

The Relationship of Pollen Dispersal with Allergy Symptoms and Immunotherapy: Allergen Immunotherapy Improves Symptoms in the Late Period of Japanese Cedar Pollen Dispersal

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Keywords

Dispersed pollen quantity · Allergic rhinitis · Severity · Sublingual immunotherapy

Abstract

Background: The severity of symptoms of pollen-induced allergic rhinitis is affected by the amount of scattered pollen. However, the relationships between the pollen dispersal pattern, symptom severity, and treatment efficacy are not clear. **Methods:** Between 2007 and 2012, we performed 4 randomized, placebo-controlled studies of sublingual immunotherapy (SLIT) on patients with Japanese cedar-induced allergic rhinitis who lived in or around Chiba, Japan. The participants were asked to avoid using rescue medicines during the cedar pollen season as much as possible and to record their nasal symptoms in allergy diaries. The amount of pollen dispersed daily was quantified using the Durham method, and the season was divided into early and late periods based on the pollen count. **Results:** A total of 721 patients were enrolled in the 4 studies during the 6-year study period. In the placebo group ($n = 349$), a correlation was observed between the amount of pollen dispersed and the severity of symptoms in the early but not late period of pollen

dispersal. Treatment with SLIT ($n = 372$) significantly improved symptom severity in the late but not early period. **Conclusion:** For patients with Japanese cedar pollen-induced allergic rhinitis, the fluctuation of daily pollen dispersal had a minimal effect on the severity of symptoms during the late period. SLIT was remarkably effective in alleviating symptoms during this period but not in the early period.

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Introduction

The severity of symptoms of pollen-induced allergic rhinitis is affected by the quantity of scattered pollen, and pollen avoidance using protective face masks and ventilation systems with appropriate filters are important methods of symptom control [1–3]. However, even when patients are exposed to the same amount of pollen, symptom severity varies greatly. The carry-over effect caused by repeated exposure to pollen also has a significant effect on symptoms. In our previous studies using an environ-

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mental pollen exposure chamber to simultaneously expose patients to the same quantity of pollen, we found that symptom severity differed among the patients, and repeated pollen exposure exacerbated the symptoms [4–7]. In addition, the relationships between the pollen dispersal pattern, timing of the pollen season, and the nasal symptoms have not been investigated in detail. Thus, understanding the relationship between the amount of pollen dispersed daily and symptom severity during a natural pollen dispersal season is useful for providing the patient with instructions to avoid pollen exposure and for determining the appropriate treatment.

In Japan, Japanese cedar (*Cryptomeria japonica*) pollen is the major causal allergen of pollen-induced allergic rhinitis [1, 6]. Cedar forests cover nearly 18% of the total forested area of Japan, and they produce enormous quantities of pollen annually. Although the timing of the pollen seasons varies slightly from year to year according to the climate, cedar pollen dispersal begins in early to mid-February and peaks between late February and early March. Cedar pollen can travel distances of more than 100 km and easily reaches major cities, including Tokyo and Osaka, thereby causing widespread pollen-induced allergic rhinitis [8].

It is difficult to examine the relationship between the pattern of cedar pollen dispersal and nasal symptoms because patients usually begin treating symptoms after the start of the pollen dispersal season. In previous placebo-controlled comparative studies that we have conducted to develop sublingual immunotherapy (SLIT) with cedar pollen extracts, the participants were asked to refrain from taking rescue drugs as much as possible [9–13]. In the present study, we put the 4 studies together and examined the relationship between the daily amount of pollen dispersed and the nasal allergy symptoms of patients in the placebo group. In addition, we examined the efficacy and agendas of the cedar pollen extract that is available in Japan in terms of the relationship described above.

Materials and Methods

Between 2007 and 2012, we performed 4 randomized, placebo-controlled studies to examine the efficacy and safety of SLIT in patients with Japanese cedar-induced allergic rhinitis. The studies were as follows: an 18-month study of 88 patients conducted from September 2006 to May 2008 [9], a 5-month study of 52 patients from November 2008 to March 2009 [13], a 5-month study of 64 patients from November 2009 to March 2010 [13], and an 18-month study of 517 patients from October 2010 to March 2012 [10]. There were no duplicate participants across these trials because each study excluded patients who had received previous im-

muno-therapy for any allergic disease, including Japanese cedar-induced allergic rhinitis. The total number of patients receiving placebo and SLIT was 349 and 372, respectively. The study was approved by the Ethics Committee of Chiba University (approval No. G201204). All patients provided written, witnessed, informed consent.

