



Clinical relevance of *Alternaria alternata* sensitization in patients within type 2-high and type 2-low asthma

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ABSTRACT

Alternaria sensitization is correlated with persistent asthma. Type 2 (T2)-asthma endotypes are characterized by the release of eosinophils. However, the prevalence and sensitization patterns in patients with *Alternaria* asthma between T2-high and T2-low endotypes are unknown. We retrospectively reviewed 582 patients with *Alternaria* asthma and divided them into T2-high ($n = 376$) and T2-low ($n = 206$) groups with a threshold of 300 cells/ μ L in blood eosinophil counts. Data for basic information, skin test or IgE detection results, and blood eosinophil counts were collected. The age of patients in the T2-high group (13.66 ± 13.23) was lower than that of the T2-low group (18.02 ± 15.03). Patients with T2-high asthma had relatively higher rates of taking inhaled corticosteroids (ICS) and positive family history than the T2-low group. Pet keepers and allergen immunotherapy (AIT) patients were comparable between these groups. In the T2-high group, patients had higher levels of total serum IgE (T-IgE) and showed a significant positive correlation with eosinophil counts ($r = 0.166$, $P = 0.001$), followed by higher *Alternaria*-specific IgE (sIgE) levels (median, 13.7; range, 4.86–25.3). Compared to the T2-low group, the frequency of poly-sensitized patients and the rate of each allergen among the nine common allergens were all higher in the T2-high group; the statistical differences mainly focused on pollens such as birch ($P = 0.005$), firmiana ($P = 0.004$), and mugwort ($P = 0.005$). Young, male patients had a high prevalence of T2-high *Alternaria* asthma, along with higher rates of T-IgE, sIgE levels, and poly-sensitized patterns.

1. Introduction

Asthma is a common chronic and complex inflammatory disease of the airways, and there is marked heterogeneity in the clinical symptoms that affect 339 million people worldwide [1]. Sensitization to perennial inhalant allergens, such as mold, dust mite, and animal epithelia, are frequently observed in asthmatic patients [2]. Notably, previous studies have established that sensitization to *Alternaria alternata* is a significant risk factor for asthma severity and hospitalization [3]. A survey found that indoor *Alternaria* odor in the workplace and inner-city increases the risk of asthma [4,5]. In China, dampness and mold at home are believed to contribute to asthma, allergic rhinitis, and eczema in preschool children [6]. Hence, *Alternaria* are considered one of the most important allergens that play a major role in the persistence of asthma. *Alternaria* is a ubiquitous mold that thrives on the ground and on plants, and while it is typically found in outdoor environments, natural ventilation and

indoor flower soil are common indoor sources [7]. *Alternaria* spores are considered the primary source of allergens, and increased humidity and warm temperatures can increase *Alternaria* spore release [8]. Consequently, despite recent advances in treatments and efforts to relieve the severity of asthma caused by *Alternaria*, patients still have high rates of complications and exacerbations.

Recently, a new classification of asthma phenotypes emphasized the importance of T2- inflammation [9], and it is now standard practice to differentiate patients with T2-high and T2-low asthma based on blood eosinophil counts [10]. Patients with T2-high asthma are more likely to elicit eosinophils and associate with type 2 innate lymphoid cells [11], while T2-low asthma might be regulated by neutrophils and T_H17 cells [9]. The overlap of allergic, eosinophilic, and T2 inflammation in asthma was demonstrated by Chen et al. [12], who revealed that 60.8% of allergic patients had T2-asthma, although allergic asthma was the most prevalent phenotype. Meanwhile, several investigations have

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Table 1

Baseline demographic and characteristics of the T2-high and T2-low endotypes (N = 582).

Characteristics	T2-high (n = 376)	T2-low (n = 206)	P values
Age (y), (Mean $\pm$ SD)	13.66 $\pm$ 13.23	18.02 $\pm$ 15.03	< 0.001
Male, n (%)	273 (72.6)	122 (59.2)	0.001
Family history, n (%)	122 (32.4)	57 (27.7)	0.260
ICS, n (%)	208 (55.6)	97 (47.6)	0.068
AIT, n (%)	99 (26.9)	52 (25.7)	0.843
Pets, n (%)	31 (8.2)	15 (7.3)	0.750
T-IgE (kU/L), median (range)	387 (165–903.5)	260 (113–722.5)	< 0.001

T2, Type 2; ICS, Inhaled Corticosteroids; AIT, Allergen Immunotherapy; T-IgE: Total Immunoglobulin E.

identified a significant association between microbiota composition in bronchial and T2-type asthma. Sharma et al. [13] found that the fungal microbiome was diverse among different asthma endotypes in airways and further identified *Fusarium*, *Cladosporium*, and *Aspergillus* enriched in T2-high asthma. Moreover, in severe asthma, Vandenberg et al. [14] illustrated that higher fungal taxa existed during asthma exacerbation when compared to the clinical stability period.

