

# Age, Sex, and Symptom-Dependent Variations in Total IgE and Eosinophils in Atopic Patients: A Five-Year Retrospective Study

Lei Zhao\*, Yiting Wu<sup>ID</sup>\*, Hui Zhu\*, Yanmei Lin, Hang Su, Jingwei Hu, Min Zhang, Wuping Bao

Department of Respiratory and Critical Care Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

\*These authors contributed equally to this work

Correspondence: Wuping Bao; Min Zhang, Department of Respiratory and Critical Care Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, NO. 100 haining Road, Hongkou District, Shanghai, 200080, People's Republic of China, Tel +0086 21 63240090 3091, Email wupingbao1982@163.com; zhangmin@sjtu.edu.cn

**Background:** Total immunoglobulin E (t-IgE) and eosinophils as central effector molecular and cells are crucial biomarkers for allergic diseases. Age, sex and allergic symptoms may influence these biomarkers and the relationship among t-IgE, eosinophils, and atopic diseases remained unknown.

**Methods:** A five-year retrospective study enrolled 7654 patients with allergic symptoms underwent allergen testing. We collected age, sex, symptoms, and complete blood counts and explored variations in t-IgE and eosinophil across demographic and clinical parameters.

**Results:** Among atopic patients, t-IgE levels peaked in children, decreasing with age, with a slight increase in the elderly, mirroring eosinophil trends. Females consistently exhibited lower t-IgE and eosinophil levels than males, regardless of atopic status. High t-IgE and eosinophils were the most common in patients  $\geq 12$  years with respiratory symptoms. Dust mites often caused respiratory symptoms. Egg allergies typically affected the ear, nose, and throat (ENT). Seafood/peanut allergies were linked to skin symptoms. Interestingly, elevated t-IgE and eosinophil levels did not consistently correlate with positive allergen tests. Among atopic patients, 44.5% exhibited normal t-IgE, and 41.4% had normal eosinophils. In the subgroup (22.8%) with both markers normal, more were females (62.5%). Sensitization to allergens like soy, shrimp, mold combination, *Artemisia argyi*, common ragweed, and willow/aspen/elm may fail to elevate t-IgE and eosinophils.

**Conclusion:** Characteristics such as older age, female, skin and ENT symptoms, and allergens may correlate with lower t-IgE and eosinophils in the allergic populations which means we should pay more attention to these population, even if their t-IgE and eosinophils are normal, the allergen test is necessary for treatment strategies.

**Clinical Trial Registration:** ChiCTR2300067700 (Date of Registration: 2023-01-18).

**Keywords:** atopy, IgE, eosinophil, age, sex

## Background

The increasing prevalence of allergic diseases, characterized by heightened immune system sensitivity to harmless environmental substances, presents a significant public health challenge. In industrialized nations, these conditions affect over 25% of the population, with their incidence continues to rise in developing regions.<sup>1-3</sup> The underlying physiology often involves a type 2 immune response, orchestrated by Th2 cells, eosinophils, mast cells, group 2 innate lymphoid cell (ILC2), and M2 macrophages, resulting in clinical symptoms that significantly impact the well-being of affected individuals.

In clinical practice, total immunoglobulin E (t-IgE) and eosinophil counts serve as vital biomarkers for diagnosing and managing allergic conditions. They are preferred due to their convenience, cost-effectiveness, and practicality.

Furthermore, correlations exist between t-IgE levels and other markers of allergic inflammation, such as exhaled nitric oxide and airway hyperresponsiveness (AHR),<sup>4</sup> which are crucial in asthma pathophysiology. Eosinophils, as central effector cells in allergic inflammation, also hold evaluating and therapeutic value in allergic diseases.<sup>5–7</sup> Elevated t-IgE, increased eosinophils and elevated nitric oxide levels, often indicate poorly controlled asthma as independent risk factors,<sup>8</sup> emphasizing the importance of t-IgE and eosinophils in clinical assessment and treatment decisions.<sup>9</sup> Nevertheless, it is crucial to acknowledge that t-IgE and eosinophil counts are influenced by various factors, including age, sex, BMI, and smoking history. Therefore, relying solely on a single test result can sometimes lead to inaccurate assessments of the atopic status, contributing to both misdiagnosis and misevaluation.<sup>10,11</sup>

Despite their utility, interpreting these biomarkers requires an understanding of the complexities of immune responses and the modifying effects of demographic factors such as age, sex, and symptoms. Our previous research has revealed variability in t-IgE and eosinophil counts,<sup>12,13</sup> suggesting that these variations may be attributed to a range of elements, including allergic symptoms, allergens, and the presence of concurrent allergic conditions. However, within the realm of allergic diseases, limited research exists on the influence of factors like age and sex on t-IgE and eosinophils levels. The predictive value of both biomarkers for allergic diseases remains insufficiently recognized. Given these complexities, the primary objective of this study is to elucidate the correlations between t-IgE and eosinophil levels with allergen type and severity in patients displaying allergic symptoms. Additionally, we aimed to explore age and sex-related variability in these biomarkers, establish connections between clinical symptoms and biomarker levels, uncover any seasonal patterns, identify allergens causing atopy without significantly elevating t-IgE and characterize atopic patients with normal t-IgE levels, thereby highlighting potential diagnostic challenges. These insights are paramount for improving diagnostic accuracy and advancing the management of allergic diseases.

## Methods

### Study Design

We conducted a retrospective, single-center study of patients with allergic symptoms, such as cough, wheezing, nasal congestion, runny nose, itchy eyes, itchy skin, and wheals, who underwent serum allergen specific-IgE (s-IgE) testing between January 2013 and December 2017. A total of 7,654 patients were enrolled, with 5,066 having sIgE and peripheral eosinophil counts measured, and 1,863 undergoing sIgE and t-IgE testing. Among them, 1,371 patients had concurrent testing for s-IgE, t-IgE and eosinophil counts. Baseline data, including age, sex, onset date, s-IgE, t-IgE, and complete blood count results, were collected.