Participants

The subjects consisted of patients with Japanese cedar-induced allergic rhinitis who lived in or around Chiba, Japan. Patients met the following inclusion criteria: male or female, 12–74 years of age with serum cedar-specific IgE levels of class 2 or higher (ImmunoCAP method; Phadia, Tokyo, Japan) and symptoms of cedar-induced allergic rhinitis present for the previous 2 years. The exclusion criteria were perennial allergic rhinitis, drug-induced rhinitis, nonallergic rhinitis requiring treatment, previous immunotherapy for any allergic disease (including Japanese cedar-induced allergic rhinitis), an asthma attack within the previous 5 years, or current pregnancy. Those who had nasal allergic symptoms outside of the cedar pollen dispersal season or who were negative for specific IgE antibodies to Japanese cedar pollen were excluded.

Pollen Measurement

Pollen counts were measured using the gravimetric method with a Durham sampler placed in Chiba City [14, 15]. Throughout the 4 studies, the pollen captured with the Durham sampler was stained and counted using a microscope. In this way, the cedar pollen was selectively measured. We divided the cedar pollen dispersal season into an “early period,” defined as day 1 of the cedar pollen season to the day when the integrated pollen count reached 500 grains/cm², and a “late period,” lasting from that day until March 31.

Study Drugs

Doses of 20, 200, and 2,000 Japanese allergy units (JAU)/mL of standardized Japanese cedar pollen extract (Torii Pharmaceutical, Tokyo, Japan) were used. These solutions were formulated by the dilution of the standardized Japanese cedar pollen extract original solution of 10,000 JAU/mL (7.3–21 µg/mL Cry j 1).

Study Protocols

Patients in the SLIT group ($n = 372$) received standardized Japanese cedar pollen extract, and the placebo group ($n = 349$) received inactive 50% glycerol in saline, as previously described [10, 13]. The dosing regimen in the SLIT group is shown in Table 1. Patients who participated in the first study (November 2006 to March 2008) were administered 0.2–1.0 mL of 20 JAU/mL during week 1, 0.2–1.0 mL of 200 JAU/mL during week 2, 0.2–1.0 mL of 2,000 JAU/mL during week 3 (induction phase), and 1 mL of 2,000 JAU/mL from week 4 onward (maintenance phase). Patients who participated in the other studies were administered 0.2–1.0 mL of 200 JAU/mL during week 1, 0.2–1.0 mL of 2,000 JAU/mL during week 2 (induction phase), and 1 mL of 2,000 JAU/mL from week 3 onward (maintenance phase). Patients received the maintenance dose weekly in the first study [9] and daily in the other studies [10, 13]. The extract was placed sublingually, kept in place for 2 min, and then swallowed. Patients were instructed to place nothing in their mouth and not to gargle for 5 min after swallowing. Although participants were asked to refrain from taking rescue drugs as much as possible, the daily use of the following relief drugs was al-

lowed for unbearable symptoms (a nasal symptom with a score of 4): fexofenadine hydrochloride (a 60-mg tablet) and tramazoline hydrochloride (a 0.118% nasal solution). The use of each rescue drug was scored as 0 (not used), 3 (use either one) or 6 (use both of them). The participants were asked to record their nasal symptoms and the use of rescue drugs in a paper diary daily from February 1 to March 31.

The patients were divided into SLIT and placebo groups. A group allocation number was given to each patient, and this number was closely guarded by the controller and a member of the ethics committee who was not directly involved in the studies, until accessed with the key after the completion of the study.