However, the clinical features of patients with asthma sensitized by

Alternaria in T2-high and T2-low endotypes are still unknown, and understanding these manifestations in the two different groups based on blood eosinophil counts would be useful in guiding treatment decisions and optimizations. Therefore, our study aimed to characterize both the potential clinical relevance and prevalence of *Alternaria* sensitization asthma patients in T2-high and T2-low endotypes, to better clarify the diversity of endotypes in the *Alternaria*-induced asthma population.

2. Materials and methods

2.1. Study design and patients

We conducted a retrospective analysis of 582 patients aged 1–72 years with a diagnosis of asthma treated in the outpatient allergy department of Peking Union Medical College Hospital (PUMCH) between January 2016 and January 2021. The diagnosis was determined by an experienced allergist according to the Global Initiative for Asthma guidelines. As this was a retrospective study with anonymous data collection, informed consent and ethical approval were required rapidly by the International Review Board of PUMCH (No. S-K1672).

This study used patient charts as the primary source of information regarding outpatient visits. Data were collected for laboratory results

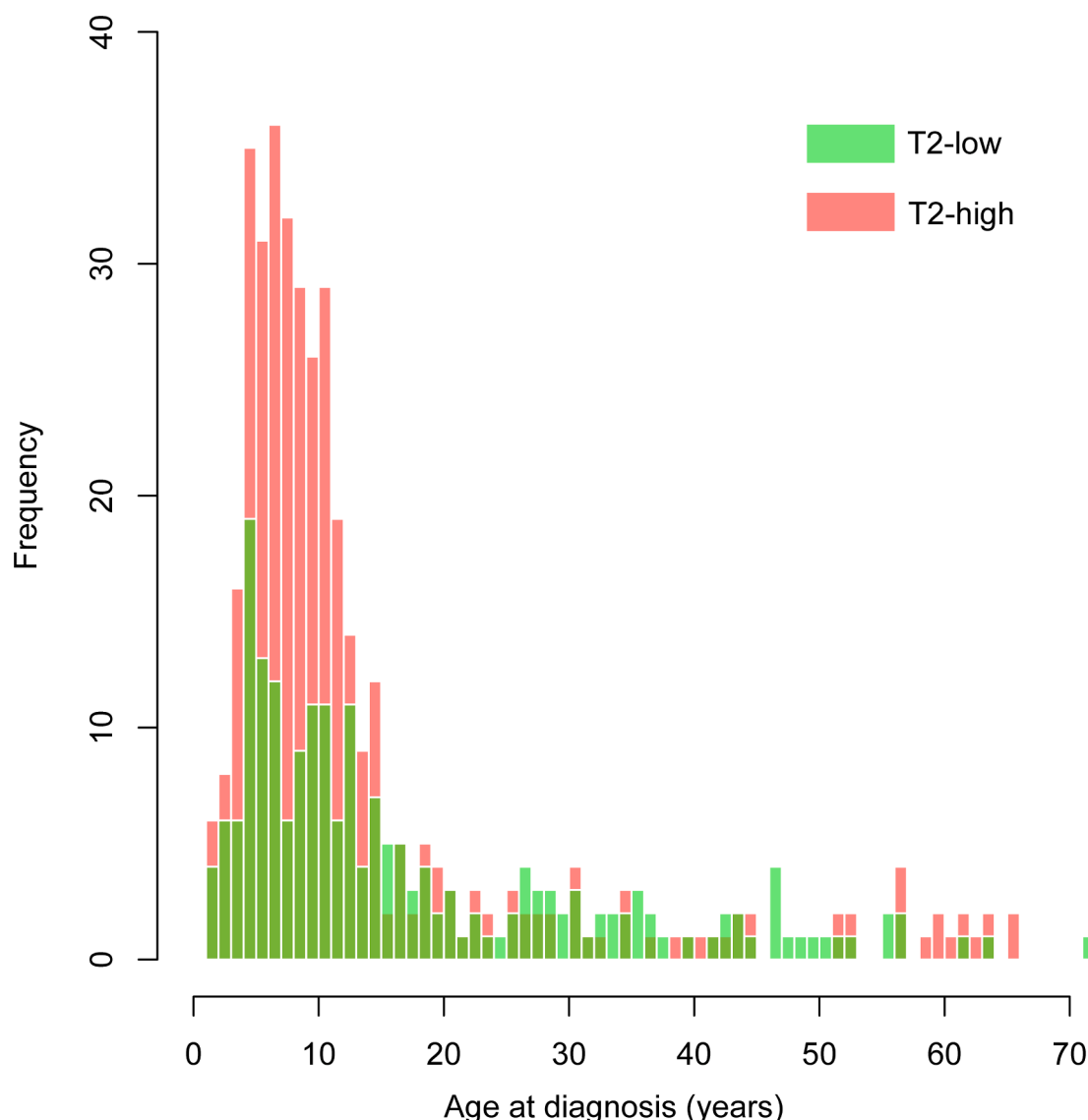


Fig. 1. Age at diagnosis and distribution in the T2-high and T2-low endotypes (n = 582). **Abbreviations:** T2, Type 2.

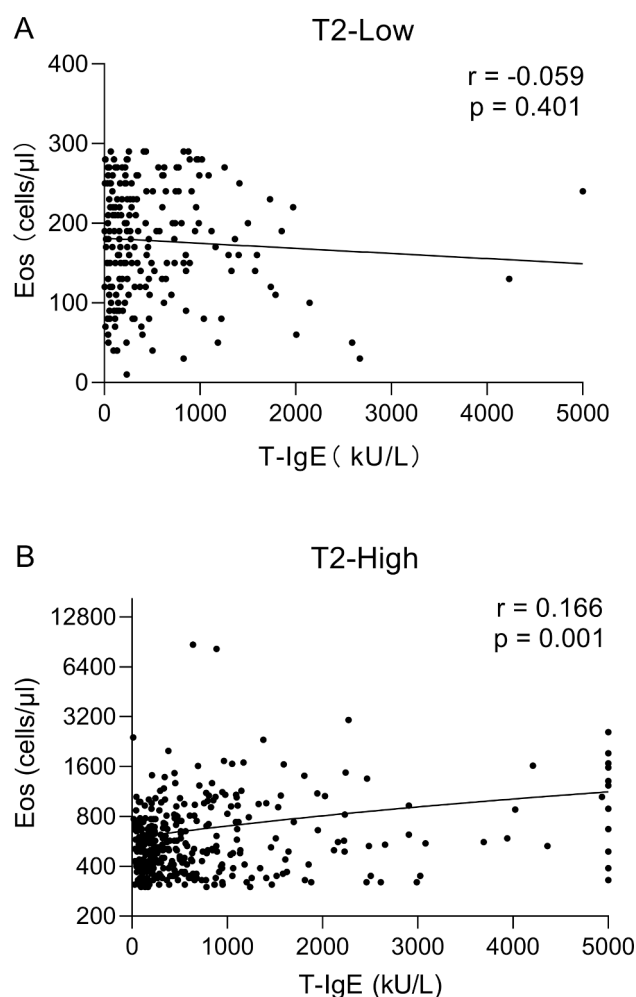


Fig. 2. Correlation between T-IgE and blood eosinophil counts in the T2-high and T2-low endotypes ($n = 582$). (A) A negative correlation between T-IgE and blood eosinophil counts in the T2-low endotypes ($n = 206$). (B) A positive correlation between T-IgE and blood eosinophil counts in the T2-high endotypes ($n = 376$). **Abbreviations:** T-IgE: Total Immunoglobulin E; Eos: Eosinophil; T2, Type 2.

(blood cell counts, skin test, and IgE detection for *Alternaria* and other common aeroallergens) and medical history during the study period. Patients who had negative tests for *Alternaria*, incomplete data, other severe debilitating chronic diseases, and dermatographism were excluded from the study. Altogether, 582 patients with asthma induced by *Alternaria* were classified as T2-high ($n = 376$) and T2-low ($n = 206$) according to blood eosinophil counts (≥ 300 cells/ μ L or < 300 cells/ μ L).