Patients were categorized into five age groups (1–17, 18–36, 37–52, 53–69, and 70–100 years) based on our previous study.<sup>12</sup>

The study protocol was approved by the ethics committee, with a waiver of informed consent (number: 2017KY159). Detailed information about allergen testing and biomarker evaluation was provided in our previous study.<sup>12</sup>

### S-IgE, t-IgE and Complete Blood Count Evaluation

s-IgE levels against 17 allergen extracts and cross-reactive carbohydrate determinants (CCD) were assessed using the EUROLINE Atopy (China7) (IgE) Assay Kit, a semi-quantitative immunoblot method. These 17 allergens were categorized into two groups: 10 aeroallergens (willow/aspen/elm, common ragweed, *Artemisia argyi*, dust mites, house dust, cat, dog, *Blattella germanica*, *Penicillium notatum*/branch spore wood/*Aspergillus fumigatus*/*Alternaria*, and *Humulus*) and seven food allergens (egg albumen, milk, peanuts, soy, cod/lobster/scallops, shrimp, and crab).

The severity of atopy was quantified into seven grades based on s-IgE concentrations, corresponding to sensitive index (SI) values of 0–6 for ranges of 0–0.35, 0.35–0.69, 0.70–3.49, 3.5–17.49, 17.5–49.99, 50–99.99, and  $\geq 100$  kU/L. A test was considered positive if s-IgE levels were  $\geq 0.35$  kU/L. Atopy was defined as the presence of at least one positive s-IgE result. To minimize bias, individuals with positive anti-CCD s-IgE were excluded from the analysis.

Total IgE (t-IgE) was measured using the UniCAP 1000 system and classified as elevated for values exceeding 60 IU/mL.

Peripheral eosinophil counts were determined using an automated complete blood count with differential, reported as both absolute counts and percentages. An absolute eosinophil count  $>0.15 \times 10^9$  cells/L was considered elevated.

## Statistical Analysis and Data Visualization

Descriptive statistics were used to summarize demographic and clinical characteristics. The Shapiro–Wilk test assessed the normality of continuous variables. Data were presented as mean  $\pm$  standard error of the mean (SEM) for normally distributed variables and as median with interquartile range (IQR) for non-normally distributed variables.

For multiple group comparisons, Dunn's test was applied following nonparametric ANOVA. The Chi-square test evaluated associations between categorical variables (eg, sex and atopic symptoms), while Spearman correlation coefficient and rank correlation tests assessed monotonic relationships and rank order between continuous variables.

Quadrant scatter plots and heatmaps visualized biomarker relationships and allergen-specific sensitizations. The Boruta algorithm identified the relative importance of allergens in influencing t-IgE levels and eosinophil counts.

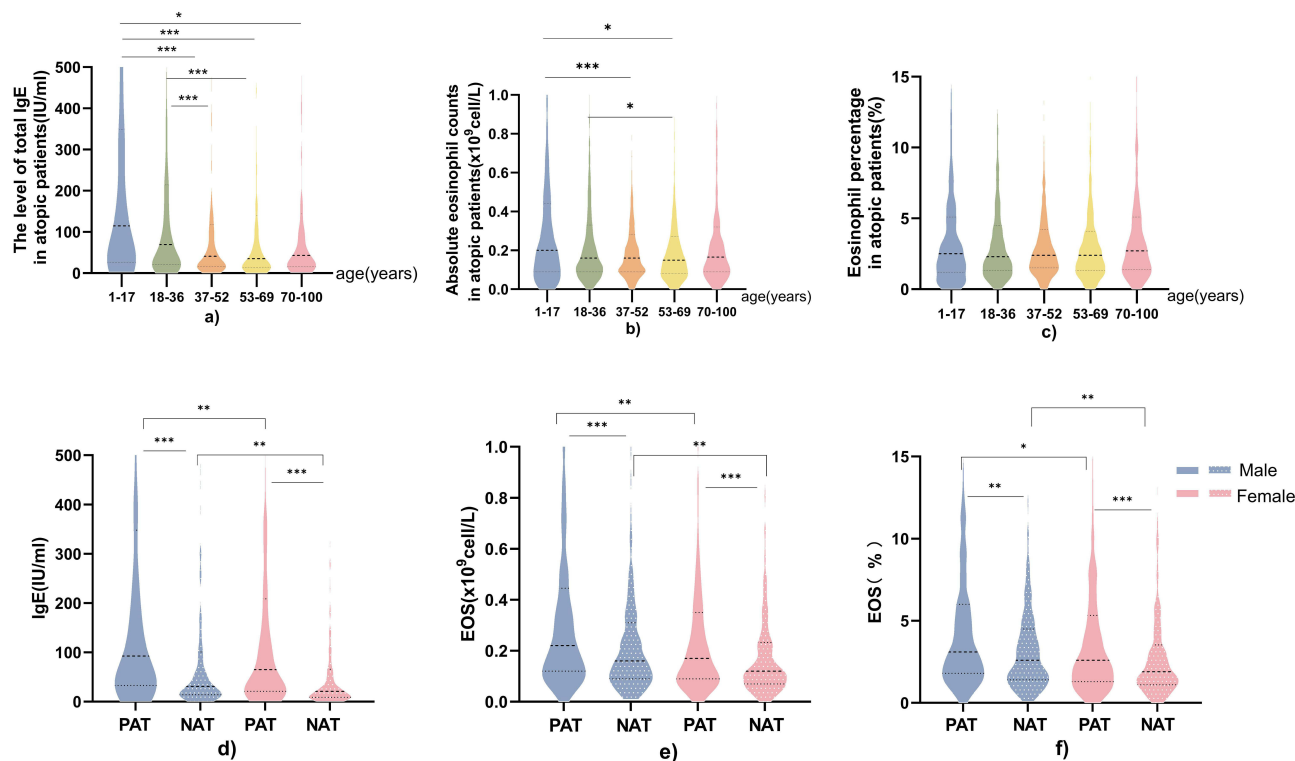
A p-value  $< 0.05$  was considered statistically significant. Statistical analyses were performed using SPSS (Version 27), OriginPro 2022, Python (Version 3.11), and GraphPad Prism (Version 9.0).

