

# Prevalence of sensitization to specific allergens in allergic patients in Beijing, China: A 7-year retrospective study

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## Abstract

**Background:** Knowledge of the prevalence of common sensitizing allergens may aid in overall management of allergic disease in a specified area.

**Objective:** The aim of this study was to identify and analyse the prevalence of common inhaled and food sensitizing allergens in Beijing.

**Methods:** This was a retrospective study, analysing demographic data and serum sIgE antibody test results from 59057 outpatients who presented to Beijing TongRen Hospital, from January 2013 to December 2019.

**Results:** 28879 patients (48.9%) showed positive sIgE test results; with significantly more males aged under 16 years sensitized to at least one allergen than females, and most patients (53.62%) were sensitized to multiple allergens. The first inhaled sensitizing allergens was *Artemisia* grass (11910 (41.24%)); and the first food allergens was crab (3547 (12.28%)). For *Artemisia* sensitized patients, sIgE levels were mostly at level 5. The number of patients with ragweed allergy is increasing year by year. The detection rates for sIgE to *Artemisia*, common ragweed, and *Humulus* grass allergens were significantly higher in August and September. R package ggplot2 analysis, demonstrated strong correlations between tree allergens and common ragweed and *Humulus* grass allergens (phi coefficients = 0.50 and 0.46, respectively; both  $P < 0.01$ ).

**Conclusion:** The prevalence of sensitization to different allergens in Beijing showed *Artemisia* grass was the most commonly inhaled sensitizing allergen, and the number of patients with ragweed grass allergy was increasing by year.

**Key words:** Inhaled allergens, food allergens, serum specific IgE, R package ggplot2, Chinese patients, sensitization prevalence, *Artemisia*, dust mites, crab, peanut

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## Introduction

Allergic disease is a group of common of immune-mediated disorders; including allergic rhinitis (AR), asthma, allergic conjunctivitis, atopic dermatitis (AD), and food allergy; which are mainly caused by an immunoglobulin E (IgE)-dependent immunological reaction to an innocuous environmental antigen (allergen) according to the site of contact with the allergen. Diagnostic tests are based on the demonstration of allergen-specific IgE in the skin (skin prick tests) or in the serum (serum specific IgE test), and the immunoblot assay is most commonly used for semi-quantitative detection of the allergen/s. Allergic disease is continually rising worldwide,<sup>1</sup> and is a common health problem that affects a substantial number of individuals worldwide; often negatively impacting the quality of life of the affected individual.

AR is a common allergic disease of the nasal mucosa that affects around 10 to 40% of the population worldwide.<sup>2,3</sup> A recent study from China has demonstrated that the self-reported prevalence rate of AR in 18 major cities in China is 17.6%.<sup>4</sup>

Allergic diseases are closely related to demographic and environmental factors, and immune dysfunction.<sup>5-7</sup> Although genetic, gender, age, and obesity are major factors that lead to the development of allergic diseases;<sup>8</sup> information on the prevalence of the common allergens that trigger allergic disease may additionally provide a clinical rationale for better overall management of allergic diseases in any specified area. Thus, the aim of the present study was to determine the prevalence of common inhaled and food allergens in Beijing, China, employing a retrospective analysis of data obtained for sIgE antibody testing over a period of past seven years in Beijing.

## Materials and Methods

### Patients

This study included allergic patients who had presented to the Department of Allergy in Beijing TongRen Hospital between January 2013 to December 2019. Patients with symptoms such as sneezing, nasal itching, runny nose and nasal congestion, or patients with symptoms such as cough, chest tightness and wheezing suspected allergic rhinitis or asthma, and patients with itching and rash suspected atopic dermatitis were recruited continuously in Allergy Clinic of Beijing Tongren Hospital, by which the sensitization to relevant allergens was assessed by sIgE. All of the patients had resided in Beijing for at least 1 year and undergone allergy testing for specific allergens when attending the Department of Allergy in Beijing TongRen Hospital. Patients not from Beijing or with nasal infections or tumors were excluded. Patients with drug allergies were excluded. The present study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Beijing TongRen Hospital (TRECKY2020-076).

### Serum sIgE test

Blood samples collected from each of the 59057 patients was analysed for sIgE using the AllergyScreen test (EUROBlotOne, Beijing, China), an immunoblot assay used to semi-quantitatively detect circulating allergen-specific immunoglobulin E (IgE) in human serum. The sIgE antibody level was evaluated using a panel of the most prevalent local inhalant and food allergens; of which the common inhaled allergens included grass pollen (including *Humulus*, *Artemisia*, common ragweed), mold (including penicillium aspergillus, herb bud branch mold, smoke *Aspergillus* and *Alternaria*), *blattella*, animal dander (including cat and dog), house dusts, dust mites (including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and tree pollen (including *Salix babylonica*, *Populus*, *Ulmus pumila*); and the common food allergens included crab, shrimp/prawn, marine fishes (cod, lobster, scallops), lamb, beef, soybean, peanut, cow's milk, and egg.