2.2. Skin test, total IgE, and allergen-specific IgE

Patients underwent intradermal skin tests with *Alternaria* extracts and nine other common aeroallergens: dust mite, cat dander, dog hair, mugwort, sabina, birch, humulus, firmiana, and ragweed. All extracts were provided by the Allergen Manufacturing and Research Center (PUMCH, Beijing, China). Intradermal skin tests were performed by a well-trained nurse under the supervision of allergists. Histamine phosphate (0.01 mg/mL) [15,16] and allergen diluent were used as positive and negative controls, respectively. The results were evaluated by the nurse or clinic allergists 15 min after application of the allergen. A wheal size of at least 5 mm larger than the negative control was defined as a positive skin response. The grading system was used according to our previously published article [17]: (1) negative = wheal and erythema < 5 mm or no erythema; (2) class 1 = wheal 5–10 mm and erythema

10–20 mm; (3) class 2 = wheal 10–15 mm and erythema 20–30 mm; (4) class 3 = wheal ≥ 15 mm and erythema ≥ 30 mm or with pseudopod formation; (5) class 4 = wheal ≥ 20 mm and erythema ≥ 30 mm or with systemic allergic reaction.

T-IgE and sIgE levels were measured using the ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden) adhering to the manufacturers. All 582 patients were tested for total IgE, and 483 patients had sIgE results for *Alternaria*. Threshold values higher than 0.35 kUA/L were considered positive. Sensitization types were divided into *Alternaria* mono-sensitization and poly-sensitization (*Alternaria* and at least one of the other allergens).

2.3. Data collection

We collected and entered patients' basic demographic data, blood eosinophil counts, T-IgE results, pets, ICS, AIT, and family history of allergy into an Excel spreadsheet and analyzed the data. We further collected skin tests and sIgE levels. Vitamin D values were also determined, if available.

2.4. Statistical analysis

All data were analyzed using SPSS (version 24.0; IBM Inc., Chicago, IL, USA), Prism 9.0 (GraphPad, California, USA), and R packages (R Foundation for Statistical Computing, Vienna, Austria). A descriptive analysis was performed, and the results are presented in a table. Continuous variables are presented as mean (SD) or median (interquartile range). Categorical data were summarized using percentages and frequency counts. Correlations were calculated using Spearman's correlation analysis. Statistical significance was set at $P < 0.05$.

3. Results

A total of 582 patients aged 1–72 years with asthma (mean age, 15.2 ± 14.04) were included and categorized into T2-high ($n = 376$) and T2-low ($n = 206$) groups depending on the eosinophil counts in blood (Table 1). Overall, 395 (67.9%) were male, and the age of all patients ranged from 2 to 18 years, the T2-high and T2-low groups had a similar trend in age distribution (Fig. 1). The number of males was higher than that of females in both groups, and the percentage of males in the T2-high group (72.6%) was significantly higher than that in the T2-low group (59.2%). The age of patients in the T2-high group (13.66 ± 13.23) was significantly younger than that in the T2-low group (18.02 ± 15.03), with a P -value < 0.001 .

As for the family history of allergy, in the T2-high group, 122 (32.4%) patients had a relatively higher rate of positive family history than the T2-low group (27.7%). Similarly, the rates of taking ICS were higher in the T2-high group (55.6%) than in the T2-low group (47.6%), although the difference was not significant. The number of patients with pets was comparable between both groups (8.2% vs. 7.3%). Meanwhile, patients in the T2-high group (26.9%) who accepted *Alternaria* subcutaneous immunotherapy after diagnosed asthma also corresponded to the T2-low group (25.7%).

T-IgE levels ranged from 3 to 5000 kU/L (median, 337 kU/L). The T2-high group had higher levels of T-IgE (median, 387 kU/L; range 165–903.5 kU/L) than the low group (median, 260 kU/L; range 113–722.5 kU/L). A statistically significant positive correlation was observed between increasing eosinophil counts and T-IgE levels in the T2-high group ($r = 0.166$, $P = 0.001$), whereas no significant association was observed in the T2-low group ($r = -0.059$, $P = 0.401$) (Fig. 2). From all 582 patients, sIgE levels (≥ 0.35 kUA/L to *Alternaria alternata*) were detected in 483 patients, and the values in 415 patients were more than 0.7 kUA/L. *Alternaria* sIgE levels measured in T2-high patients (median, 13.7; range 4.86–25.3 kUA/L) were significantly higher than those of T2-low patients (median, 10.1; range 4.02–19 kUA/L) (Fig. 3A). The consistency of *Alternaria* sIgE levels and skin test results was evaluated

