allergy is observed in countries that are significantly infested by this plant, such as Hungary and France [1]. On the other hand, there is almost no sensitization to *Ambrosia* pollen allergens in Scandinavia (a region with negligible local sources). The estimated prevalence of sensitization to *Ambrosia* pollen

in Poznan (3.5%) is similar to that observed in Scandinavia (i.e. 2.3% in Finland), and lower than in neighbouring countries, e.g. 14.4% in Germany [1] and around 20% in Czech Republic [34]. It is also lower than the rate of sensitization to *Ambrosia* pollen allergens observed in other Polish cities, e.g. 10.8% in Lodz (Central Poland) [1], and almost 50% of patients reacted to *Ambrosia* pollen allergens in Krakow (Southern Poland) [35]. These two cities are located further south than Poznan, and as such are nearer to the northern distribution range of *Ambrosia* in Europe [17]. Moreover, in Southern Poland *Ambrosia artemisiifolia* occurs in several well-established populations (> 1000 individuals), becoming a distinct local source of *Ambrosia* pollen [36].

It should, however, be noted that exposure to pollen is not only related to the plant's distribution area but may also depend on the ability of pollen to be transported over long distances. Episodes of long-distance transport (LDT) of *Ambrosia* pollen from infested to "ragweed-free" areas, such as Poznan, have been detected over the last

decades [19–21]. A recent study [37] also revealed that these pollen grains maintain their allergic potency during the atmospheric transport and therefore are equipped to induce allergic reactions in sensitized patients. However, it is still unclear whether this may affect the overall prevalence of *Ambrosia* pollen allergy. For instance, Cecchi *et al.* [38] observed that the prevalence of *Ambrosia* sensitization remains low (< 10%) in Northern Italy, where people are only exposed to LDT *Ambrosia* pollen. The same situation can be observed in Poznan. We found that the probability of recording high atmospheric concentrations of *Ambrosia* pollen in Poznan is low or very low ($p < 0.30$). In addition, as previous studies revealed [20, 22], episodes of LDT of pollen are short (lasting 1–2 days) and usually not very intense. It therefore seems that LDT episodes are too intermittent to markedly influence the prevalence of allergy. On the other hand, the distribution range area of *Ambrosia* in Europe is changing, mainly expanding into northerly areas [39]. We may therefore expect that the frequency and magnitude of LDT episodes will increase in areas such as Poznan as a product of reduced distance to the pollen source. As a consequence, it may cause the prevalence of sensitization to *Ambrosia* pollen allergy to increase, as seen in Germany [1].

Due to the cross-sensitization between *Artemisia* and *Ambrosia* pollen allergens, the expansion of ragweed may be particularly important for people already sensitized to *Artemisia*. In our study, almost 75% of patients sensitized to *Artemisia* pollen extract showed positive reactions to *Ambrosia* pollen extract. In addition, possible cross-sensitization was observed in 55% of patients with positive reactions to *Artemisia* and *Ambrosia* pollen extract (Subgroup I). The majority of these patients were primarily sensitized to *Artemisia*. As a result, we should expect that over half of patients showing hypersensitization to *Artemisia* pollen might also present symptoms during elevated episodes of LDT of *Ambrosia* pollen. This is most likely to occur at the end of August and beginning of September, i.e. the period with the highest probability of recording high concentrations of *Ambrosia* pollen in the air. Although the risk is still low, this should be taken into account when investigating unusual occurrences of allergy symptoms in sensitized patients during the late *Artemisia* pollen season.

Six of the patients (27.3%) belong to Subgroup I (Art+/Amb+), suggesting cross-sensitization, likely to pan-allergens such as profilins (e.g. Amb a 8 and Art a 4) or calcium-binding proteins (e.g. Art v 5, Amb a 9 and Amb a 10) [40]. In contrast to the high prevalence of cross-sensitization among investigated patients with *Artemisia* and *Ambrosia* pollen allergy, the frequency of possible co-sensitization was rather low (15%). In addition, as we did not investigate the reactivity to Art v 6 (which is a homologue of Amb a 1), we cannot exclude the possibility that these patients were in fact cross-sensitized. The low prevalence of co-sensiti-

zation observed in Poznan concurs with the findings from other European studies. For instance, in Switzerland, the observed sensitization to *Ambrosia* was in most cases a consequence of a primary sensitization to *Artemisia* [41], whilst no co-sensitized patients have been found in Northern Italy [8].

Conclusions

Western Poland is currently a ragweed free area, without local plant populations, and the majority of pollen grains recorded in Poznan are transported from very far away. However, in recent decades the distance from the nearest *Ambrosia* sources has been gradually decreasing. *Ambrosia* has infested areas in Northern Europe; it has been observed in Eastern Germany and Southern Poland. The “ragweed pollen noose” is slowly tightening... how this situation will affect the allergic population of Poznan is still uncertain. However, it seems that nowadays, *Ambrosia* pollen does not pose a high allergenic risk in Poznan. The probability of recording elevated mean daily *Ambrosia* pollen levels is still low and limited to a 20-day period (in late August and early September). In addition, the chances of observing high concentrations of *Artemisia* and *Ambrosia* pollen in the air at the same time are even lower, which should be helpful information for physicians choosing appropriate allergy immunotherapy. Finally, the prevalence of *Ambrosia* pollen allergy is very low, and the co-sensitization to both investigated species is a rather rare phenomenon. This positive information should not, however, lull our sense of security. *Ambrosia* produces one of the most potent aeroallergens and its distribution range is gradually expanding; it surely requires the highest attention.

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Conflict of interest

The authors declare no conflict of interest.

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