A concentration of any sIgE antibody at least 0.35 IU/mL was considered positive sensitization. Based on the concentration of the antibody detected, the semi-quantitative test results of the sIgE was classified into one of the following six allergic levels: level 0: < 0.35 IU/mL; level 1: 0.35–0.69 IU/mL; level 2: 0.7–3.49 IU/mL; level 3: 3.50–17.49 IU/mL; level 4: 17.50–49.99 IU/mL; level 5: 50.00–100.00 IU/mL; level 6: > 100.00 IU/ml.<sup>9</sup>

### Statistical analysis

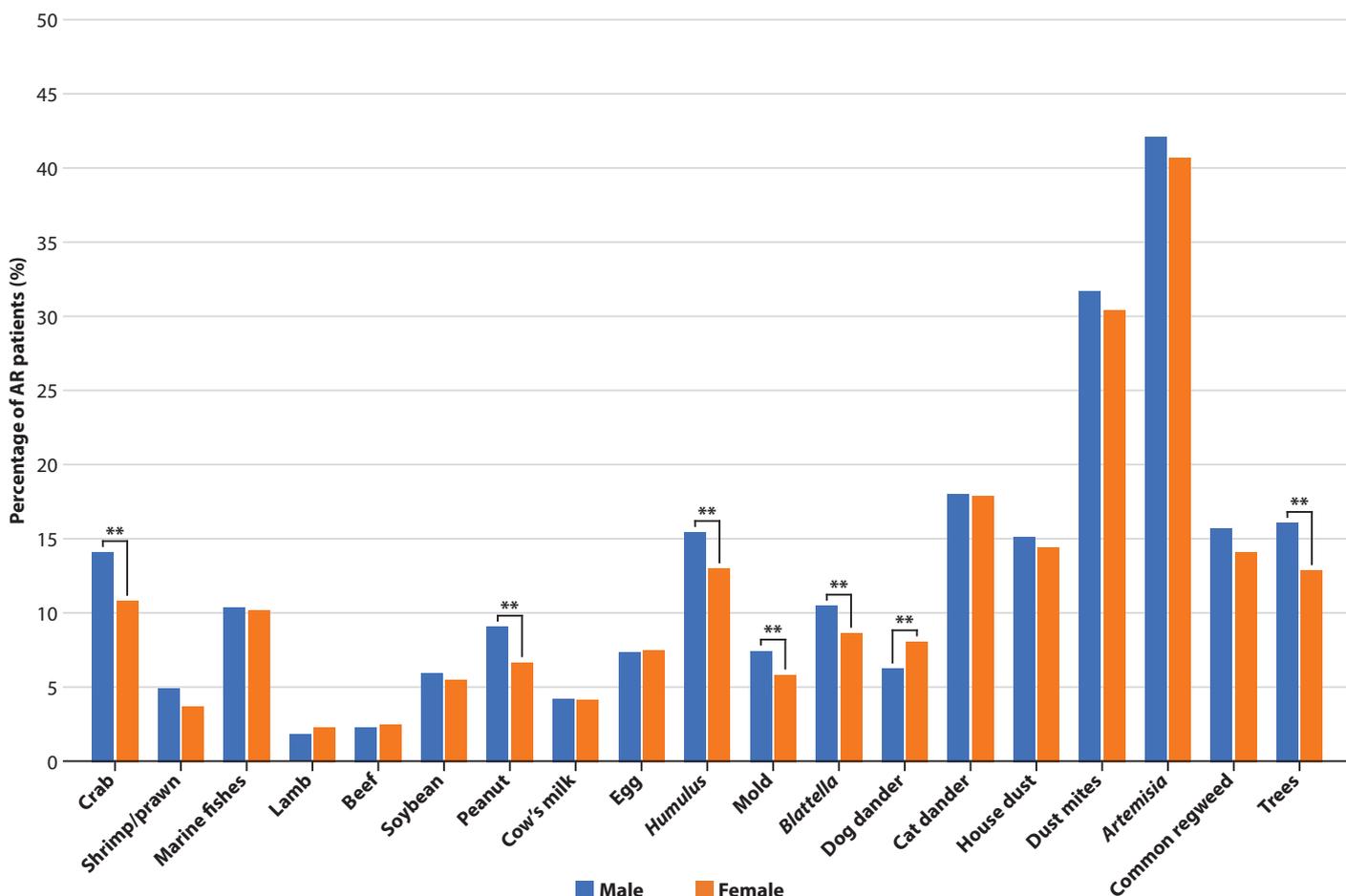
Statistical analyses were performed using the SPSS 25.0 statistical software (IBM Co., Armonk, NY, USA). Significance of differences in gender and ages was compared using the Chi-square test and correlations between serum sIgE and age were analysed by Spearman's rank-order test. Values of  $P < 0.05$  were considered to be statistically significant.

Sector graphs and correlation analysis were performed using GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, CA, USA). The heatmap of the association between two allergens was illustrated using an R package ggplot2. Phi coefficient was applied to measure the correlation between two allergens in all sIgE-positive patients; with values of Phi coefficient  $\geq 0.70$  considered to be a very strong correlation, between 0.40 and 0.69 considered to be a strong correlation, between 0.30–0.39 considered to be moderate correlation, between 0.16–0.29 considered to be weak correlation, and  $\leq 0.15$  considered to be negligible correlation.

## Results

### Characteristics of the study population

A total of 59057 patients had undergone allergy testing for specific allergens during the period from January 2013 to December 2019. The age of the patients ranged from 1-95 years (mean  $\pm$  SD: 32.60  $\pm$  6.26), and the patients were therefore grouped according to nine age groups; including  $\leq 5$  years, 6-10 years, 11-15 years, 16-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, and  $\geq 61$  years. Assessment of data from these patients demonstrated that 28879 (48.90%) patients had positive sIgE test results (i.e. 0.35 IU/mL to > 100 IU/ml), and 30178 (51.09%) negative sIgE test results (< 0.35 IU/mL). Among the patients with positive sIgE test results, 15391 (53.29%) patients were male, and 13488 (46.71%) patients were female. Analysis of gender differences among patients who tested positive for sIgE in each age group demonstrated that significantly greater numbers of males in age groups  $\leq 5$  years, 6-10 years and 11-15 years, had positive sIgE levels than females ( $\chi^2 = 23.03$ ,  $\chi^2 = 18.23$ ,  $\chi^2 = 12.24$ ;  $P < 0.01$ ,  $P < 0.01$ ,  $P < 0.01$ , respectively). However, no gender difference was detected in patients aged over 15-years. Similarly, analysis of allergen-positivity by age demonstrated significantly greater numbers of patients aged 21-30 years and 31-40 years to be sIgE positive than patients in other age groups ( $P < 0.01$ ,  $P < 0.01$ ).