## Results

### Variability in t-IgE and Eosinophil Counts by Age and Sex

In our study, 7654 patients performed the allergen test to identify the level of s-IgE, in which, 5066 patients underwent eosinophil count testing, while 1863 patients underwent t-IgE testing. Significant variations in t-IgE and eosinophil levels were observed across different age groups and between sexes.

Children and adolescents (1–17 years) displayed the highest t-IgE ( $458.50 \pm 95.47$  IU/mL), progressively decreasing with age. Mean t-IgE for adults (18–36 years), middle-aged adults (37–52 years), older adults (53–69 years), and the elderly (70–100 years) were  $267.20 \pm 22.59$ ,  $205.80 \pm 26.91$ ,  $207.40 \pm 21.31$ , and  $227.60 \pm 45.61$  IU/mL, respectively (Figure 1a). This highlights a significant age-related decline in t-IgE, with a slight increase noted in the oldest age group. Conversely, eosinophil distribution, influenced by atopic conditions, showed a distinct pattern, with notable increases



**Figure 1** Variety about the value of t-IgE (a) and eosinophil (b and c) in atopic patients by the five age categories. Comparative analysis of t-IgE levels (d) and eosinophil counts (e and f) based on sex, between patients who tested positive for allergens and those who tested negative. Data was expressed as Mean  $\pm$  SEM. EOS, eosinophil. \* $< 0.05$ , \*\* $< 0.01$ , \*\*\* $< 0.001$ .

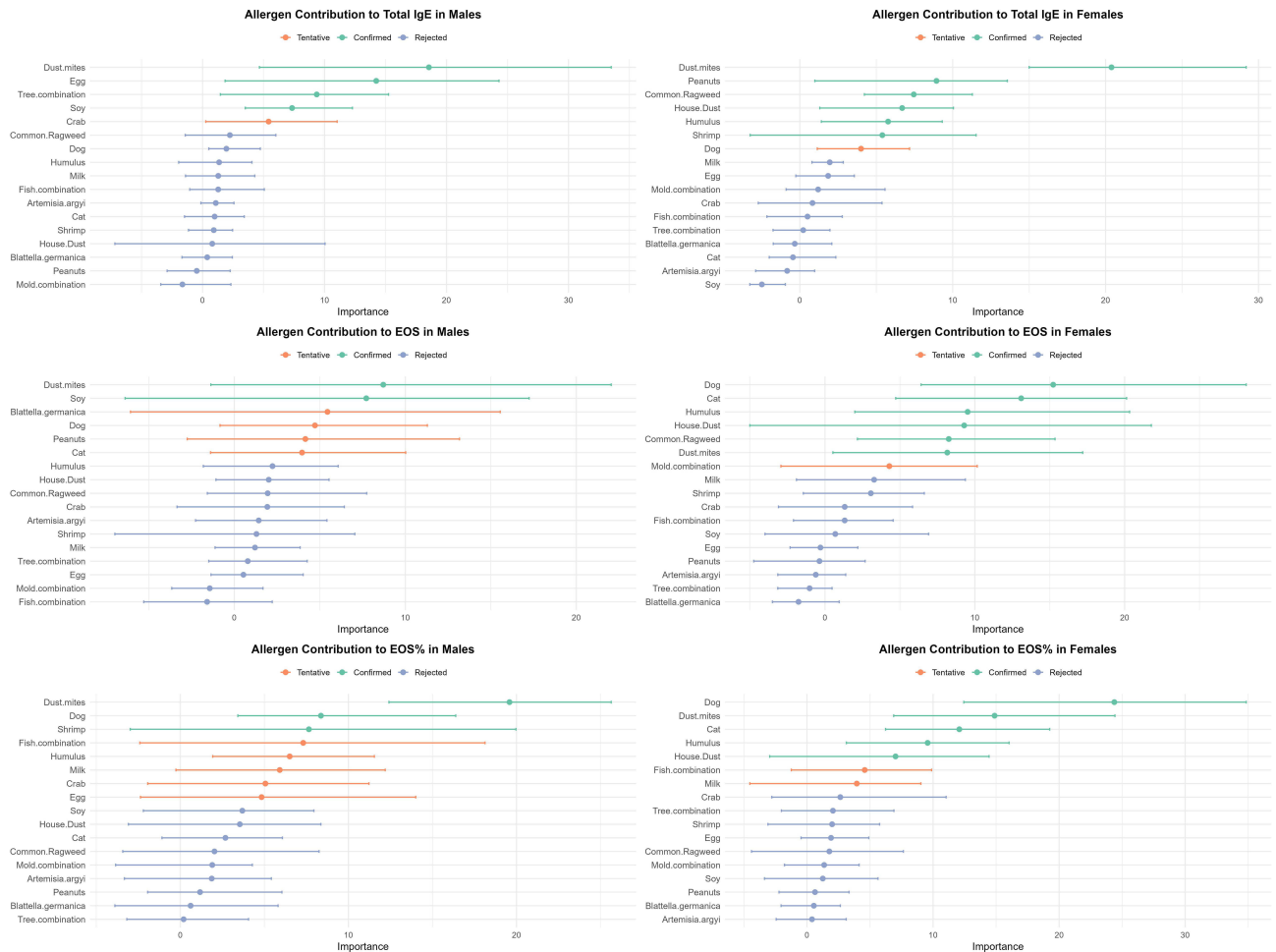
observed in teenagers and the elderly, particularly in the 70–100 years age group (Figure 1b and c). The 37–52 age group exhibited lower percentages and absolute values of eosinophils compared to other age groups.

Sex-based analysis revealed significantly higher t-IgE in males ( $282.20 \pm 23.44$  IU/mL for atopic and  $143.40 \pm 23.77$  IU/mL for non-atopic patients) compared to females ( $194.20 \pm 19.53$  IU/mL for atopic and  $73.82 \pm 8.09$  IU/mL for non-atopic patients), regardless of allergen test results (Supplementary Table 1, Figure 1d–f). Eosinophil counts were also elevated in males, suggesting a potential sex-related influence on these biomarkers.