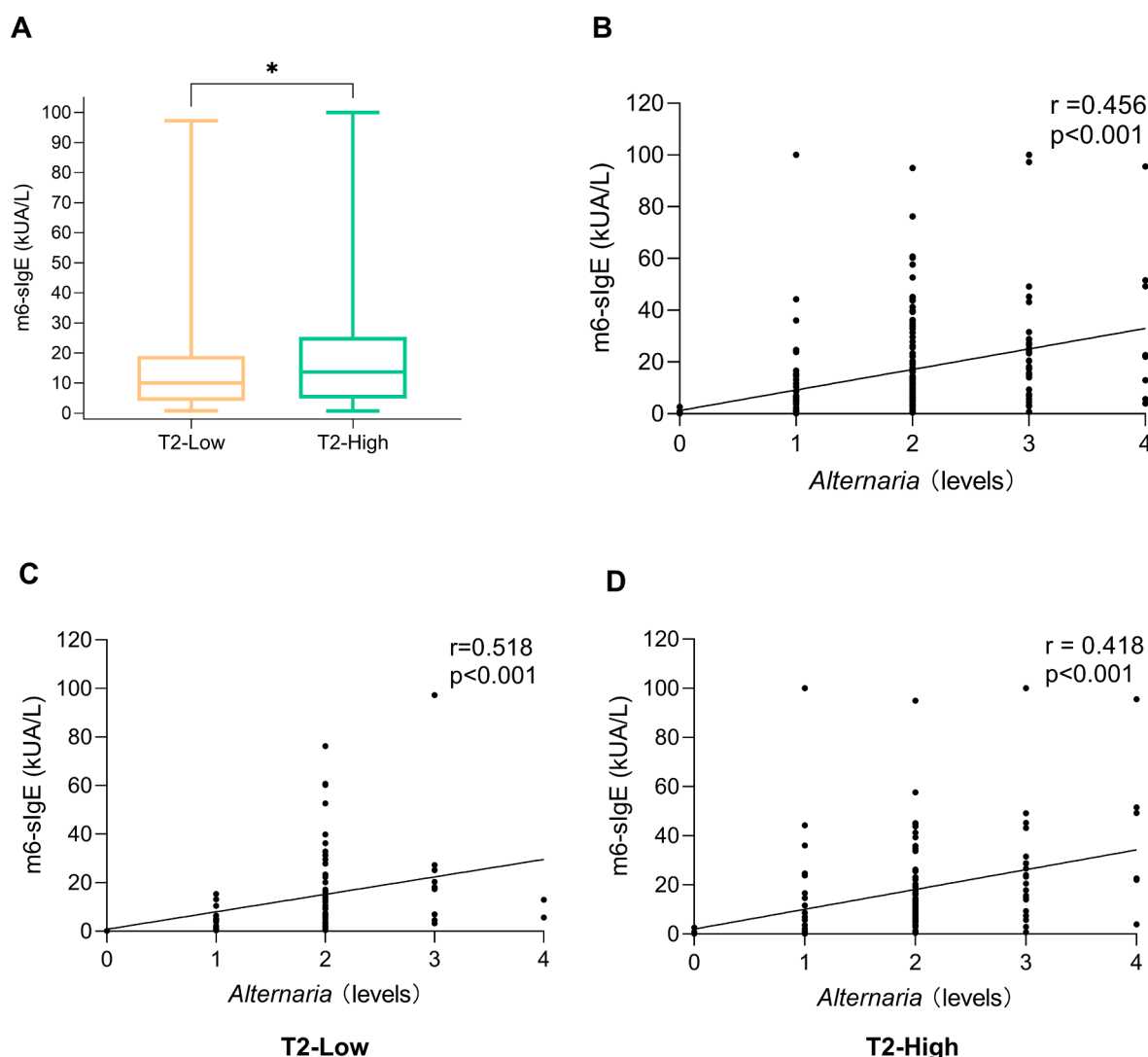


Fig. 3. The levels of m6-sIgE and its consistency with *Alternaria* skin test results in 483 patients and in the T2-high, T2-low endotypes. (A) m6-sIgE levels in the T2-high and T2-low endotypes (* $P = 0.044$). (B) The consistency of m6-sIgE levels and *Alternaria* skin test results in 483 patients. (C) The consistency of m6-sIgE levels and *Alternaria* skin test results in the T2-low group. (D) The consistency of m6-sIgE levels and *Alternaria* skin test results in the T2-high group. **Abbreviations:** sIgE: Specific Immunoglobulin E; T2, Type 2.

using Spearman correlation analysis, and the total correlation coefficient in 582 patients was 0.456, which was lower than 0.518 in the T2-low group, but was comparable to that in the T2-high group (0.418) (Fig. 3B, C, D).