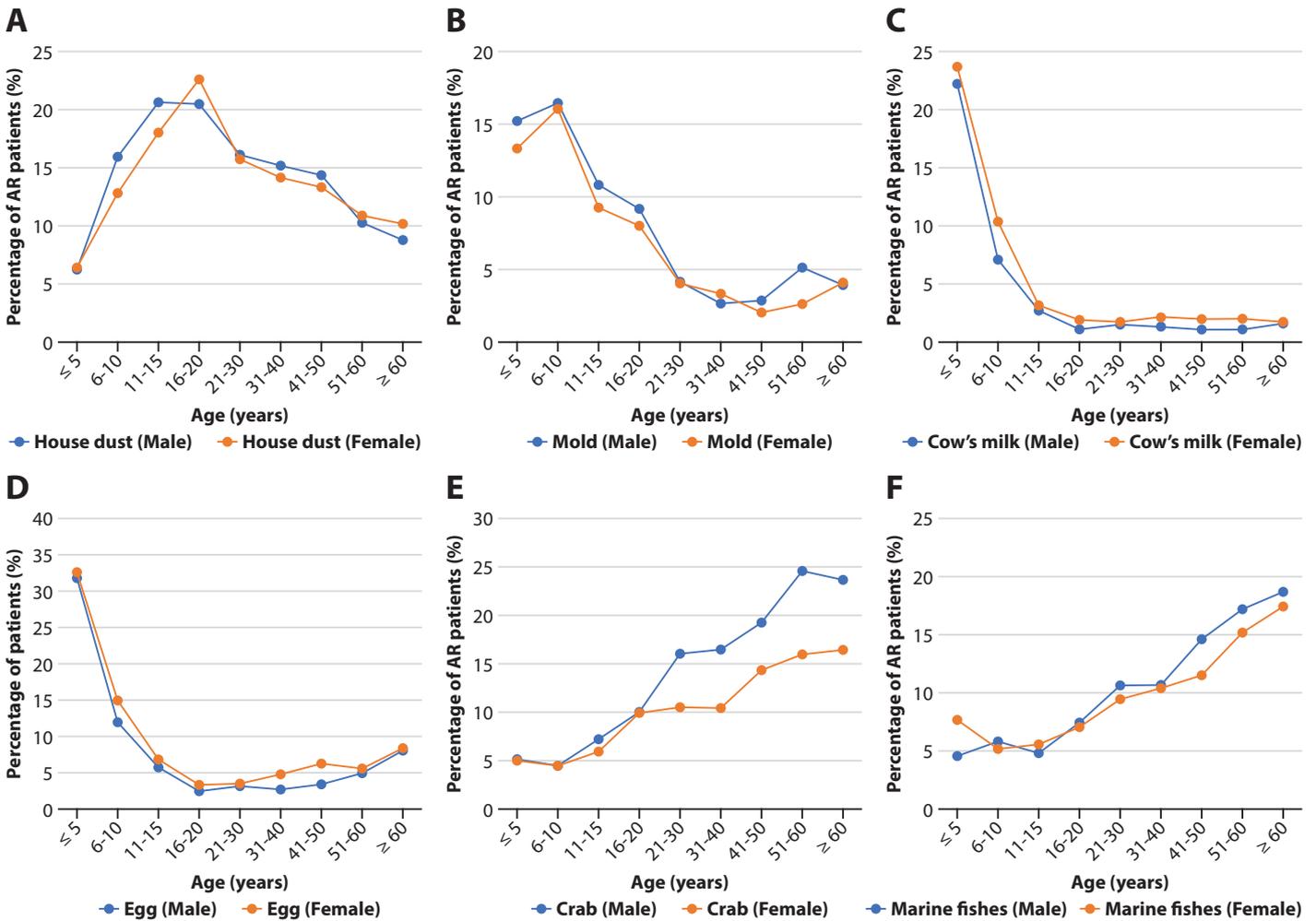
**Figure 1.** The percentage of total patients sensitized to specific allergens. Blue bar diagram shows male allergen-positive patients and Red bar diagram shows female allergen-positive patients. P value was calculated using the chi-squared test. \* $p < 0.05$ , \*\* $p < 0.01$ .

