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ORIGINAL RESEARCH

Efficacy of *Artemisia annua* Sublingual Immunotherapy for Seasonal Allergic Rhinitis in Relation to Variable Pollen Exposure: A Multicenter Study

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Purpose: Evaluating allergen immunotherapy efficacy amidst natural pollen fluctuations is complicated by pollen variation. This study evaluates the relationship between pollen exposure and therapeutic impact of *Artemisia annua* sublingual immunotherapy (SLIT) in seasonal allergic rhinitis (SAR).

Patients and Methods: In a two-year multicenter, controlled trial, SAR patients sensitized to *Artemisia* received SLIT or symptomatic medication. Daily assessments of combined symptom and medication score (CSMS), total nasal symptom score (TNSS) and daily medication score (DMS), were conducted across two pollen seasons. Linear mixed-effect models (LMMs) were utilized to evaluate treatment effects while considering pollen variability. Exploratory analyses of immunoglobulins in serum and nasal secretions were performed.

Results: Pollen fluctuations correlated positively with CSMS, TNSS, and DMS in both groups. SLIT significantly reduced the mean daily CSMS ($\beta = -0.34$, $q < 0.001$), TNSS ($\beta = -1.01$, $q < 0.001$), and DMS ($\beta = -0.09$, $q < 0.001$) compared to the control group. These effects were consistent across both pollen seasons. During the first pollen season, a significantly negative impact of group × time × pollen interactions on the average daily CSMS was observed in SLIT group ($\beta = -0.11$, $P = 0.03$). LMM analyses revealed significant group × time interactions for serum *Artemisia*-specific IgG4 and IgA with nasal IgA showing isolated significance.

Conclusion: SLIT provided early and sustained symptom relief in SAR, taking into account the annual and seasonal pollen fluctuations. SLIT efficacy may be influenced by pollen exposure, with effect size increasing in correlation with higher levels of pollen exposure. SLIT elevated allergen-specific IgG4 and IgA in systemic and local compartments.

Keywords: seasonal allergic rhinitis, sublingual immunotherapy, pollen exposure, symptom severity, efficacy

Introduction

Seasonal allergic rhinitis (SAR) is primarily caused by exposure to airborne pollen. The overall global prevalence of pollen allergies is estimated to be 14.4%, with a significantly higher rate of up to 30.9% in high income countries.[1]

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A study conducted in the grassland regions of northern China has found that the incidence rate of pollen-induced allergic rhinitis can reach up to 31.4%.[2] SAR incidence and severity are increasing, exacerbated by the higher pollen activity and prolonged pollen seasons, which intensify SAR symptoms compared to perennial allergic rhinitis.[3,4] This heightened exposure to pollen significantly elevates the risk associated with SAR compared to indoor allergens,[5,6] leading to a substantial increase in outpatient visits throughout the season.[7,8] *Artemisia* is a highly allergenic plant for pollen-induced rhinitis, afflicting 32.35% to 58.2% of people with allergic rhinitis.[9–11] SAR is a growing health issue with a notable impact on affected populations, necessitating increased attention to the role of pollen exposure in the management of SAR.

Pollen exposure should be considered a critical variable in the therapeutic outcome assessment. The position paper from the European Academy of Allergy and Clinical Immunology (EAACI) emphasizes the importance of an appropriate definition of seasonal pollen exposure impact on the severity of symptoms in pollen allergen immunotherapy (AIT) trials.[12] Durham et al have revealed that the efficacy of AIT is highly variable due to year-to-year and in-season fluctuations in pollen exposure, with the treatment's impact potentially influenced by both its efficacy and the actual pollen exposure experienced.[13] Individuals with severe pollen allergies may have intermittent symptoms during the pollen season, sometimes with little to no symptoms at all, due to variations in pollen levels. Symptom variations attributable to yearly and daily variations in pollen exposure are often overlooked in the analysis of AIT efficacy, as they are averaged over predefined time points, thus obscuring the day-by-day changes. Long-term clinical benefits can be induced by AIT after repeated doses of the sensitizing allergen are administered for at least 2 to 3 years.[14–16] However, there is a notable absence of emphasis on pollen exposure in pollen AIT trials, with scant researches incorporating pollen exposure into the analysis of treatment efficacy.[14,15] This oversight not only diminishes the internal validity of clinical studies on AIT outcomes but also leads to discordance and non-comparability among studies.[12,13] Merely providing a definition of the pollen season is insufficient; incorporating pollen concentration into the assessment of AIT efficacy for SAR appears to provide a more accurate description of AIT's therapeutic effects.[17,18]

Consequently, to better assess the correlation between pollen exposure and the therapeutic efficacy of *Artemisia annua* allergens (SLIT) in patients with SAR, we initiated this study in Beijing, China.

Patients and Methods

Study Design and Participants

This study was conducted from March 2022 to October 2023 at the Department of Otolaryngology Head and Neck Surgery and Department of Allergy of three hospitals in Beijing (Beijing Tongren Hospital, Beijing Shijitan Hospital, and Peking University People's Hospital). The inclusion criteria for the participants, were as follows: (i) aged from 18 to 60 years; (ii) a history of least two years of SAR related to *Artemisia* based on the international diagnostic criteria of AR guidelines (Allergic Rhinitis and its Impact on Asthma, ARIA);[19](see detailed criteria in the Supplementary Information) (iii) the sum of total nasal symptom score during the last peak pollen period (PPP) reached or exceeded 6; (iv) dominant sensitization to *Artemisia annua* confirmed by the serum specific immunoglobulin E (sIgE; ≥3.5 kU/L). Exclusion criteria included higher sIgE against *ragweed or Humulus* than *Artemisia*, a clinical history of perennial AR, chronic rhinitis, or chronic rhinosinusitis, contraindications of AIT, previous immunotherapy with pollen extracts and living outside of Beijing during the pollen season. The study was approved by the Medical Ethics Committee of Beijing Tongren Hospital (TREC2022-KY029). All of the patients gave their written consents to participate after being made aware of the objectives and protocols of the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

After obtaining written informed consent and screening, baseline data including demographics, medical history, and nasal and ocular symptom scores and the use of symptomatic medication during the last PPP were collected. Eligible participants were allowed to self-select their treatment group based on personal preference, with those opting for SLIT assigned to Group 2, and those choosing symptomatic medication alone assigned to Group 1. The inclusion criteria were identical for both groups to ensure comparability at baseline. The self-reported symptoms of SAR and the corresponding medication usage were systematically tracked on a daily basis via electronic diary throughout the pollen season. During the research, follow-up assessments were meticulously carried out via in-person consultations, telephonic interviews, or

video conferences. This approach ensured comprehensive data acquisition, encompassing evaluations at the commencement and conclusion of each pollen season, a minimum of one assessment during the pollen season, and at least one evaluation every three months during the non-pollen season.