Allergen sensitization patterns were determined by positive sIgE levels or skin test results in 582 patients. A total of 111 (19.1%) patients were sensitized to *Alternaria*, while the remaining 471 (89.9%) were poly-sensitized. The progress of asthma was longer in poly-sensitized patients (median, 36 months; range, 12–72 months) than in mono-sensitized patients (median, 24 months; range, 2–48 months). In a poly-sensitization pattern, almost 70% ($n = 330$) were positive for dust mites (Fig. 4A), followed by mugwort ($n = 267$, 56.7%) and birch ($n = 244$, 51.8%), and in 175 (37.2%) patients, the rates of poly-sensitization to ragweed, firmiana, sabina, humulus, and dog hair were 30.1%, 28.7%, 23.6%, 22.9%, and 14%, respectively. Compared to the T2-low group, the frequency of poly-sensitized patients was significantly higher in the T2-high group ($P = 0.002$) (Fig. 4B). The rate of each allergen among the nine allergens in the T2-high group was also higher (Fig. 4C), and the significant differences were mainly in the data for spring and autumn pollens, such as birch ($P = 0.005$), firmiana ($P = 0.004$), and mugwort ($P = 0.005$).

We further analyzed the vitamin D levels of 187 patients in the T2-high ($n = 115$) and T2-low ($n = 72$), respectively; however, the difference was not significant ($P = 0.135$).

4. Discussion

To the best of our knowledge, this retrospective study is the first to report that patients with *Alternaria*-induced asthma exhibit distinct characteristics according to T2 endotypes. Based on blood eosinophil counts, we compared age, sex, family history, T-IgE, sIgE, ICS, AIT, and sensitization profiles and described the differences between the T2-high and T2-low groups.

Age distribution seems to play a vital role in *Alternaria*-associated asthma. We found that regardless of whether in total patients or in the T2-high and T2-low group, the predominant sensitization ages were focused at 2–18 years and peaked at 6–10 years, which is similar to the results of other surveys conducted by Lehmann [18], Nollés [19] and Kowal [20]. Although adult asthma patients were included in the current research regarding T2-high and T2-low endotypes [12], the 6–10-year-old *Alternaria*-associated asthma patients should be specifically considered because of their persistence and refractory nature. Similarly,