Evaluation of Symptoms

In the Japanese practical guideline for allergic rhinitis, the severity of allergic rhinitis symptoms is classified using the three symptoms of sneezing, rhinorrhea, and nasal congestion; we complied with this classification in the study. A 5-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe) for each symptom was used according to the Japanese guidelines for allergic rhinitis [1, 3]. The total nasal symptom score (TNSS) was obtained by summing the scores for those three symptoms. The TNSS score ranged from 0 to 12, and nasal symptoms were defined as mild, moderate, and severe when the TNSS was ≤ 3 , 4–8, and ≥ 8 , respectively. The Japanese cedar pollen dispersal season usually starts in February and peaks in March (Fig. 1). We evaluated the severity of Japanese cedar-induced allergic rhinitis between the start of the dispersal and the end of March. The first day of cedar pollen dispersal was considered to be day 1 of the first 2 consecutive days after January 1 on which the pollen count was $\geq 1/\text{cm}^2/\text{day}$, as measured by the Durham method. Patients were asked not to use any medications for rhinitis prophylaxis or treatment from the first day of cedar pollen dispersal until March 31. However, a daily dose of fexofenadine hydrochloride (60-mg tablet) or tramazoline hydrochloride (0.118% nasal solution) as rescue medication was allowed if one of the nasal symptoms became unbearable (e.g. score of sneezing = 4). The use of each relief medication was scored as 0 (not used) or 3 (used), giving possible scores of 0, 3, or 6.

Statistical Analyses

Data analyses were performed as described previously [9, 10]. In brief, Student's *t* test was used to compare the mean TNSS between the SLIT group and the placebo group during the Japanese cedar pollen dispersal seasons.

Results

A total of 721 patients were enrolled in 1 of 4 randomized, placebo-controlled studies during a 6-year study period (Table 2). Figure 1 shows the cedar pollen dispersal pattern and the severity of nasal symptoms (total TNSS) of the placebo groups for each season. The use of rescue drugs was low in all seasons; the mean rescue score was <0.1 (on a scale of 0–6), even during the peak periods of pollen dispersal, and there was no significant difference

Table 1. Dosage regimen (mL) of cedar pollen extract during the induction and maintenance phases

a The indicated volumes and concentrations of extract were administered for the first 3 weeks (induction phase) and from week 4 onward (maintenance phase) in the first study (November 2006 to May 2008)

	Week 1 20 JAU/mL	Week 2 200 JAU/mL	Week 3 2,000 JAU/mL	Week 4 2,000 JAU/mL
Day 1	0.2	0.2	0.2	1.0
Day 2	0.4	0.4	0.4	none
Day 3	0.6	0.6	0.6	none
Day 4	0.8	0.8	0.8	none
Day 5	1.0	1.0	1.0	none
Day 6	none	none	none	none
Day 7	none	none	none	none

b The indicated volumes and concentrations of extract were administered for the first 2 weeks (induction phase) and from week 3 onward (maintenance phase) in the remaining studies

	Week 1 200 JAU/mL	Week 2 2,000 JAU/mL	Week 3 2,000 JAU/mL
Day 1	0.2	0.2	1.0
Day 2	0.2	0.2	1.0
Day 3	0.4	0.4	1.0
Day 4	0.4	0.4	1.0
Day 5	0.6	0.6	1.0
Day 6	0.8	0.8	1.0
Day 7	1.0	1.0	1.0

JAU, Japanese allergy unit.

in the medication score between the placebo and the SLIT groups (data not shown). There was considerable variation in the total amount of cedar pollen dispersed per season (total amount of dispersed pollen during the observed period was 1,560/cm² in 2007, 4,590/cm² in 2008, 4,290/cm² in 2009, 2,070/cm² in 2010, 7,430/cm² in 2011, and 3,240/cm² in 2012), and in the starting date of the pollen dispersal season (ranging from February 5 to 21; Fig. 1). Overall, the number of patients with moderate-severe symptoms (TNSS of ≥ 4 points) increased rapidly at the start of the dispersal season and decreased gradually toward the end. However, the amount of pollen dispersed did not always cause comparable TNSS, even in the same season. Thus, there were daily and seasonal variations in the relationship between the cedar pollen count and the percentage of patients in the placebo group with moderate-severe nasal symptoms throughout the pollen dispersal season.