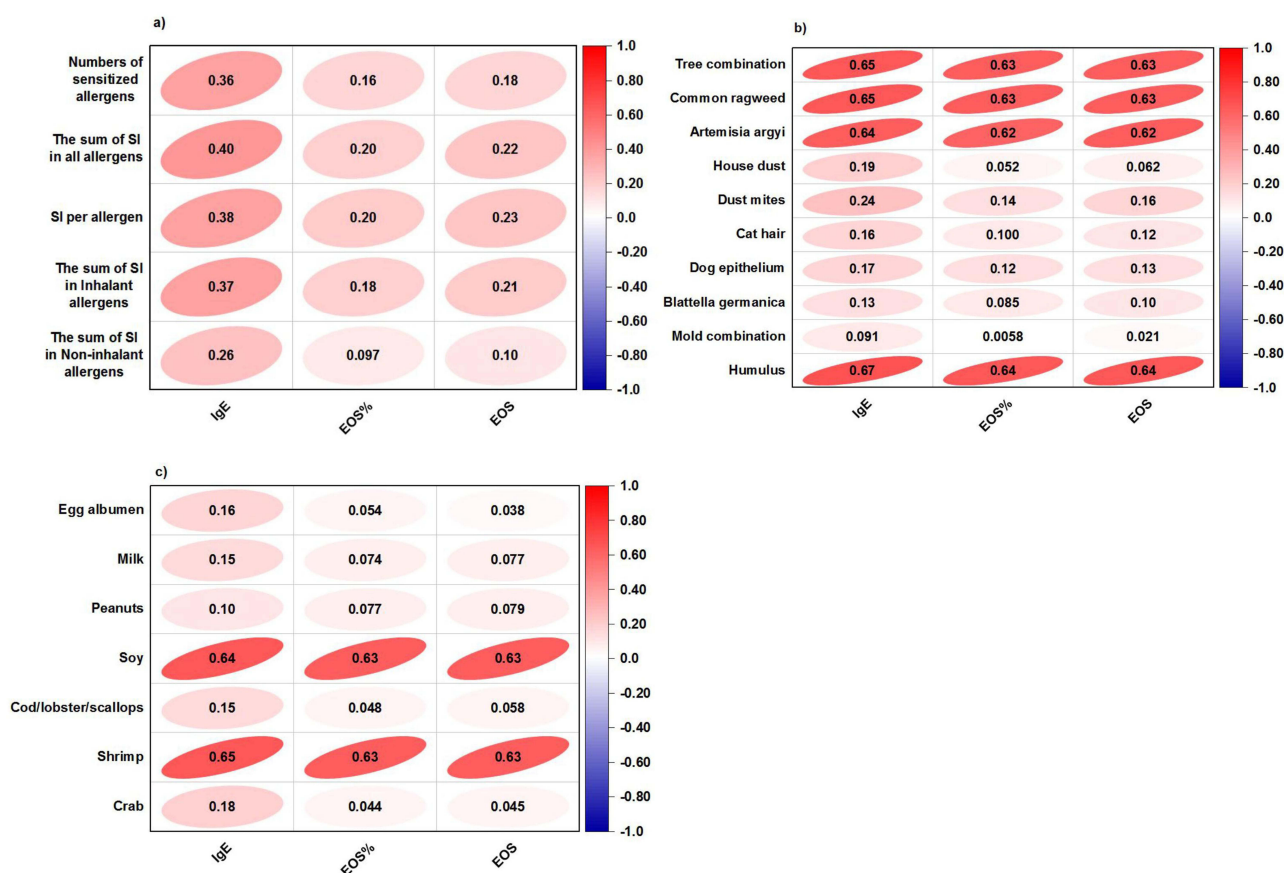
## Differential Responses in t-IgE and Eosinophils to Different Allergens

Our analysis of specific allergen responses revealed significant variability in t-IgE and eosinophil counts (Supplementary Table 2). Patients allergic to cat and Humulus exhibit the highest t-IgE, while those sensitized to *Artemisia argyi* and peanuts have the lowest t-IgE. Absolute eosinophil values of patients allergic to cat and dog are the highest. Conversely, patients sensitized to mold and soybeans have the lowest eosinophil values. The results of the Boruta algorithm demonstrated that dust mites had the highest importance in increasing total IgE levels across all populations. For eosinophil counts, dust mites showed the highest importance in males, while in females, the cat and dog allergens were identified as the most influential (Figure 2).

On the other hand, correlation analyses further confirmed the significant relationships between t-IgE, eosinophil counts, and the sensitive index (SI) of allergens involved. A significant positive correlation was found between t-IgE and the number of allergens, as well as their SI (Figure 3). Eosinophil levels also correlated positively, albeit to a lesser



**Figure 2** The relative importance of different allergens on t-IgE and eosinophil counts analyzed using the Boruta algorithm in males and females. The x-axis represented feature importance, while green indicates confirmed influence on t-IgE or eosinophil, blue represents rejected and Orange represents tentative. EOS, eosinophil.



**Figure 3** Representation of the Spearman correlation coefficient matrix and Spearman's rank correlation tests applied to various parameters: incidence of sensitization, sensitive index (SI), t-IgE, and peripheral eosinophil (a–c). Positive correlations are highlighted in red, negative correlations in blue, with color intensity indicating the strength of the Spearman  $r$  values, which are numerically displayed within the ellipses. EOS, eosinophil.

extent. Aeroallergens had a more substantial impact on t-IgE levels compared to food allergens, as evidenced by higher correlation coefficients (0.37 and 0.26, respectively). Notably, strong positive correlations between t-IgE, eosinophils, and the SI of specific allergens were particularly observed in atopic patients for specific allergens like willow/aspens/elm, common ragweed, *Artemisia argyi*, *Humulus*, soy and shrimp (all  $r > 0.60$ ; Figure 3). The extent of allergen exposure, indicated by the number and SI of allergens, was directly associated with higher t-IgE and, to a lesser extent, eosinophil counts.

## Correlation of IgE and Eosinophil Counts with Symptomatology

In our patient cohort, individuals aged 12 years and older primarily reported ear, nose, and throat (ENT) symptoms (1251 patients), respiratory symptoms (1725 patients), and dermatologic symptoms (2815 patients) (Supplementary Table 3). Interestingly, those primarily reporting respiratory symptoms were, on average, older ( $46.00 \pm 16.71$  years) compared to those reporting ENT symptoms ( $43.08 \pm 19.50$  years) (Supplementary Table 3).