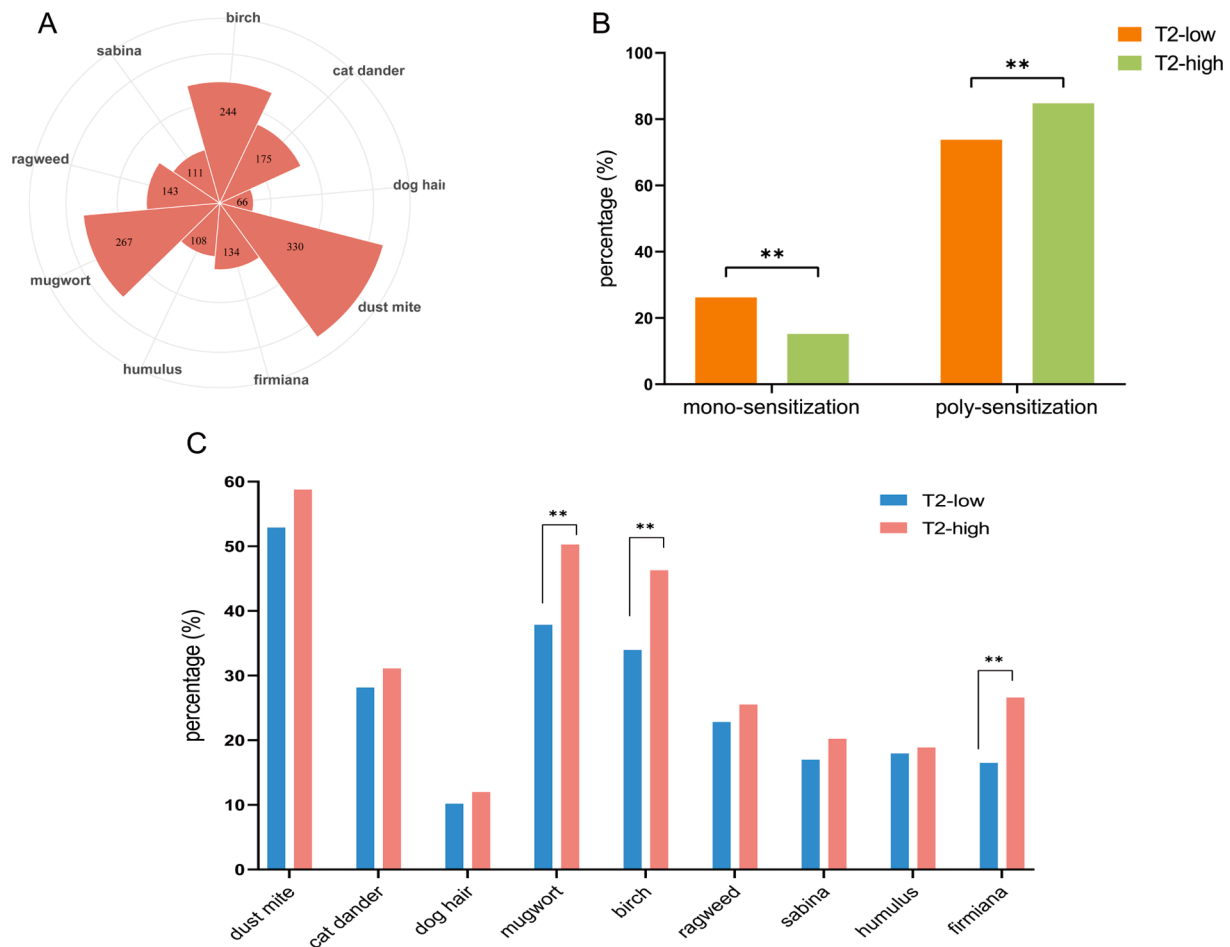


Fig. 4. Allergen sensitization patterns in all patients and the T2-high, T2-low endotypes. **(A)** An overall of allergen sensitization patterns in 582 patients. **(B)** The profile of mono and poly-sensitization in the T2-high and T2-low endotypes (** $P < 0.01$). **(C)** Nine common allergens distribution in the T2-high and T2-low endotypes (** $P < 0.01$). **Abbreviations:** T2, Type 2.

a study published by Tran et al. [21] concluded that eosinophilic and T2-high asthma appeared to be more prevalent in children than in adults. In addition, an earlier onset age was observed in the T2-high group, which indicated that asthma intervention measures might be warranted in advance.

Furthermore, we found a male predominance of both endotypes in patients with *Alternaria*-associated asthma. Several studies have confirmed a significantly higher prevalence of *A. Alternata* sensitization related to the male sex [22,23]. The frequency and distribution of sex in our studied endotypes were likely affected by the selected cutoff values used. For example, when eosinophil cutoff points of 150 or 300 cells/ μ L were used to define T2-high asthma [21], it reached the same distribution in sex as the present study, but if investigators chose 400 cells/ μ L as a cutoff point, the frequency of females might be higher than that of males [12]. We used this cutoff based on the selection of patients in clinical trials with anti-IL-5 therapies [24].

Genetic and environmental factors influence the progress of asthma [25]. Although we did not determine the significance of family history in both groups, a trend was observed in the T2-high group. A previous study conducted in Qatar revealed that 44% of asthmatic patients had a positive family history [26]. Patients in Zahradin's survey showed that a positive family history differed from asthma to allergic rhinitis that was sensitized with *Alternaria* [27]. These findings indicate that family history may promote earlier diagnosis. Current guidelines recommend ICS and AIT as two main therapy methods for asthmatic patients, and the clinical efficacy and safety of AIT for *Alternaria* have been verified by a number of studies [28–31]. However, patients in the T2-high group

preferred to take ICS rather than AIT, two reasons may explain it. On the one hand, patients in our study accepted AIT with *Alternaria* crude extracts which were produced by the Allergen Manufacturing and Research Center (PUMCH, Beijing, China), that needed a long period, always 3–5 years in this therapeutic process, it was difficult for them to corporation and persistence. On the other hand, we constructed subcutaneous immunotherapy, this complex operation may also explain this lower frequency in children. Despite its effectiveness and good safety, a small number of patients selected *Alternaria* AIT.