Procedures

After a screening period up to 3 days, the eligible participants in SLIT group underwent *Artemisia annua Allergens* Sublingual Immunotherapy Drops (Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd., Zhejiang, China), beginning approximately 14.6 weeks (range 10 to 21 weeks) before the summer-autumn pollen season in 2022. The *Artemisia annua* SLIT consists of two phases: the 5-week up-dosing phase and the continuous maintenance phase. The *Artemisia annua* drops were manufactured as five vials of specific *Artemisia annua* extracts with increasing concentrations ranging from 25 to16000BU/mL. Drops No. 1 to No. 5 are used for the dose escalation phase, while No. 5 is used for the maintenance phase (Supplementary Information Table S1). Participants both in SLIT and control groups were permitted to use symptomatic treatments specifically for rhinoconjunctivitis, which included loratadine tablets, nasal budesonide spray, and/or methylprednisolone tablets, all administered under the guidance of the research team following standardized dosing regimens.

Pollen Concentration Monitoring

During the pollen season, daily data on pollen concentration were gathered using a gravity-settling pollen trap from Beijing's pollen monitoring stations located in 12 different districts. The sampling sites, located in Changping, Chaoyang, Fangshan, Fengtai, Haidian, Huairou, Mentougou, Miyun, Pinggu, Shijingshan, Shunyi, and Yanqing, were strategically situated near residential areas, covering 12 of the city's 16 districts. This distribution ensured the data reflects the general levels of pollen exposure experienced by residents throughout Beijing. In addition to total pollen concentration, Artemisia pollen levels were also recorded, as Artemisia is the predominant pollen during the summer-autumn season, with high concentrations and a significant sensitization rate. The displayed values are the average count per unit area (grains/1000 mm^2) for all sampling locations. The pollen season is delineated as the period commencing from the first to the last day of three consecutive days where pollen concentration is equal to or exceeds 100 grains per 1000 mm^2. Similarly, the Peak Pollen Period (PPP) is characterized from the first to the last day of three consecutive days where pollen levels meet or surpass a threshold of 300 grains per 1000 mm^2.[20]

Monitoring of Symptom Severity

Symptom and medicine scores were assessed throughout the pollen season of 2022 and 2023, defined as Y1 and Y2, respectively, when all participants recorded daily nasal/ocular symptoms and medication consumption, in an electronic diary. Each individual nasal and ocular symptom were rated on a 4-point scale of 0–3, including 6 rhinoconjunctivitis symptoms (rhinorrhea, nasal congestion, nasal itching, sneezing, ocular itching/grittiness/redness, and watery eyes). Daily medication score (DMS) was calculated as follows: 0 = no use of rescue medication, 1 = use of only antihistamines and/or antileukotrienes; 2 = use of intranasal corticosteroids; 3 = use of oral corticosteroids. The total nasal symptom scores (TNSS), which range from 0 to 12, represent the sum of four-point scales measuring four nasal symptoms (detailed scoring criteria are provided in the Supplementary Information). Total ocular symptom scores (TOSS), ranging from 0 to 6, represent the sum of four-point scales measuring two ocular symptoms. Combined symptoms medication score (CSMS), ranging from 0 to 6, was calculated with the following formula: CSMS = TNSS/4 + DMS.[21–23]

Outcome

The primary clinical endpoint of this study was the CSMS, which included both nasal symptom severity and medication use. It is the most used AIT clinical endpoints recommended by the EAACI.[23] Secondary endpoints included nasal (TNSS) and ocular (TOSS) symptoms, as well as medication intake (DMS).

Serum and Nasal Samples Collection and Measurement of Immunoglobulins

Blood and nasal samples were collected at three time points: baseline (T1) and two pollen peak periods (T2, T3). Both T2 and T3 corresponded to sample collection during the pollen peak seasons. Nasal secretions were collected as described

by Xu et al.[24] The total IgE (tIgE), sIgE for *Artemisia* (Art-sIgE), and *Artemisia vulgaris 1* (Art v 1-sIgE) in both serum and nasal secretions were measured using the ALLEOS 2000 system (HYCOR Biomedical). The *Artemisia*-specific IgG4 (Art-sIgG4) and IgA (Art-sIgG4) were quantified using commercially available ELISA kits (Thermo Fisher Scientific). Briefly, plates were coated with 5μg/mL of *Artemisia* antigen.

Statistical Analysis

To evaluate the comparability of baseline characteristics between the two groups, categorical variables were analyzed using the chi-square (χ^2) test, while continuous variables were assessed with either the Mann–Whitney *U*-test or Student's *t*-test, depending on the data distribution. Categorical data were presented as frequencies (percentages), and continuous variables mean ± standard deviation (SD).

A generalized additive model (GAM) was applied to explore the relationship between symptom severity and pollen concentration. The efficacy of SLIT for SAR in relation to variable pollen exposure was assessed by fitting linear mixed-effect models (LMMs) with CSMS, TNSS, TOSS, or DMS as the dependent variables. Fixed effects included group (SLIT vs control), daily pollen concentration, time (consecutive days of observation during the pollen season), and the interactions of group × time and group × time × pollen (The "×" symbol was used to denote statistical interaction terms between variables). The inclusion of interaction terms allowed for the evaluation of whether the SLIT group demonstrated a significant improvement in CSMS, TNSS, TOSS, or DMS over time compared to the control group (group × time interaction), and also examined if there was a differential change in the effect of pollen concentration on CSMS, TNSS, TOSS, or DMS between the two groups over time (group × time × pollen interaction). Participant served as a random effect. Each variable underwent z-score transformation. The fixed effect coefficient β indicated the estimated change in the response variable for a standard deviation change in the explanatory variable.

Statistical analysis was performed via the SPSS version 27.0, and the R packages "dplyr", "mgcv", and "nlme" were employed for data preprocessing and GAM and LMM analysis in the R software (version 4.3.3). All tests were two tailed, and the significance level was set at 0.05. The Benjamini–Hochberg correction was applied to reduce the bias caused by multiple comparisons, with a significance level of $q < 0.05$.