**Patients with inhaled-and food allergen-sensitization in different gender/age groups**

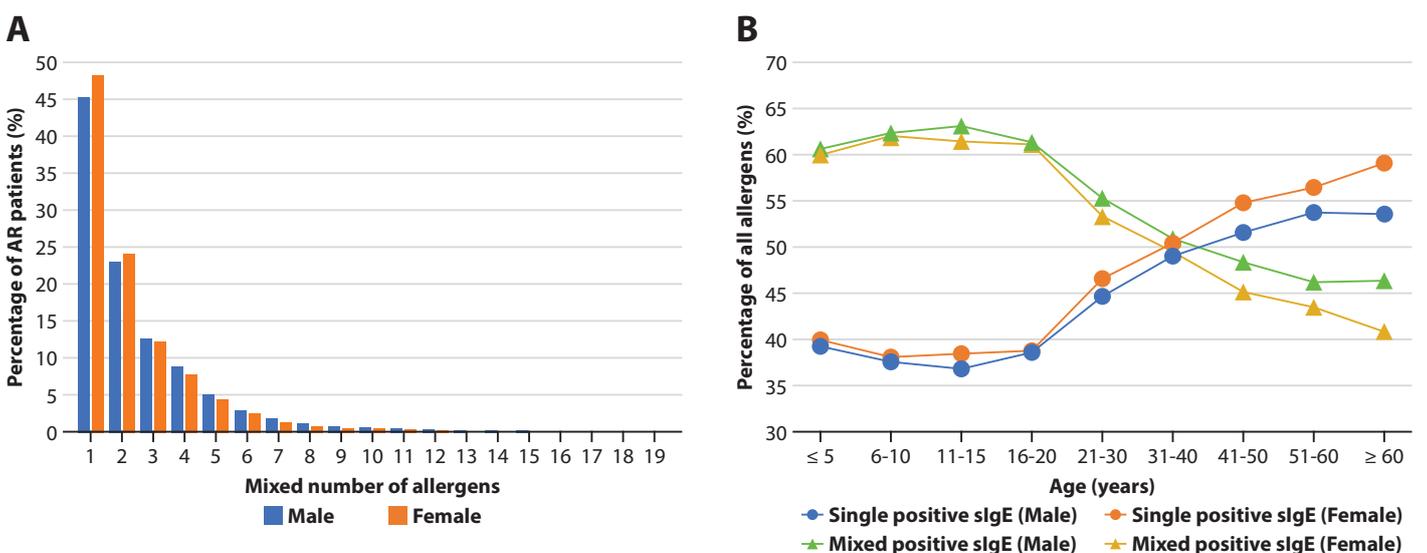
The distribution of patients sensitized to specific allergen is shown in **Figure 1**. Overall, 25077 cases (86.83%) showed positive sIgE to inhaled allergens and 10470 cases (36.25%) showed positive sIgE to food allergens. The top six inhaled allergens among patients were *Artemisia* grass (11910, 41.24%), dust mites (8921, 30.89%), cat dander (5114, 17.71%), common ragweed grass (4239, 14.68%), house dust (4190, 14.51%) and trees (4134, 14.31%); and the top three food allergens were crab (3547, 12.28%), marine fishes (2895, 10.02%), and peanut (2216, 7.67%) (**Figure 1**).

Significant gender differences were found in patients with sensitivity to six allergens; with more males than females being sensitized to crab ( $\chi^2 = 71.69, P < 0.01$ ), trees ( $\chi^2 = 60.41, P < 0.01$ ), peanut ( $\chi^2 = 58.76, P < 0.01$ ), *Humulus* grass ( $\chi^2 = 35.54, P < 0.01$ ), mold ( $\chi^2 = 30.94, P < 0.01$ ), and *Blattella* ( $\chi^2 = 28.43, P < 0.01$ ). In contrast, significantly higher number of females were sensitised to only dog dander compared to males ( $\chi^2 = 30.94, P < 0.01$ ) (**Figure 1**).

The distribution of allergen-positive patients in different age groups is shown in **Figure 2**. The highest percentage of house dust sIgE-positive patients were mainly distributed in age groups 11-15 years and 16-20 years (**Figure 2A**); whereas mold-sensitized patients were significantly higher in age groups < 5 years and 6-10 years, compared to other groups ( $P < 0.01, P < 0.01$ , respectively, **Figure 2B**). In the case of food allergens, the percentage of cow's milk and egg-sensitized patients were mainly distributed in the age group < 5 years old, and progressively decrease with increasing age (**Figure 2C and 2D**). In contrast, the percentage of patients sensitized to crab and marine fishes showed an increasing trend with age after age six (crab: male  $R = 0.97, P < 0.01$  and female  $R = 0.97, P < 0.01$ ; marine fishes: male  $R = 0.98, P < 0.01$  and female  $R = 0.90, P < 0.01$ , respectively) (**Figure 2E and 2F**). However, no age-specific distribution was noted for any other allergen.



**Figure 2.** Percentage of patients positive to specific allergens in different age groups. (A) house dust, (B) mold, (C) crab, (D) marine fishes, (E) cow's milk, (F) egg. Blue line, male patients; Red line, female patients. The correlations between serum sIgE and age were analysed by Spearman's rank-order test.



**Figure 3.** Distribution of patients sensitized to one or more allergens. (A) Percentage of patients with sIgE to different numbers of allergens. Blue bar diagram, male patients; Red bar diagram, female patients. *P* value was calculated using the chi-squared test. (B) Percentage of patients with sIgE to single and multiple allergens in different age groups. Blue line, male patients with single positive allergen; Green line, male patients with multiple positive allergens; Red line, female patients with single positive allergen; Yellow line, female patients with multiple positive allergens. \**p* < 0.05, \*\**p* < 0.01.