Patients experiencing respiratory symptoms displayed a significantly elevated average SI for aero-allergens when compared to patients with the other two types of symptoms (Supplementary Figure 1a). Notably, 46.6% of patients with respiratory symptoms exhibited sensitization to aeroallergens. Within this subgroup of patients, the average SI for food allergens was also the highest, albeit the discrepancy between these patients and those with skin symptoms or ENT symptoms was relatively minor (Supplementary Figure 1b).

Analyzing the symptomatology in individuals aged 12 and above, we found higher t-IgE levels in those primarily reporting respiratory symptoms ( $464.20 \pm 236.80$  IU/mL) compared to dermatologic symptoms ( $343.00 \pm 159.80$  IU/mL).

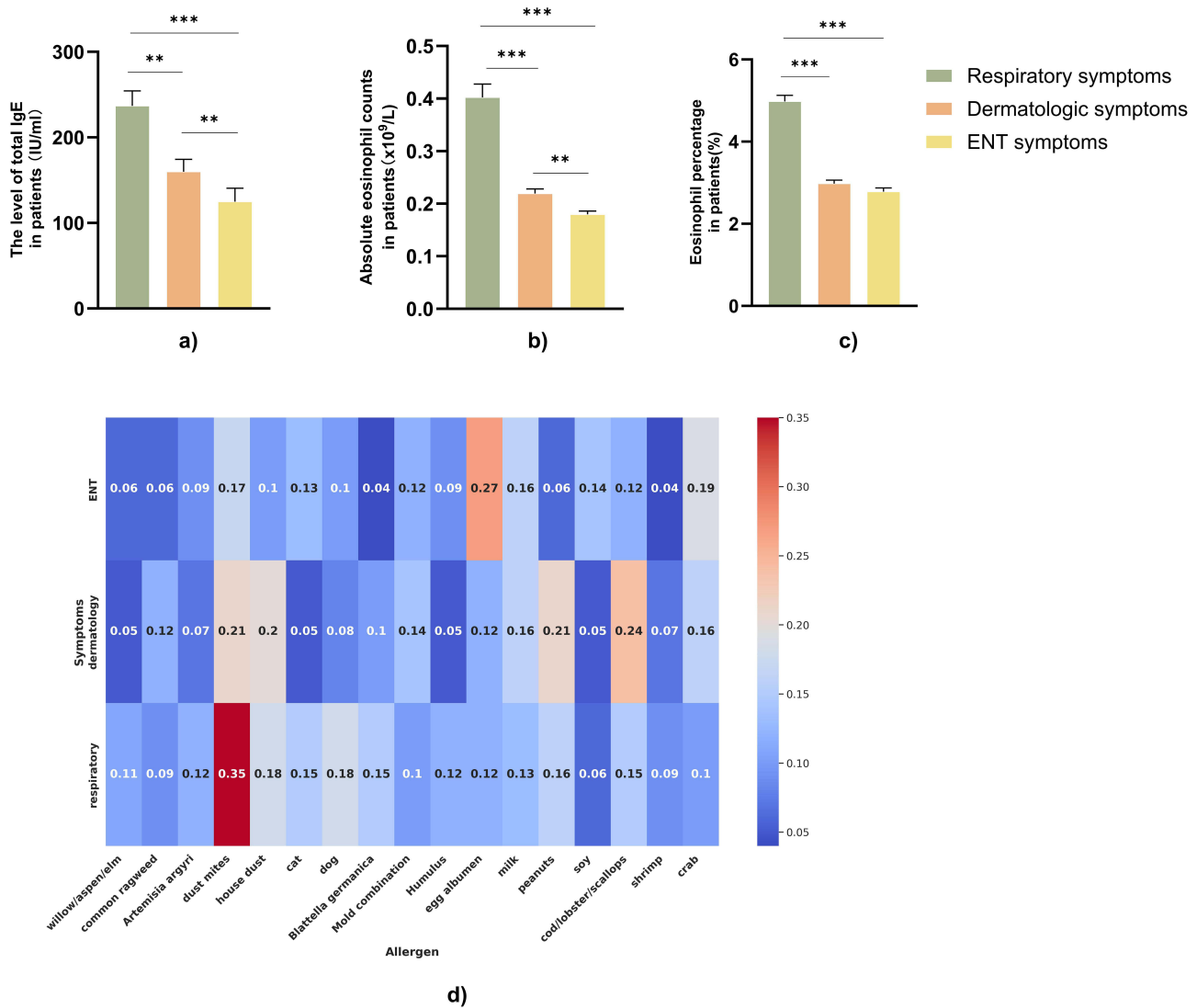
or ENT symptoms ( $332.50 \pm 125.00$  IU/mL) (Figure 4a). Eosinophil counts exhibited a similar trend, with the highest levels in patients primarily affected by respiratory symptoms (Figure 4b and c).

Supplementary Figure 1c illustrates the prevalence of elevated t-IgE or increased eosinophil counts within different symptomatic groups. Notably, among patients with respiratory symptoms, a larger proportion exhibited elevated t-IgE compared to those with increased eosinophil counts. Conversely, for those with dermatologic or ENT symptoms, a higher percentage demonstrating increased eosinophil counts.

Significant variations in the prevalence of specific allergens exist among patients with different primary complaints (Figure 4d). For instance, dust mites were commonly linked to respiratory symptoms, with approximately 35% of allergic patients sensitized to dust mites. Patients sensitized to house dust, peanuts, or fish appeared more prone to developing dermatologic symptoms. Among patients with ENT symptoms, 27% showed sensitization to egg.