Results

Study Population

Following the screening process, a total of 68 participants were enrolled in the SLIT group, while 12 participants were assigned to the control group. After a two-year period encompassing two summer-autumn pollen seasons, 82.4% (56/68) of participants in the SLIT group and 81.7% (11/12) of participants in the control group completed the study (Figure 1). There were no significant differences in age and ratio of males between the SLIT and control group. Both groups were comparable in co-morbidity of allergic conjunctivitis and asthma at baseline (Table 1). Patients with SAR demonstrated comparable levels of moderate to severe symptoms during the previous pollen season, as evidenced by CSMS, TNSS, TOSS, and DMS between the two groups. All participants met the inclusion criteria of having an IgE level of 3.5 kU/L or higher; however, the average level of serum sIgE against to *Artemisia* was higher in the SLIT group compared to the control group. By the time of the peak pollen period in 2023, the average duration of immunotherapy received by the SLIT group participants was 16.3 months (ranging from 15.2 to 17.8 months).

Relationship Between Pollen Concentrations and SAR Symptoms in SLIT and Control Group

During the two-year duration of pollen monitoring in this study, we observed both daily and annual fluctuations in the total pollen counts (Figure 2). Despite the annual fluctuations, a pronounced peak in pollen levels was consistently recorded in both years, ensuring that participants were exposed to substantial pollen concentrations over each season.

In this study, we identified a significantly positive, non-linear correlation between pollen concentration and SAR symptoms as indicated by CSMS, TNSS, DMS, as well as individual nasal and ocular symptoms scores (all $q < 0.001$) (Supplementary Information Figure S1 and Table S2). Notably, a predominantly linear effect was observed throughout

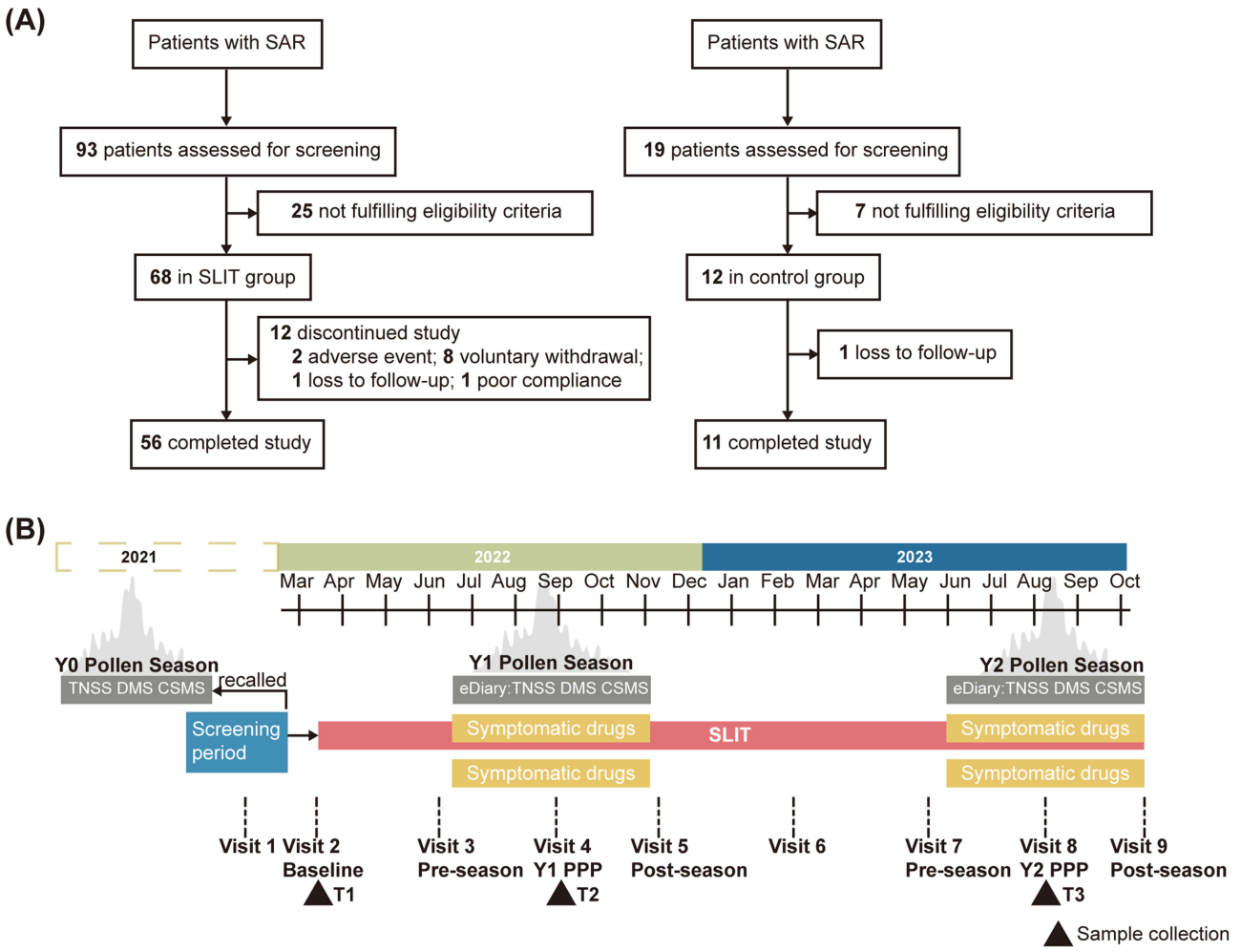

Figure 1 Overview of patient enrollment (**A**) and study design (**B**).
Abbreviations: CSMS, combined symptom and medication score; DMS, daily medication score; PPP, peak pollen period; SAR, seasonal allergic rhinitis; SLIT, sublingual immunotherapy; TNSS, total nasal symptom scores.

a substantial portion of the observation period, which is consistent with findings from prior research.[25,26] GAM analysis revealed that this substantial association was evident in both the SLIT and control groups (Supplementary Information Figure S1 and Table S2).

The Efficacy of SLIT Was Enhanced with Elevated Pollen Exposure Over the Initial Pollen Season

To investigate the impact of SLIT on SAR symptom over time and to assess the influence of pollen concentration in this therapeutic evaluation, LMMs were constructed using data from summer-autumn pollen season 2022 and 2023, as well as

Table 1 Demographic Characteristics of the Participants in Both Groups

Variables	SLIT (n=56)	Control (n=11)	*P* value
Age (year)	35.73 (8.42)	37.09 (9.61)	0.634
Sex, n (%)			0.953
Male	26 (46.43%)	5 (45.45%)	
Female	30 (53.57%)	6 (54.55%)	

(*Continued*)

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Table 1 (Continued).