**Patients with single/multiple positive allergens**

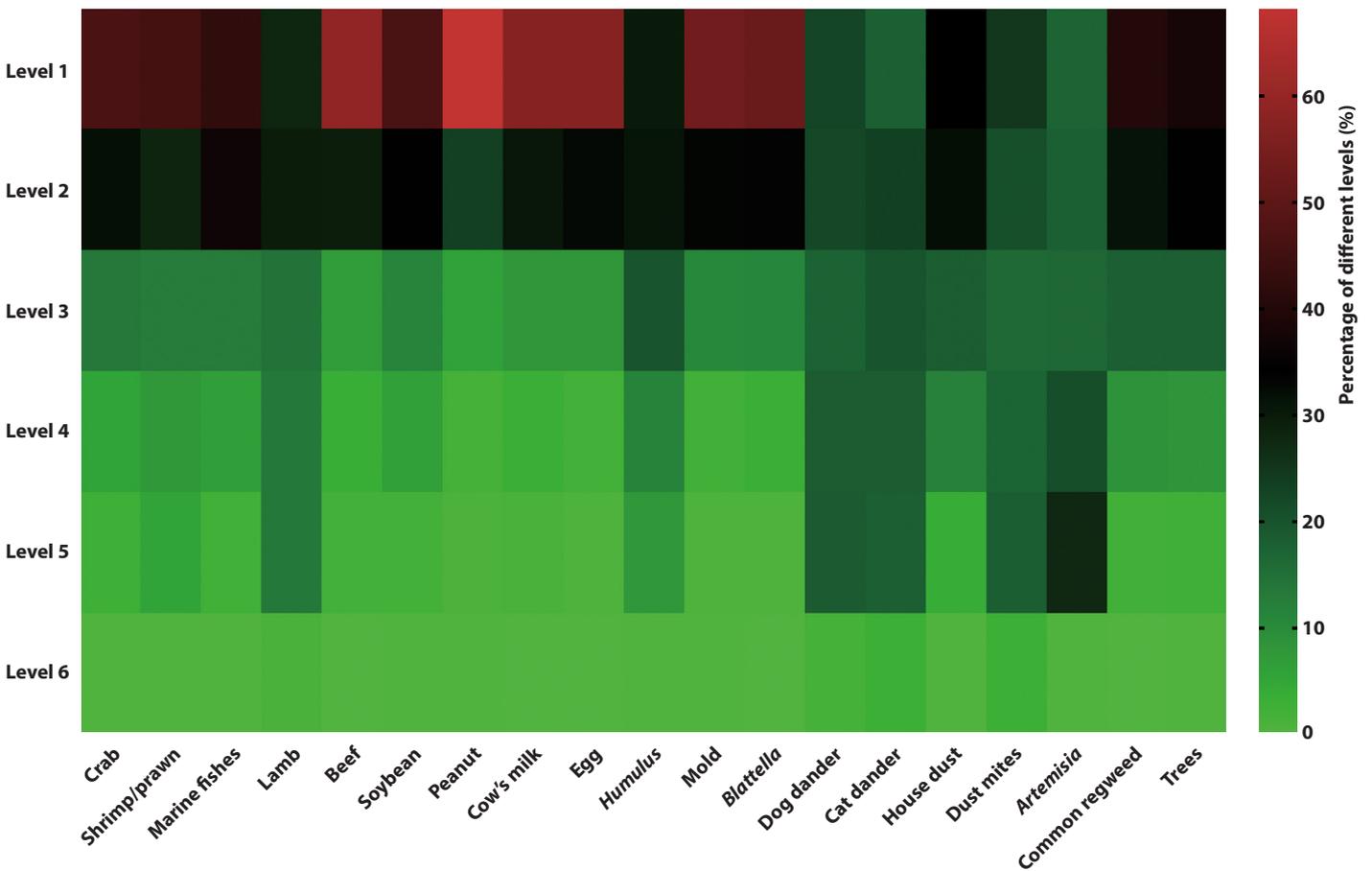
Assessment of the number of different allergens the patients were sensitized to showed that 46.38% patients (male 44.98%, female 47.98%) were sensitized to a single allergen, and 53.62% patients were sensitized to multiple allergens (Figure 3A).

The proportion of patients with multiple allergens sensitivity was higher than that of patients with single allergen before 31-40 years old, but after 31-40 years old the proportion of patients with single allergen was higher than that of patients with multiple allergens (Figure 3B). Moreover, the proportion of patients with multiple allergens decreased

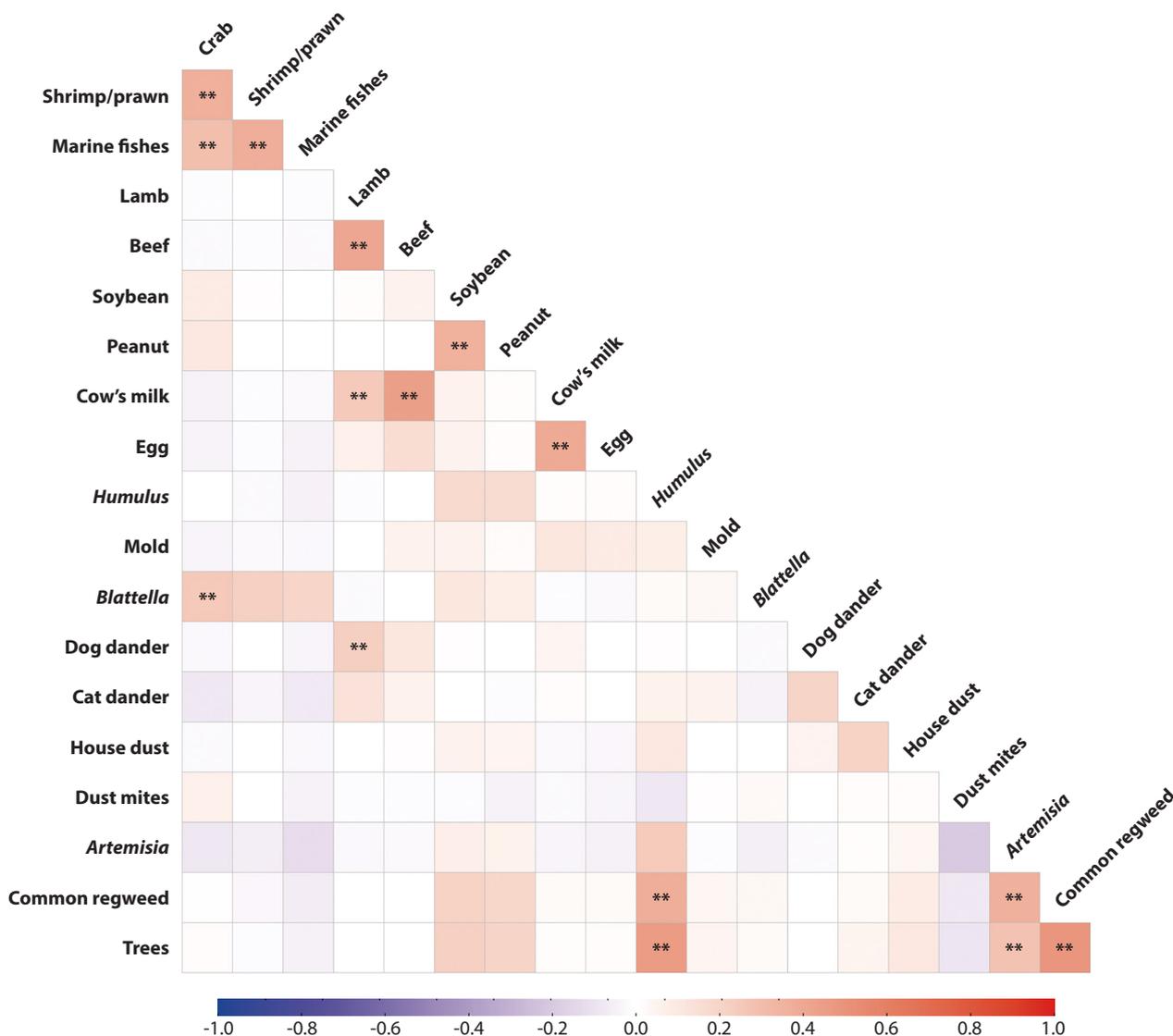
gradually from age 21-30 years (male  $R = 0.95, P < 0.01$ ; female  $R = 0.97, P < 0.01$ ); whereas the proportion of patients with single allergens are opposite (male  $R = -0.95, P < 0.01$ ; female  $R = -0.97, P < 0.01$ ) (Figure 3B).