Notably, an increase in T-IgE level was identified as a potential risk factor for high levels of *Alternaria*-induced asthma or hospitalizations [18]. We analyzed the levels of T-IgE and its relationship with eosinophil counts in two different groups; consequently, a higher and positive correlation was observed in the T2-high asthma group. Although the levels of T-IgE could be influenced by many parameters, such as parasites infection, allergen types, medications, and even chronic diseases, according to the interpretation of the news report on the epidemiological investigation of parasites in our country, the prevalence of parasites was already decreased to <6% in 2018 [32], more importantly, most parasites were prevalent in the south of China that many rivers and lakes were located in there, while our study mainly focused on the patients in northern China. Consequently, our findings were consistent with the findings of a previous study investigating the fungal and bacterial microbiota in asthma endotypes [13], where serum IgE levels were significantly higher in T2-high patients. In another study that focused on the overlap among the three subtypes, T-IgE levels did not correspond to asthma subtypes or eosinophil levels; it was only used for determining

omalizumab dosing [12]. We further compared *Alternaria* sIgE levels and their consistency with skin test results. As mentioned above, compared to the T2-low group, patients in the T2-high endotype showed higher levels and better correlation with skin test results, despite the low number of patients. However, it remains to be elucidated whether Alt a 1-sIgE detection would improve the sensitivity and specificity of allergen assays [3,33,34], since clinical study confirmed that Alt a 1 has been associated with an increased rate of diagnosing *Alternaria alternata* sensitization [35]. But due to the limitation of components detection in our hospital, we did not carry out Alt a 1 levels in these patients.

In addition, we found that 89.9% of *Alternaria*-sensitized asthma patients were poly-sensitized. These poly-sensitivities among patients with asthma or allergic rhinitis have also been reported by other authors [22,36]. A reason for this may be that there is an extremely high prevalence of epidemiologic allergic rhinitis in northern China [37], and rhinitis has a “triggering” role in the further development of poly-sensitization. More specifically, asthma diagnosis occurred later in this particular population, which suggested that poly-sensitivity patients were more likely to misdiagnose or delay identification, and not seeking medical treatment might explain this phenomenon. Moreover, when we further analyzed the profile of sensitization in all patients or both endotypes, we surprisingly found that the majority of *Alternaria*-induced asthma patients still suffered from dust mite sensitization, followed by birch and mugwort pollen. In a birth cohort study [38], exposure to perennial allergens (e.g., house dust mite, cat, and dog hair) in the first 3 years of life was associated with impaired lung function at school age. In addition, birch and mugwort were the most typical spring and autumn pollens in northern China, and a multistage and clustered epidemiological study conducted by Wang et al. [37] revealed that the prevalence of pollen-induced allergic rhinitis was extremely high due to high seasonal pollen exposure. All evidence confirmed our results and had an implication for clinical practice that assessed sensitization to other high frequency allergens, such as dust mites, birch, mugwort, or grass mix.

A number of investigations have shown that vitamin D3 supplementation reduces severe childhood exacerbations [39,40]. Hence, we especially analyzed the vitamin D levels of patients, but there was no significance between T2-high and T2-low patients, although a larger population and further screening for vitamin D in clinical practice are necessary for patients and clinicians.

One of the major limitations in our study was lacking the comparison in respiratory function results between these two groups. Unfortunately, due to the retrospective study design, not all patients reserved these results or could be found in the medical records system. Besides, the testing instrument and results of respiratory function were differed from hospitals or institutions, even though they shared the majority of similarities. In any case, further detections in lung function should be performed to make our results more convincing.

5. Conclusion

In conclusion, our results illustrated, for the first time, that lower age and higher number of male *Alternaria*-sensitized patients existed in the T2-high group, who presented with higher levels of T-IgE and *Alternaria* sIgE. Furthermore, there was a significant positive correlation between increasing eosinophil count and T-IgE levels in the T2-high group. The majority of T2-high patients were more likely to be poly-sensitized, and almost 70% were positive for dust mites, followed by birch and mugwort.

These findings might further underline the differences between both endotypes and present evidence for precise asthma treatment decisions and optimizations. Detailed clinical history and allergen testing data should be obtained to better explain the prevalence and mechanisms of asthma.

CRedit authorship contribution statement

Juan Liu: Conceptualization. **Junda Li:** Data curation. **Jia Yin:** Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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