Variables	SLIT (n=56)	Control (n=11)	*P* value
Height (m)	1.70 (0.07)	1.71 (0.11)	0.647
Weight (kg)	67.79 (13.97)	71.81 (12.86)	0.326
BMI (kg/m^2)	23.37 (3.36)	24.49 (3.11)	0.204
Nasal symptom scores at the Y0 PPP[a]	9.34 (1.60)	9.00 (1.10)	0.356
Sneezing	2.29 (0.73)	2.18 (0.60)	0.543
Rhinorrhea	2.89 (0.37)	2.91 (0.30)	1.000
Nasal itching	2.18 (0.74)	2.00 (0.45)	0.306
Nasal congestion	1.98 (0.73)	1.91 (0.83)	0.757
Ocular symptom scores at the Y0 PPP	3.05 (1.91)	2.73 (1.85)	0.606
Ocular itching/grittiness/redness	1.98 (1.07)	1.45 (1.21)	0.171
Watery eyes	1.07 (1.08)	1.27 (0.79)	0.386
CSMS at the Y0 PPP	4.15 (0.58)	4.16 (0.46)	0.817
DMS at the Y0 PPP	1.82 (0.43)	1.91 (0.30)	0.547
Conjunctivitis, n (%)	48 (85.71%)	10 (90.91%)	1.000
Asthma, n (%)	2 (3.57%)	1 (9.09%)	0.421
Atopic dermatitis, n (%)	8 (14.29%)	3 (27.27%)	0.371
Artemisia-specific IgE (kU/L)	33.38 (29.19)	15.68 (26.54)	0.008
Allergies to *ragweed*, n (%)	33 (58.93%)	7 (63.64%)	1.000
Allergies to *Humulus*, n (%)	13 (23.21%)	4 (36.36%)	0.451
Allergies to *ragweed* and *Humulus*, n (%)	10 (17.86%)	4 (36.36%)	0.223

Notes: Data were presented as n (%) or mean (standard deviation). [a]Y0 PPP represented the peak pollen period of the year prior to enrollment.
Abbreviations: BMI, body mass index; CSMS, combined symptom and medication score; DMS, daily medication score; IgE, immunoglobulin E; PPP, peak pollen period; SLIT, sublingual immunotherapy.

data encompassing both seasons, respectively (Table 2). SLIT significantly reduced the mean daily CSMS, TNSS, TOSS, and DMS compared to the control group at the onset of two pollen seasons (group effect: CSMS $\beta = -0.82$, $P < 0.001$; TNSS $\beta = -1.22$, $P = 0.02$; TOSS $\beta = -0.60$, $P = 0.08$; DMS $\beta = -0.52$, $P = 0.003$) and consistently across all seasons

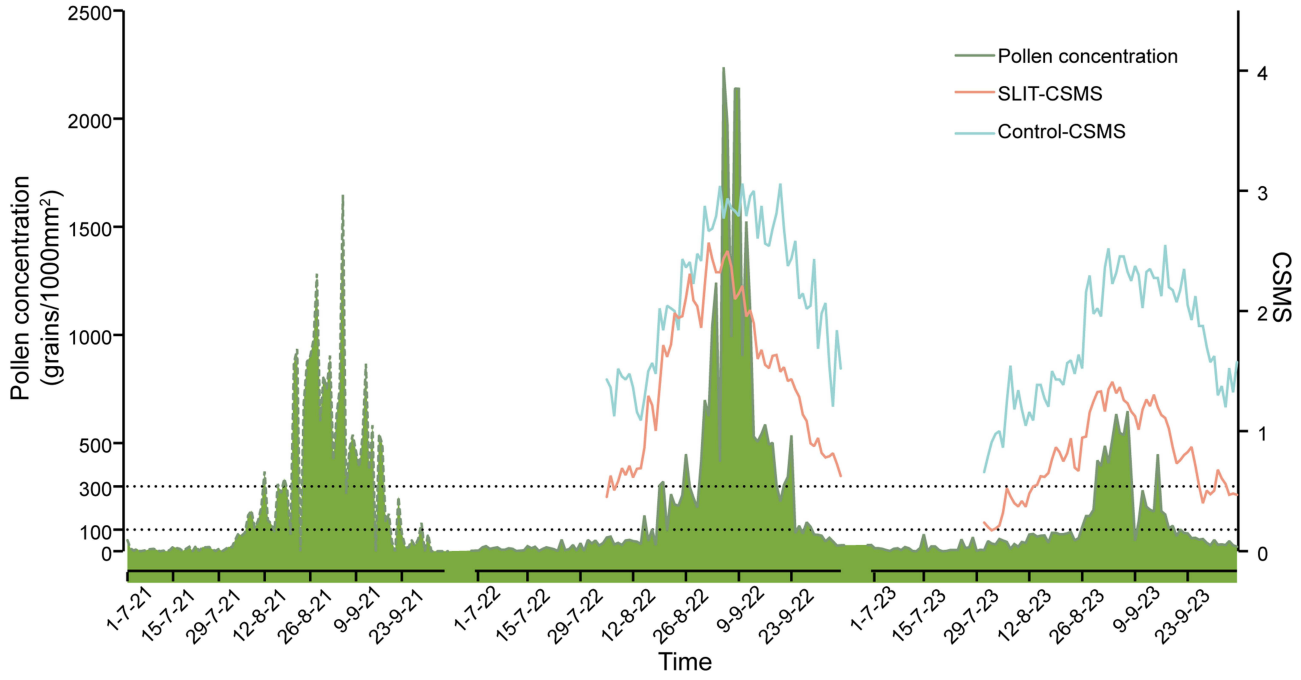

Figure 2 Pollen concentration of Beijing in 2021, 2022, and 2023 and CSMS of SLIT and control group during pollen season of 2022 and 2023.
Abbreviations: CSMS, combined symptom and medication score; SLIT, sublingual immunotherapy.