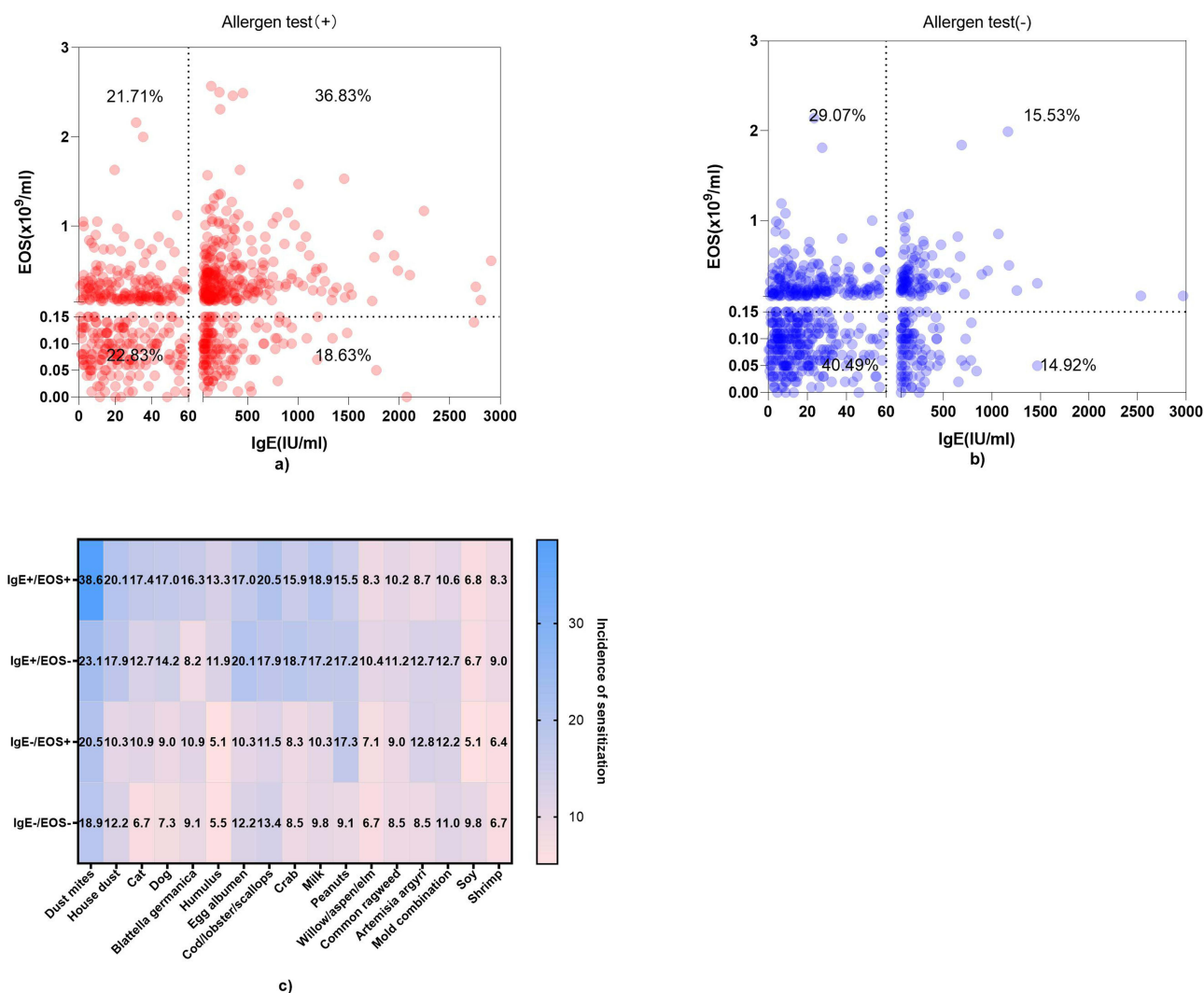
# Assessment of t-IgE and Eosinophil Consistency with Allergen Test Outcomes

Quadrant scatter plot analysis (Figure 5a and b) indicated that over half of patients with positive allergen tests had elevated t-IgE or eosinophil, but only 36.8% exhibited elevations in both biomarkers. In contrast, 22.8% displayed normal levels for both. Among those with negative allergen tests, 40.4% had normal t-IgE and eosinophil, whereas 15.5%



**Figure 4** Characteristics of t-IgE and eosinophils are presented across different symptom categories (a–c). Percentage of positive response to allergens, categorized by the primary symptom are detailed (d). Data was expressed as Mean  $\pm$  SEM. ENT, ear, nose, and throat. \*\*< 0.01, \*\*\*< 0.001.





**Figure 5** Quadrant scatter plots depicting the distribution of t-IgE and eosinophil levels in patients with positive (a) or negative (b) allergen test results. For various allergens, the incidence of sensitization in four categories grouped by t-IgE and eosinophil counts was indicated in (c). Each analysis is stratified based on t-IgE levels to provide a comprehensive view. EOS, eosinophil.

showed elevations in both. Importantly, irrespective of allergen test results, higher t-IgE were frequently associated with elevated eosinophils, a statistically significant finding ([Supplementary Figure 2](#)). Quadrant scatter plot analysis revealed that elevated t-IgE and eosinophil did not consistently coincide with positive allergen tests, indicating a complex relationship between these biomarkers and atopic sensitivity.

In patients with evaluated t-IgE and eosinophil counts, dust mites sensitization had the highest incidence. Most allergen sensitization seemed to induce an increase in t-IgE and eosinophil. However, some aero-allergens like tree combination and mold combination, and food allergens like soy and shrimp, had a weak capacity to induce an increase in t-IgE or eosinophil ([Figure 5c](#)).

Further categorization based on t-IgE concentrations into four groups (<60, 60–499, 500–999, and ≥1000 IU/mL) revealed a trend where higher t-IgE was associated with increased eosinophil counts, a greater number of sensitized allergens identified, and a heightened SI ([Supplementary Figure 2](#)).