**sIgE levels of different allergens**

Analysis of the level of sensitization to specific allergens demonstrated that patients with positive food allergens and house dusts, mold, *Blattella*, common ragweed and trees allergens mainly presented with sIgE at levels 1 and 2; whereas *Artemisia* grass allergen-positive patients mainly presented with sIgE at level 5 (Figure 4).



**Figure 4.** The level of sIgE detected in different allergen-positive patients. Percentage of patients with sIgE levels are shown according to a specific color chart; with a large percentage displayed in red, small percentage displayed in green, and color intensity proportional to the percentage.



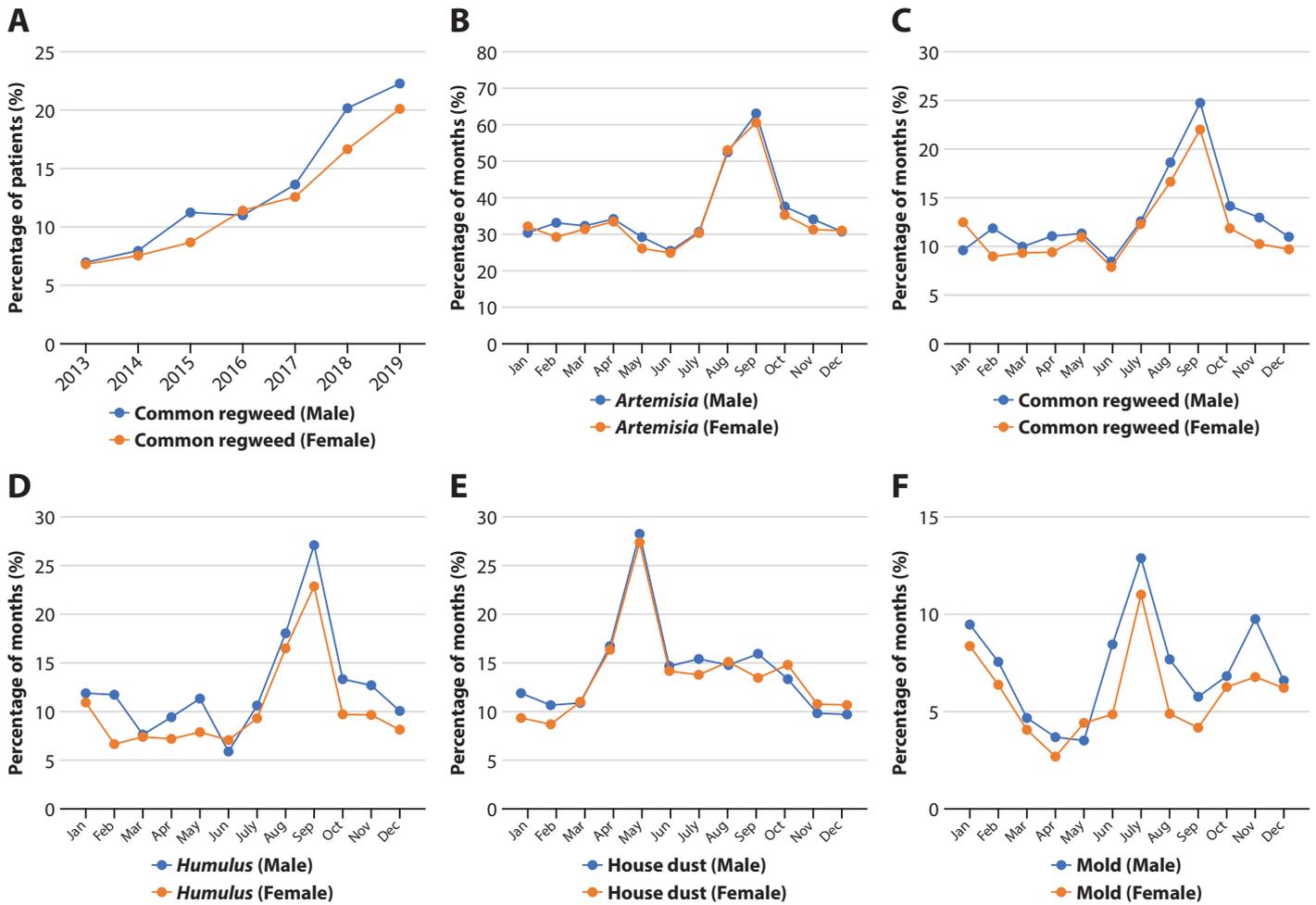
**Figure 5.** Correlations between allergens. The heatmap is illustrated using an R package ggplot2. Phi coefficient is expressed as a color chart; with positive correlations displayed in red, negative correlations displayed in blue, and color intensity proportional to the correlation coefficients. Phi coefficient  $\geq 0.70$  was considered a very strong correlation, 0.40-0.69 a strong correlation, 0.30-0.39 a moderate correlation, 0.16-0.29 a weak correlation, and  $\leq 0.15$  a negligible correlation. \* $p < 0.05$ ; \*\* $p < 0.01$ .