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Table 2 Linear Mixed Effects on Clinical Outcomes for Year-Specific and Combined Data

		Model 1	Model 2	Model 3
CSMS	Group2	−0.64* (0.25)	−1.05*** (0.23)	−0.82*** (0.22)
	Time	0.15 (0.08)	0.07 (0.06)	0.04 (0.03)
	Pollen	0.22*** (0.02)	0.11*** (0.02)	0.18*** (0.01)
	Group2 × time	−0.45*** (0.09)	−0.09 (0.06)	−0.34*** (0.04)
	Group1 × time × pollen	0.06 (0.12)	−0.02 (0.07)	0.02 (0.05)
	Group2 × time × pollen	−0.11* (0.05)	0.02 (0.03)	−0.03 (0.03)
TNSS	Group2	−0.61 (0.65)	−2.15*** (0.57)	−1.22* (0.50)
	Time	0.39# (0.21)	0.30* (0.12)	0.29** (0.09)
	Pollen	0.52*** (0.04)	0.21*** (0.04)	0.41*** (0.06)
	Group2 × time	−1.07*** (0.22)	−0.42** (0.13)	−1.01*** (0.10)
	Group1 × time × pollen	0.20 (0.30)	0.07 (0.15)	−0.02 (0.13)
	Group2 × time × pollen	−0.21# (0.13)	−0.04 (0.07)	−0.06 (0.08)
TOSS	Group2	−0.30 (0.40)	−1.04** (0.34)	−0.60# (0.34)
	Time	0.11 (0.09)	0.23*** (0.06)	0.16*** (0.04)
	Pollen	0.28*** (0.02)	0.13*** (0.02)	0.21*** (0.03)
	Group2 × time	−0.45*** (0.10)	−0.20** (0.06)	−0.44*** (0.04)
	Group1 × time × pollen	0.27* (0.14)	0.10 (0.07)	0.09 (0.06)
	Group2 × time × pollen	−0.05 (0.06)	−0.04 (0.03)	−0.03 (0.04)
DMS	Group2	−0.49* (0.19)	−0.52** (0.17)	−0.52** (0.17)
	Time	0.06 (0.05)	−0.004 (0.04)	−0.04 (0.02)
	Pollen	0.09*** (0.01)	0.05*** (0.01)	0.08*** (0.01)
	Group2 × time	−0.18** (0.06)	0.01 (0.04)	−0.09*** (0.02)
	Group1 × time × pollen	0.01 (0.08)	−0.04 (0.04)	0.02 (0.03)
	Group2 × time × pollen	−0.06# (0.03)	0.03 (0.02)	−0.01 (0.02)

Notes: Model 1, Model 2, and Model 3 were based on data from the 2022 pollen season, the 2023 pollen season, and the combined data from both years, respectively. Group 1 and Group 2 represent the control group and the SLIT group, respectively. The values indicated the coefficients of the fixed effects (β) with standard errors (SE) shown in parentheses. # $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. The "×" symbol was used to denote statistical interaction terms between variables.
Abbreviations: CSMS, combined symptom and medication score; DMS, daily medication score; TNSS, total nasal symptom scores; TOSS, total ocular symptom scores.

(Figure 3) (group × time interaction: CSMS $\beta = -0.34$, $P < 0.001$; TNSS $\beta = -1.01$, $P < 0.001$; TOSS $\beta = -0.44$, $P < 0.001$; DMS $\beta = -0.09$, $P < 0.001$). The findings indicated that SLIT could offer early symptom relief and reduced medication use at the onset of the pollen season (group effect), as well as sustained symptom alleviation and continued reduction in medication consumption over the course of the two-year pollen seasons (group × time interaction effect). In addition, the pollen levels at the onset of pollen seasons demonstrated significantly positive effects on symptoms (pollen effect), consistent with the aforementioned results of the GAM analysis. The longitudinal analysis of treatment and pollen interactions (Table 2) demonstrated a significantly negative effect of the group × time × pollen interactions on the average daily CSMS within the SLIT group ($\beta = -0.11$, $P = 0.03$), as opposed to the control groups, and this was observed exclusively during the initial pollen season (Supplementary Information Figures S2 and S3).

Similarly, the group × time × pollen interaction for TOSS in the control group during the initial pollen season was significant ($\beta = 0.27$, $P < 0.05$), indicating a greater symptom increase with rising pollen levels compared to the SLIT group. This suggested that elevated levels of pollen exposure were positively associated with the efficiency of SLIT during the initial pollen season.

Sensitivity analysis was conducted to assess the robustness of our results by specifically considering Artemisia pollen concentrations, which are the predominant allergens during the summer-autumn season. This analysis was particularly important because some participants had mixed allergies to both Artemisia and other pollen types, which could potentially confound the findings if total pollen counts were used. Thus, we conducted a sensitivity analysis using