**Table 1** Variability of Age, Sex, t-IgE, and Allergens Sensitized of Patients with Positive Allergen Tests: Grouped by t-IgE and Peripheral Eosinophil Counts

Characteristic	t-IgE(+)EOS(+)	t-IgE(+)EOS(-)	t-IgE(-)EOS(+)	t-IgE(-)EOS(-)	P value
Age (years) <sup>#</sup>	41.81 ± 18.29	43.74 ± 17.97	48.75 ± 17.23	47.51 ± 16.64	<0.001
1–17, n(%)	18 (7.0%)	9 (7.0%)	3 (2.0%)	5 (3.0%)	-
18–36, n(%)	110 (42.0%)	45 (34.0%)	46 (30.0%)	41 (25.0%)	-
37–52, n(%)	53 (20.0%)	31 (23.0%)	37 (24.0%)	45 (28.0%)	-
53–69, n(%)	61 (23.0%)	38 (28.0%)	54 (35.0%)	59 (36.0%)	-
70–100, n(%)	20 (8.0%)	10 (8.0%)	15 (9.0%)	13 (8.0%)	-
Male, n(%)	146 (55.5%)	67 (50.3%)	83 (53.5%)	61 (37.5%)	-
Sensitized Allergen types <sup>#</sup>	2.64 ± 1.95	2.42 ± 1.68	1.78 ± 1.27	1.65 ± 1.05	<0.001
SI of all allergens <sup>#</sup>	5.70 ± 4.49	4.26 ± 3.20	2.73 ± 2.70	2.21 ± 2.05	<0.001
SI of aeroallergens <sup>#</sup>	3.73 ± 3.54	2.37 ± 2.53	1.65 ± 2.41	1.19 ± 1.33	<0.001
SI of food allergens <sup>#</sup>	1.97 ± 2.52	1.88 ± 1.94	1.07 ± 1.28	1.02 ± 1.57	0.001

**Note:** <sup>#</sup>Data was expressed as Mean ± SEM.

**Abbreviations:** NAT, negative allergen test; PAT, positive allergen test; SI, sensitive index; t-IgE: total immunoglobulin E; t-IgE(+), t-IgE ≥ 60IU/mL; t-IgE(-), t-IgE < 60IU/mL; EOS(+), peripheral eosinophils ≥0.15×10<sup>9</sup>cell/L; EOS(-): peripheral eosinophils <0.15×10<sup>9</sup>cell/L;

## Characteristics of Atopic Patients with Normal t-IgE Levels

Analyzing the characteristics of patients with atopic patients with normal t-IgE levels is crucial for identifying populations at potential risk of overlooked diagnoses of allergic diseases, emphasizing the necessity for additional allergen testing.

Among patients with positive allergen tests, 44.5% had normal t-IgE levels, and 41.4% had normal eosinophil counts. Patients with normal t-IgE levels were, on average, older (48.12 ± 0.95 years) than those with elevated t-IgE (42.46 ± 0.91 years) ([Supplementary Table 4](#)). In patients with normal t-IgE levels, eosinophil counts (0.23 ± 0.01×10<sup>9</sup> cells/L) were lower than in patients with elevated t-IgE (0.46 ± 0.07×10<sup>9</sup> cells/L) ([Supplementary Table 4](#)). Notably, patients with high t-IgE and eosinophil counts were the youngest (41.81 ± 18.29 years) among those undergoing allergen testing, t-IgE, and eosinophil count testing ([Table 1](#)).

Patients with elevated t-IgE generally showed higher sensitization rates to most allergens, except for mold, grass and tree pollen, peanuts, and soy, where no significant differences were observed between groups. Without allergen testing, diagnoses may overlook patients allergic to these specific allergens due to normal t-IgE.

Females with elevated eosinophil counts and normal t-IgE levels had a similar proportion to the overall population with elevated t-IgE. Intriguingly, 63.5% of females with allergic symptoms but normal t-IgE and eosinophil levels still had the risk of atopy ([Table 1](#)), indicating that normal t-IgE and eosinophil levels do not rule out sensitization, especially in females.

Symptomatically, patients reporting skin and ENT symptoms were comparable between t-IgE normal and elevated groups. Without allergen tests, diagnoses may miss patients with only skin or ENT symptoms due to the absence of elevated t-IgE.

Analyzing the timing of clinic visits and allergen tests in atopic patients revealed that from April to June, most patients exhibited normal t-IgE levels ([Supplementary Table 4](#)). Conversely, in other months, those with elevated t-IgE predominated. This highlights the need to consider seasonal factors in allergen tests and diagnoses.

## Discussion

Our extensive study on 7,654 patients (1 to 100 years old) illuminates the significant influence of age, sex, and primary symptoms on immunological parameters, utilizing variable t-IgE levels and eosinophil counts as crucial biomarkers for allergic conditions. These findings reveal patterns crucial for guiding clinical practices and interpreting diagnostic tests. Amid the current landscape of allergy research, our investigation meticulously scrutinizes the immunological profiles across diverse age groups, sex, symptoms, and responses to various allergens.



Previous studies have consistently reported elevated t-IgE in children and adolescents, reflecting heightened immunological reactivity in these younger groups.<sup>14,15</sup> This aligns with observations of increased allergic reactions or conditions like eczema associated with elevated t-IgE in children.<sup>16</sup> Similar traits are found in eosinophils.<sup>10</sup> A study from Austria to explore level of the blood eosinophil count in the general population, it was found that age  $\leq 18$  years, asthma, current smoking, positive skin prick test and male sex were significantly associated with high blood eosinophil counts. We also observed the same characteristics, which the level of eosinophil counts in 1–17 years was highest in our research. Interestingly, patients in 70–100 years showed a increasing trend on eosinophils although there had no significant difference in our research. Notably, the chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is a cell surface protein that can reduce tissue eosinophilia and decreases with age,<sup>17,18</sup> possibly leading to elevated levels of eosinophils in the elderly. Changes in CD44 and CD54 with age can also affect the eosinophil counts level.<sup>17</sup> This notable observation, coupled with insights into changes in specific cell surface molecules associated with eosinophils, suggests potential targets for more tailored allergic diseases treatments across different age groups.