### Correlation between allergens

The association between two allergens was analysed using an R package ggplot2 and Phi coefficient was applied to measure the correlation between two allergens in 28879 sIgE-positive patients. In the case of inhaled allergens, trees and common ragweed/*Humulus* grass exhibited a strong correlation (phi coefficients 0.50 and 0.46, respectively; both  $P < 0.01$ ) (Figure 5). Similarly, beef and cow's milk, beef and lamb, and cow's milk and egg allergens were strongly correlated positively (phi coefficients were 0.45, 0.41, and 0.40, respectively; both  $P < 0.001$ ) (Figure 5). Additionally, common ragweed and *Humulus*, common ragweed and *Artemisia*, Crab and Marine fishes, crab and shrimp/prawn, and Soybean and Peanut also showed moderate positive associations (phi coefficients were 0.38, 0.38, 0.37, 0.37 and 0.36 respectively; all  $P < 0.01$ ) (Figure 5), while crab and *Blattella* showed a weak association (phi coefficient was 0.26;  $P < 0.01$ ) (Figure 5).

### Annual and seasonality of allergen sensitivity

We analysed the variation of positive detection rates of different allergens in different years and months. The percentage of patients sensitized to common ragweed showed an increasing trend year by year (male  $R = 0.96$ ,  $P < 0.01$  and female  $R = 0.97$ ,  $P < 0.01$ ) (Figure 6A). The positive detection rates for sIgE to *Artemisia*, common ragweed, and *Humulus* grass allergens were significantly higher in August and September than in other months ( $P < 0.01$ ) (Figure 6B, C, and D). Similarly, the sIgE detection rate for house dust was significantly higher in May than in other months ( $P < 0.01$ ) (Figure 6E) and the positive detection rate for mold sIgE was significantly higher in January, February and July than in other months ( $P < 0.01$ ) (Figure 6F). No statistically significant seasonal differences were detected for any of the food allergens.



**Figure 6.** Seasonality of sensitization to specific allergens. (A) the number of patients with common ragweed allergy is increasing year by year. (B) the number of patients with *Artemisia*, (C) common ragweed, (D) *Humulus*, (E) house dust, (F) mold in each month. Blue line, male patients; Red line, female patients. The correlations between serum sIgE and months were analysed by Spearman's rank-order test.

## Discussion

Allergic diseases are inflammatory conditions induced by an immunoglobulin E (IgE)-mediated reaction in allergen-sensitized subjects. The allergic inflammation is initiated by allergen molecules cross-linking their corresponding receptor-bound sIgE molecules on the surface of mast cells and basophils. Thus, a higher sIgE reflects a higher level of allergen sIgE present on the mast cell and basophil surface, and the amount of sIgE in the serum reflects the degree of an individual's sensitivity to the antigen.<sup>10,11</sup>

Analysis of demographic data of 28879 patients with serum positive allergen sIgEs to variety of common sensitizing allergens indicated that there was a significant gender difference in sensitization to the allergens in patients under 16 years of age ( $\leq 5$ , 6–10 and 11–15 years old groups), and significantly greater numbers of males were sensitized compared to females. In terms of age, allergen-positive patients were significantly higher in the 21–30 years and 31–40 years old groups compared to other age groups.

For different allergens, house dust sIgE-positive patients were mainly distributed in 11–15 years and 16–20 years old groups and mold sIgE-positive patients in < 5 years and 6–10 years old groups. These findings are in accordance with findings of several studies,<sup>12,13</sup> which showed that younger patients were more sensitized than older patients, and serum sIgE was significantly greater in men than in women. Indeed, a study by Bartra and colleagues has demonstrated that the prevalence of *Alternaria* sensitization is significantly higher in children compared to adults, and fungal allergy, in particular *Alternaria* allergy, is a major risk factor for asthma and severe asthma, especially in children presenting genetic condition.<sup>14</sup> However, it is presently not clear whether the differences in age- and gender-associated sensitization are due to tolerance of the immune system or reflect different levels of exposure to allergen, and needs to be investigated further.