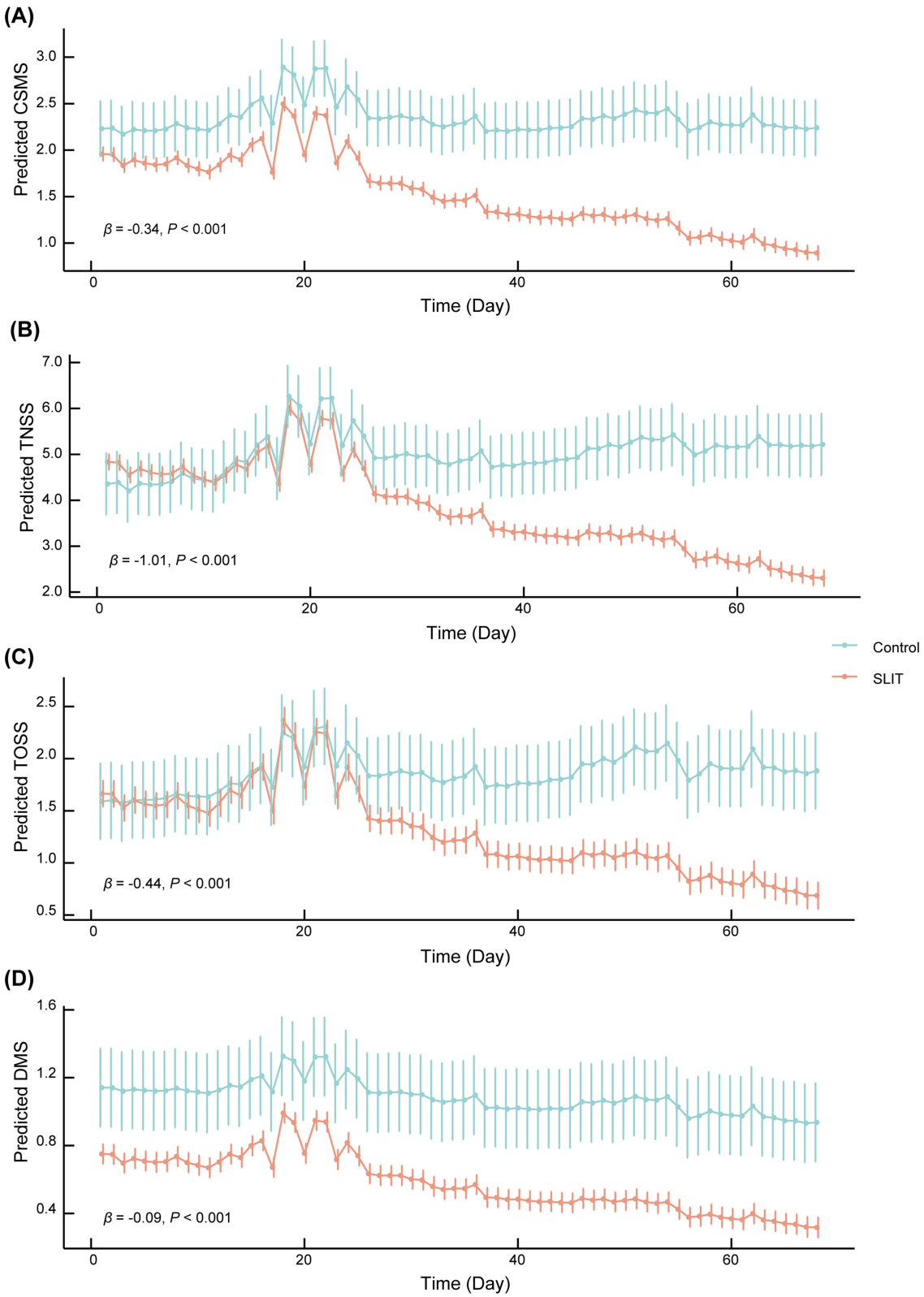

Figure 3 LMM-predicted CSMS (**A**), TNSS (**B**), TOSS (**C**), and DMS (**D**) over time for SLIT and control groups.
Notes: The data represented the combined data from both pollen seasons. The data showed the mean ± SE for the predicted values using the random-effects model of two-year data. β represented the coefficient of the group × time interaction term.
Abbreviations: CSMS, combined symptom and medication score; DMS, daily medication score; LMM, linear mixed mo; SE, standard error; SLIT, sublingual immunotherapy; TNSS, total nasal symptom scores; TOSS, total ocular symptom scores.

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only Artemisia pollen to verify if similar results would emerge when considering a single predominant allergen. The sensitivity analysis confirmed the robustness of our results, showing that Artemisia pollen levels exerted a similar effect, further supporting the validity of the primary total pollen-based analyses. The SLIT cohort showed significant negative coefficients for group × time interactions across clinical endpoints, confirming sustained therapeutic efficacy through consecutive pollen seasons (Supplementary Information Table S3). Critically, in the group × time × pollen interaction, the SLIT group exhibited markedly reduced escalation of symptom-medication scores relative to controls during high *Artemisia* pollen exposure (Supplementary Information Table S3). This directional trend indicated that as pollen concentrations increased, symptom worsening in the SLIT group was significantly less pronounced than in controls, supporting a dose-dependent enhancement of SLIT efficacy under higher allergen exposure.

Longitudinal Changes in Immunoglobulin Concentrations

LMM analyses demonstrated significant group × time interaction effects for serum Art-sIgG4 and Art-sIgA at T3 (sIgG4: $\beta = 1.70$, $q < 0.01$; sIgA: $\beta = 0.36$, $q < 0.05$) (Figure 4A and B). The SLIT group exhibited significantly elevated serum Art-sIgG4 levels compared to controls at T2 ($\Delta = 1.40$ μg/mL, $q < 0.05$), with further elevation at T3 ($\Delta = 2.23$ μg/mL, $q < 0.001$) (Figure 4A). Serum Art-sIgA showed rapid elevation in the SLIT group by T2 (Δ=0.26 μg/mL, $q<0.05$), sustaining this elevation through T3 (Δ=0.38 μg/mL, $q<0.01$) compared with controls (Figure 4B). No significant interaction effects emerged for serum tIgE, Art-sIgE, or Art v1-sIgE (Figure 4C–E). SLIT recipients displayed transient initial increases followed by decreases in serum Art-sIgE, Art v1-sIgE, and sIgE/tIgE ratios (Figure 4D–F).

Nasal Art-sIgG4 in SLIT group exhibited only marginal elevation at T3 compared to baseline (Figure 4G). Nasal Art-sIgA demonstrated significant group × time interaction at T3 ($\beta = 1.81$, $q<0.05$) (Figure 4H), with SLIT recipients showing progressive increases from T1 to T3 (Δ=1.63 μg/mL, $q<0.001$) and superior levels versus controls at endpoint (Δ=1.74 μg/mL, $q<0.01$). No significant interaction effects emerged for nasal tIgE, Art-sIgE, or Art v1-sIgE (Figure 4I–K). Nasal IgE parameters (tIgE, Art-sIgE, Art v1-sIgE) maintained baseline levels (Figure 4I–K), though nasal sIgE/tIgE ratios in SLIT group showed significant elevation from baseline (Figure 4L). Spearman correlation analyses did not reveal any significant associations between changes in CSMS and allergen-specific IgG4 or IgA levels in serum or nasal secretions in the SLIT group (Supplementary Information Figure S4).

Discussion

This study aimed to evaluate the therapeutic effects of SLIT on SAR symptoms, with a focus on the impact of pollen concentration on efficacy assessment over two consecutive pollen seasons. Our research demonstrates that administering SLIT three to six months before the start of the pollen season can significantly alleviate symptoms and facilitate steady daily improvement throughout the initial pollen season. Additionally, we found that higher pollen concentrations were associated with more pronounced efficacy in the SLIT group. This highlighted the importance of incorporating pollen concentration into efficacy assessments. These results emphasized the need for future clinical trials to account for pollen concentration fluctuations in efficacy evaluations, ensuring more accurate and reliable outcomes.