Sex disparities in t-IgE and eosinophil levels are evident, with females consistently exhibited lower levels than males, regardless of atopic status, as found in previous research.<sup>10</sup> Even among allergic females, nearly half exhibit normal t-IgE, aligning with findings from our previous study in mild asthma patients, which demonstrated that female patients have lower FeNO than males.<sup>19</sup> These observations, supported by literature on sex hormones and immune responses,<sup>20–23</sup> emphasize the nuanced understanding needed, as allergic disease incidence and severity in males may not consistently follow patterns. Particularly, in females with allergic symptoms but normal biomarker levels, relying solely on t-IgE and eosinophil counts for diagnosis could lead to underdiagnosis. These findings advocate for heightened clinical vigilance and perhaps a lower threshold for initiating allergen testing in females, even when t-IgE levels appear normal. Various factors, including sex hormones, genetic differences, environmental exposures, and immune system differences, contribute to these disparities. X-chromosomal genotypes like Forkhead Box P3 (FOXP3), may contribute to the development of sensitization against egg and indoor allergens in girls during early childhood.<sup>24</sup> It's the possible reason that although the t-IgE levels in males are higher than in females, the incidence and severity of allergic diseases are not consistently higher in the male population.

Interestingly, elevated t-IgE and eosinophil did not consistently correlate with positive allergen tests. Notably, the SI of allergens such as willow/aspen/elm, common ragweed, *Artemisia argyi*, Humulus, soy, and shrimp exhibit strong correlations with t-IgE and eosinophil levels in allergy patients.<sup>25–29</sup> In contrast, dust mites show no significant correlation, emphasizing the need for a dual-parameter approach in clinical evaluations to avoid diagnostic inaccuracies.

Analysis of symptomatology revealed the highest t-IgE and eosinophil in patients aged 12 years and older with respiratory symptoms. These findings suggest that higher t-IgE and eosinophil counts may indicate more severe respiratory-related atopic conditions.<sup>30</sup> In the context of allergic reactivity and the induction of t-IgE and eosinophils, the upper and lower respiratory tracts exhibit distinct functions and responses due to their differing environments and exposures.<sup>31–33</sup> When allergens penetrate deeper into the lower respiratory tract, they can induce a more systemic allergic response. This explains why patients with co-morbid allergic rhinitis and asthma have higher t-IgE and eosinophil counts than those with only allergic rhinitis.<sup>34,35</sup> Clinically, heightened attention is required for patients visiting the ENT department with high biomarker levels, identifying possible comorbidities of asthma.

In our study, dust mites often cause respiratory symptoms, while egg allergies typically affect the ear, nose, and throat (ENT). Seafood and peanut allergies are usually linked to skin symptoms. This is the first time that different allergens have been linked to different primary symptoms, which is advantageous for clinical practitioners in selecting the appropriate allergen panel for screening based on various clinical presentations.

We have depicted the prevalence of patients displaying elevated t-IgE or eosinophil counts within different symptomatic groups. Notably, among patients experiencing respiratory symptoms, a larger proportion exhibited elevated t-IgE compared to those with increased eosinophil counts. Conversely, for patients with dermatologic or ENT symptoms, the situation was reversed, with a higher percentage demonstrating increased eosinophil counts. Patients with respiratory symptoms should prioritize t-IgE screening. Patients presenting with ENT and dermatological symptoms should not overlook the assessment of peripheral blood eosinophils, which helps to comprehensively identify patients' allergic traits and contributes to the further exploration of potential beneficiaries of biologic targeted therapies.

Importantly, our quadrant scatter plot analysis indicated that more than a quarter of the patients have both biological markers within the normal range. The sensitization to allergens such as soy, shrimp, mold combination, *Artemisia argyi*, common ragweed, and willow/aspen/elm was not affected by whether t-IgE and eosinophils are elevated. For this subset of patients, allergen testing is necessary to reflect sensitization, and cannot be replaced by screening with the two biological markers mentioned above. This suggests a need for more comprehensive diagnostic approaches to capture diverse immunological responses.

Finally, our findings underscore the temporal patterns of clinic visits, with peaks in normal t-IgE levels during April to June, suggesting a role for seasonal allergens in symptom elicitation without corresponding biomarker elevations.<sup>36–39</sup> This seasonal pattern emphasizes the importance of considering environmental factors when interpreting test results, with implications for the timing of allergen testing and diagnosis.

This study has several limitations that warrant discussion. Firstly, the investigation was conducted as a single-center, retrospective analysis. Future studies should adopt a multi-center, prospective design to validate and extend our results. And it was considered atopy status according to the positive serum allergen test which may be difficult to confirm using other tests. Secondly, the cohort size was limited. For a more comprehensive understanding of the allergy progression and its biomarkers across different age groups, we plan to recruit a larger and more diverse patient population. Lastly, the number of pediatric patients in our study was limited due to the lower outpatient population in the pediatric department.

## Conclusion

This study highlights the complex relationship among t-IgE, eosinophils, and atopic diseases and the limitations of traditional biomarkers in capturing the full spectrum of allergic responses. The variations we have observed in t-IgE and eosinophil counts, influenced by age, sex, symptoms, and allergen exposure. In the allergic population, characteristics such as older, female, and having dermatologic or ENT symptoms may be associated with lower t-IgE and eosinophils. It's crucial to test for allergens based on the specific symptoms presented. Furthermore, our study identifies specific populations at risk of misevaluation. Even if t-IgE and eosinophil levels are normal, for patients exhibiting allergic symptoms with these clinical features, allergen testing is necessary for treatment strategies. And we will perform a prospective research to validate the findings in the future.

## Abbreviations

AHR, airway hyperresponsiveness; CI, confidence intervals; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; ENT, ear, nose, and throat; FOXP3, Forkhead Box P3; ILC2, group 2 innate lymphoid cell; IQR, interquartile range; NAT, negative allergen test; PAT, positive allergen test; SEM, standard and mean; SI, sensitive index; Th2, T helper 2; t-IgE, total immunoglobulin E.

## Data Sharing Statement

All data generated or analysed during this study are included in this published article [and its supplementary information files].

## Ethics Approval and Consent to Participate

Given the observational nature of this study, and the minimal risk posed to participants, obtaining informed consent was deemed impracticable. The ethics committee of Shanghai General Hospital, Shanghai Jiao Tong University approved the protocol and a waiver of informed consent was given for our study. And the study complied with the Declaration of Helsinki, which was conducted in strict accordance with the principles of non-invasive data collection, and all patient data were fully anonymized to ensure the protection of privacy throughout the research process.

Our protocol was approved by the ethics committee of Shanghai general hospital and a waiver of informed consent was issued for our study (number: 2017KY159).

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare that they have no conflicts of interests.

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