The present study has also demonstrated that *Artemisia* was the most prevalent sensitizing aeroallergen (41.24%) in the 28879 patients with positive serum sIgEs, and that the *Artemisia* grass allergen sIgE was present at mostly level 5 in these patients. Furthermore, the positive rates of *Artemisia* grass allergen were significantly higher in August and September than in other months of the year. This is consistent with the findings that *Artemisia*, a common grass in Beijing, which flowers in August and September can release a large amount of pollen allergens and lead to seasonal allergic reactions.<sup>15</sup> These findings are also in accordance with other studies, which have reported sensitization to pollens from grass to be the most prevalent in patients with allergic disease,<sup>16,17</sup> and have a seasonal onset.<sup>18</sup> In fact seasonal allergic rhinitis is being the most common disease in our hospital. Similarly, the present study has indicated that *Humulus* and common ragweed, which are also common grasses in Beijing, are likely to play an important role in sensitizing individuals and impacting their quality of life. Furthermore, we found that annual sensitization to common ragweed showed an increasing trend. Common ragweed is a malignant invasive grass in China,<sup>19</sup> this change suggests that the spread of ragweed needs to be controlled in time.

In this study, dust mites were found to be the second most sensitizing inhalant allergen in Beijing, with a sensitization rate of 30.89%. This is much lower than the rate of 65% sensitization to dust mites allergens reported in southern China,<sup>20</sup> and possibly may be as a consequence of the lower humidity and temperature in Beijing than in southern China.<sup>20</sup> Similarly, cat allergen was the third most common inhaled allergen is likely to be related to the generally increased number of urban cats and treatment of cats as the most common indoor pets.<sup>21</sup> This study found that the incidence of sIgE to house dust; an indoor inhalant allergen consisting microorganisms, insects, skin flakes, hair, and fibers;<sup>22</sup> was higher in May, which might be related to the climate in Beijing.

In this study, we found that the most common food allergens in Beijing were crab, marine fishes, and peanut, which is in accordance with the study of Wang and colleagues.<sup>23</sup> Furthermore, the percentage of cow's milk and egg sIgE-positive patients were mainly distributed in the < 5 years old group and gradually decreased with increasing age. This result is in accordance with a recent study, which has demonstrated that production of allergen-specific IgE by food molecular components tend to reduce with ageing.<sup>24</sup> Some studies have considered the deficiency of gastrointestinal mucosal immune function in infants and the sensitization caused by the combination of macromolecular allergens and gastrointestinal antibodies.<sup>25,26</sup> Interestingly, our study has demonstrated that crab and marine fishes sIgE-positive patients increase with increasing age after six years old, indicating the effect of different dietary habits on food allergies, which has been demonstrated in different countries and regions.<sup>27</sup>

Our study has indicated that 46.38% patients (male 44.98%, female 47.98%) were sensitized to a single allergen, and 53.62% patients were sensitized to multiple allergens. The proportion of patients with multiple allergens sensitivity was higher than that of patients with single allergen before 31-40 years old, but after 31-40 years old the proportion of patients with single allergen was higher than that of patients with multiple allergens. These results were consistent with previous studies.<sup>28</sup> These results may be related to environmental pollution, early childhood allergen exposure, and food supplementation.<sup>29</sup> In adults, immune tolerance after repeated allergen exposure or food intake resulted in fewer allergens.<sup>30</sup>

Studies have confirmed cross-reactivity between allergens.<sup>31</sup> Assessment of associations between allergens in the current study has demonstrated that tree allergens are strongly correlated with common ragweed and *Humulus* allergens, while common ragweed allergen is moderately correlated with *Humulus* and *Artemisia* allergens. Similarly, in the case of food allergens, beef and cow's milk, beef and lamb, and cow's milk and egg allergens show a strong correlation, whereas crab and marine fishes, crab and shrimp/prawn, and Soybean and Peanut allergens show moderate correlation. Indeed, there is evidence that the major allergenic protein of *Artemisia* and common ragweed have a high level of homology, especially Art v1.<sup>32</sup> Crab, Shrimp/prawn and lobster have cross reacting antigens such as tropomyosin (TM) and arginine kinase (AK).<sup>33,34</sup> Indeed, TM and AK allergenic proteins are present in various crustaceans and mollusks such as scallops, *Blattella* and dust mites, and exhibit cross-reactivity among different species.<sup>35</sup> However, dust mites did not show an association with crab or other allergens in the present study. The correlation between allergens may be related to the close taxonomic relationship.<sup>36</sup> In contrast, although no significant association was demonstrated between plant pollen and food in the present study, a recent review by Saunders and Platt<sup>36</sup> has indicated that some pollen-allergic patients develop allergic reaction in the maxillofacial within 2~15 min after ingesting fresh plant foods (fruits and vegetables), which is an IgE-mediated allergic reaction to food.<sup>37</sup> Collectively, these studies suggest that the cross-reactivity between allergens is likely to involve complex mechanisms and further prospective studies and molecular biology experiments are needed to investigate and confirm associations between allergens. For example, long-term follow-up of patients with cross-allergens and proteomic detection of cross-reactive allergens.

In conclusion, this study presented new information about the prevalence of sensitization to different allergens in Beijing, the most inhaled sensitizing allergens were *Artemisia* grass, and the number of patients with ragweed grass allergy was increasing year by year. These results suggest that grass pollen allergens are becoming the most important allergens in Beijing. Although the incidence of allergic diseases in the Chinese population has previously been documented based on mostly self-reporting questionnaires,<sup>4,23</sup>

this large-scale study reports objectively determined sensitization rates to different allergens among patients with suspected allergic diseases in Beijing, China. The findings of this study may also be applicable to other provinces in northern China, with similar climatic conditions and lifestyles of patients; however, these findings are somewhat limited. First of all, a limited number of allergens were included and some common allergens, such as cypress and birch pollen, were omitted. Second, this was a single-center retrospective chart review study. Thus, confirmation of these findings in large scale prospective multi-center studies to determine the incidence and prevalence of allergen sensitization in Beijing and different regions of China may help in the prevention, diagnosis, and management of allergic diseases in China.

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### Disclosures

The authors declare that they have no competing interests.

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