Our study demonstrated a significant positive correlation between pollen concentration and SAR symptom severity, corroborating previous research findings.[27–29] The non-linear relationship observed in individual and combined symptom scores indicated that symptom severity initially increases with pollen concentration but reached an inflection point beyond which further increases in pollen concentration had a diminished impact on symptoms (Supplementary Information Table S2). Although the GAM showed a non-linear relationship, over 90% of the observed pollen range exhibited a linear pattern. This linear trend aligned with our subsequent analyses and reflected the primary relationship in the data. The non-linear effect appeared mainly at extreme pollen levels, where observations were sparse, ensuring that our linear model captures the key dynamics effectively. This finding underscored the complexity of the allergen-exposure response, reinforcing the need for detailed pollen monitoring in SAR research.

The therapeutic benefits of SLIT were evident in the early stages of the first year of pollen season, with further improvements observed over the subsequent period, as evidenced by the significant coefficient of group × time interaction term in the first-year data and combined dataset. The first year had a longer pollen season (41 days) and higher average daily pollen concentration. These conditions led to greater symptom variability and stronger SLIT

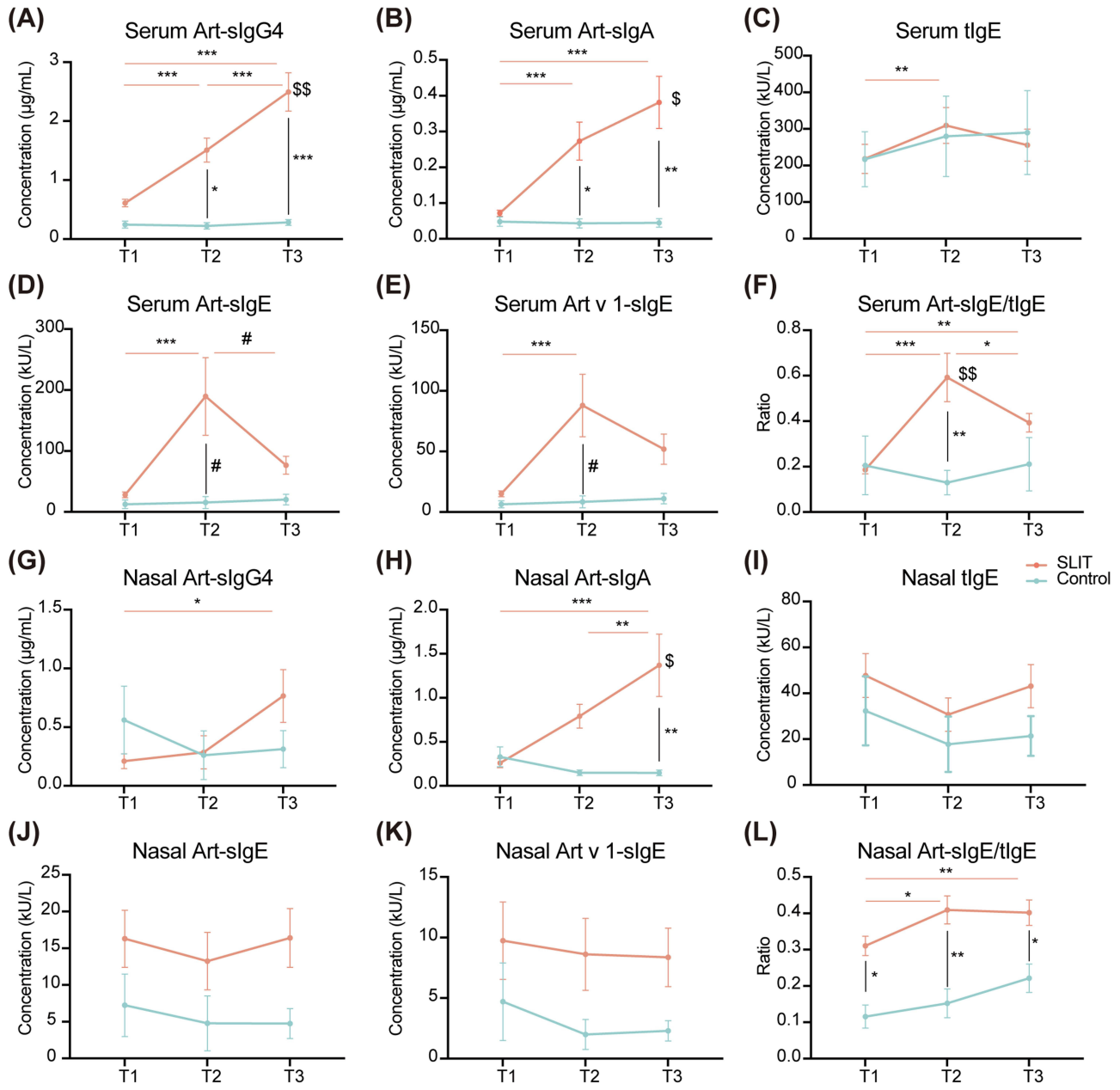

Figure 4 Longitudinal changes in serum and nasal immunoglobulin concentrations and ratios.

Notes: Longitudinal immunoglobulin measurements of Art-sIgG4 (**A**), Art-sIgA (**B**), tIgE (**C**), Art-sIgE (**D**), Art v 1-sIgE (**E**), and Art-sIgE/tIgE (**F**) in serum and Art-sIgG4 (**G**), Art-sIgA (**H**), tIgE (**I**), Art-sIgE (**J**), Art v 1-sIgE (**K**), and Art-sIgE/tIgE (**L**) in nasal secretion were expressed as mean ± standard error of the mean (SEM), with serum and nasal parameters assessed at baseline (T1), Y1 PPP (T2), and Y2 PPP (T3). Symbols represent statistical significance based on linear mixed model and post-hoc analyses: $ $q < 0.05$ and $$ $q < 0.01$ for significant group × time interaction effects (Benjamini-Hochberg corrected); # $P < 0.05$ (unadjusted) in post-hoc pairwise comparisons; * $q < 0.05$, ** $q < 0.01$. *** $q < 0.001$ in post-hoc comparisons (Benjamini–Hochberg corrected). All q values were corrected using the Benjamini–Hochberg method; *P* values are unadjusted.

Abbreviations: Art-sIgG4, Artemisia-specific Immunoglobulin G subclass 4; Art v 1, Artemisia vulgaris allergen 1.

efficacy. In the second year, however, statistical significance was observed solely for TNSS which is likely attributable to the mild intensity and limited duration of pollen exposure (27 days). TNSS, being more directly linked to symptom severity, remained sensitive to SLIT efficacy, from which we could still robustly infer that SLIT conferred sustained symptom alleviation throughout both pollen seasons. Our results, after fully accounting for variations in pollen exposure, aligned with previous studies that demonstrated the efficacy of AIT in alleviating SAR symptoms.[30–32] The efficacy of *Artemisia* pollen sublingual drop had been affirmatively established within the Chinese population, showcasing

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substantial clinical advantages.[20,33–37] A real-world study indicated that starting SLIT 2–4 months before the pollen season could be effective during the pollen season.[38] Another study also found that starting SLIT 8 or 12 weeks before the pollen season provided equivalent efficacy during the peak pollen period.[39] Our study demonstrated notable symptom diminution in the SLIT group after 3–6 months of treatment, suggesting that SLIT could facilitate early symptom improvement in the treatment process. Recent systematic reviews and meta-analyses have provided high-level evidence supporting the clinical efficacy and safety of sublingual immunotherapy for allergic rhinitis, demonstrating significant reductions in symptom scores and medication use across diverse allergens, and underscoring the clinical relevance of SLIT in immunotherapy practice.[40–42]

To elucidate the combined effects of treatment, time, and environmental exposure on clinical outcomes, we incorporated a three-way interaction term (group × time × pollen) into the linear mixed-effects model. This interaction captured the dynamic relationship whereby the therapeutic impact of SLIT may vary over time depending on pollen concentration levels. Specifically, it allowed us to assess whether the differential treatment effects between SLIT and control groups were modulated by fluctuations in ambient pollen exposure. In the study, we observed a significantly negative impact of the group × time × pollen interactions on the average daily CSMS in the SLIT group in the first pollen season. This indicated that higher pollen concentrations were associated with improved SLIT efficacy. The efficacy assessments for patients with SAR were significantly influenced by the level of natural pollen exposure, as evidenced by several studies. In a subgroup analysis of a short-term clinical trial on grass pollen AIT, it was found that participants exposed to elevated pollen concentrations demonstrated more favorable AIT outcomes than those with lower exposure levels.[43] Another study found no AIT efficacy in overall participants but observed significant benefits in high-exposure subgroups compared to placebo.[44] Notably, Durham and colleagues established a positive correlation between elevated pollen concentrations and enhanced efficacy of AIT by employing linear correlation analysis across a compilation of six clinical trials.[13,45] Incorporating pollen into the evaluation of AIT efficacy is essential; nevertheless, none of the current trial efficacy analyses accounted for this variable. Therefore, this study incorporated a three-way interaction term, group × time × pollen, and utilized daily data within an LMM framework to more precisely investigate the role of pollen fluctuations in evaluating treatment efficacy. This underscored the importance of incorporating pollen concentration into efficacy assessments, as unadjusted fluctuations in pollen levels might compromise the accurate interpretation of treatment outcomes.

Our findings demonstrated that *Artemisia annua* SLIT significantly enhanced both serum and nasal allergen-specific IgA and IgG4 levels, with nasal secretory IgA exhibiting a more pronounced fold-increase compared to IgG4. This observation aligns with evidence from a double-blind RCT investigating timothy grass pollen immunotherapy, which similarly reported SLIT-induced concurrent elevation of allergen-specific IgA and IgG4 in serum and nasal secretions, particularly demonstrating a predominant humoral response characterized by dominant allergen-specific IgA production – a pattern consistent with our data trends.[46] Peripheral immune mechanisms play a critical role in establishing and maintaining immune tolerance during allergen-specific immunotherapy. Successful SLIT has been associated with coordinated humoral and cellular responses characterized by a shift from pathogenic Th2-driven inflammation toward a more regulatory immune profile, including the generation of regulatory T cells and allergen-specific non-IgE antibodies such as IgG4 and IgA, which are thought to contribute to blocking IgE-mediated allergic responses and modulating inflammatory pathways.[47] Recent clinical studies and mechanistic overviews have demonstrated that SLIT induces significant increases in allergen-specific IgG4 and IgA levels, both systemically and locally, which may act to neutralize allergens, limit IgE binding, and support peripheral tolerance (eg, via inhibitory Fcγ receptors and mucosal immune exclusion) in the context of aeroallergen exposure.[48,49] These peripheral immunological changes complement cell-mediated tolerance processes, highlighting the multifaceted nature of immune regulation in SLIT and reinforcing the relevance of our findings within the broader landscape of allergen immunotherapy research.

However, several limitations should be acknowledged. First, the unequal sample sizes between the SLIT and control groups might have introduced potential bias. Recruiting and retaining control participants who received only symptomatic medications proved challenging, resulting in a smaller control group at the final analysis. Nevertheless, despite the imbalance (11 vs 56 in the SLIT group), power analysis indicated a high statistical power of 97.2%, with a 95% confidence interval (CI) ranging from 95.98% to 98.13% to detect an effect size (f^2) of 0.202 for assessing the efficacy of SLIT under natural pollen fluctuation conditions, at a false positive rate (a) of 0.05 for the combined dataset, supporting

the robustness of our findings, even with the unequal sample sizes. Second, as a non-randomized observational study, residual confounding cannot be fully excluded. However, key baseline characteristics, including symptom severity, medication usage, allergen sensitization profiles, and comorbid allergic conditions were comparable between groups, and all participants were enrolled during the same period using standardized data collection procedures. Finally, although all participants met the sIgE inclusion criteria, baseline serum sIgE levels were higher in the SLIT group, which may have influenced treatment responses. Additionally, spatial variability in pollen measurements across monitoring stations may have introduced environmental heterogeneity. Integrating advanced environmental monitoring techniques, such as real-time pollen tracking, could refine symptom-exposure analysis, enhancing treatment assessments. These findings highlight the necessity of incorporating pollen exposure factors in future allergen immunotherapy clinical trial designs. By integrating such environmental factors, future studies could adopt a more standardized and precise approach in evaluating treatment efficacy, leading to better-targeted interventions and improved clinical outcomes.

Conclusion

The study substantiated SLIT as an effective intervention for SAR, offering sustained symptom control even with varying pollen levels. Moreover, higher pollen concentrations were associated with more pronounced efficacy of SLIT. SLIT significantly elevated allergen-specific IgG4 and IgA levels in serum and nasal secretions. Future clinical trials should incorporate pollen concentration into their analyses to provide a more accurate and comprehensive evaluation of treatment efficacy.

Data Sharing Statement

The data used and/or analyzed during the current study are available from the corresponding author, Chengshuo Wang, on reasonable request.

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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 conflict of interest.

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