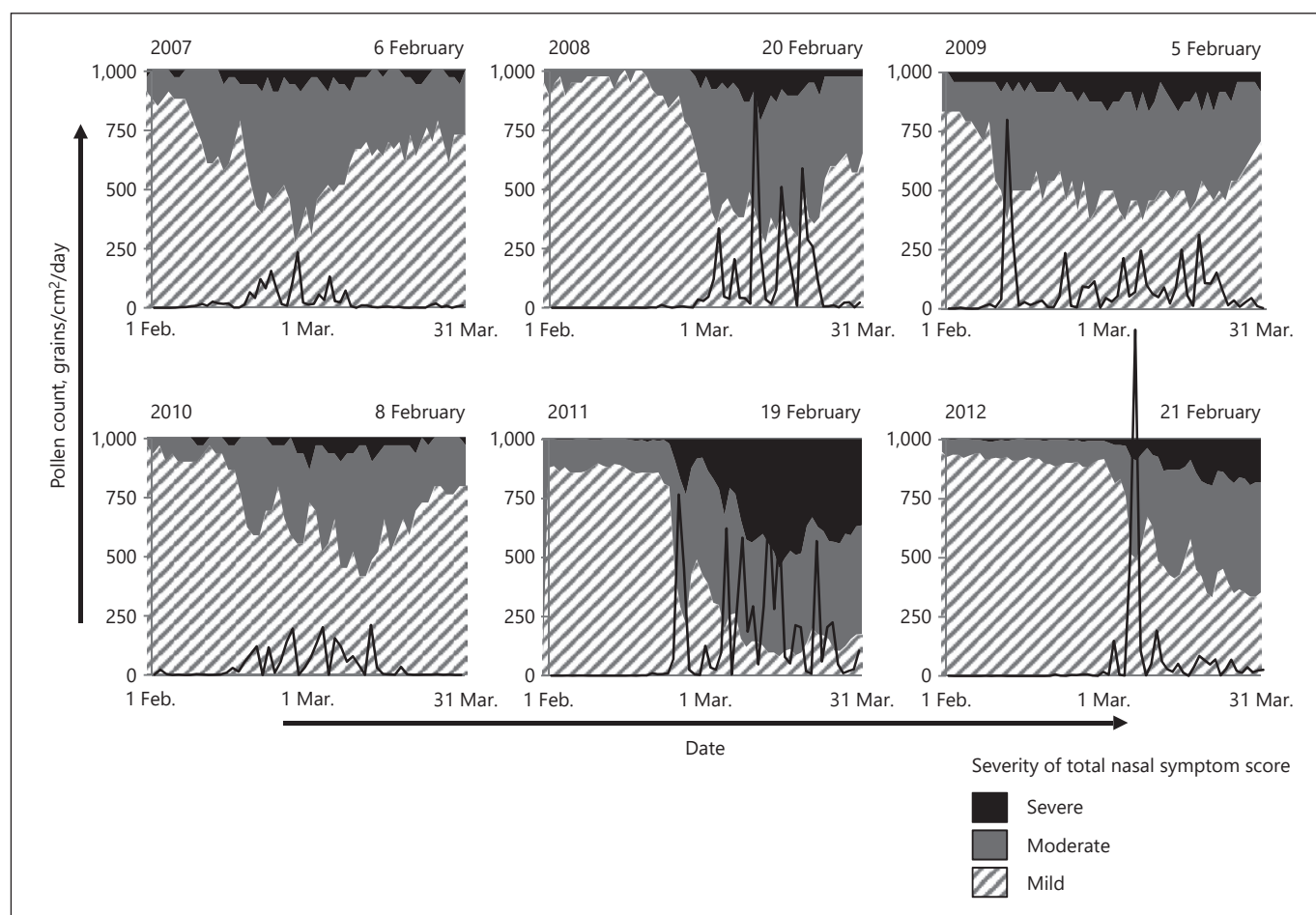


Fig. 1. Japanese cedar pollen dispersion pattern and severity of nasal symptoms in the placebo group. Black lines show the seasonal cedar pollen dispersal pattern. The shaded, light gray, and dark gray zones represent the daily percentage of patients with Japanese

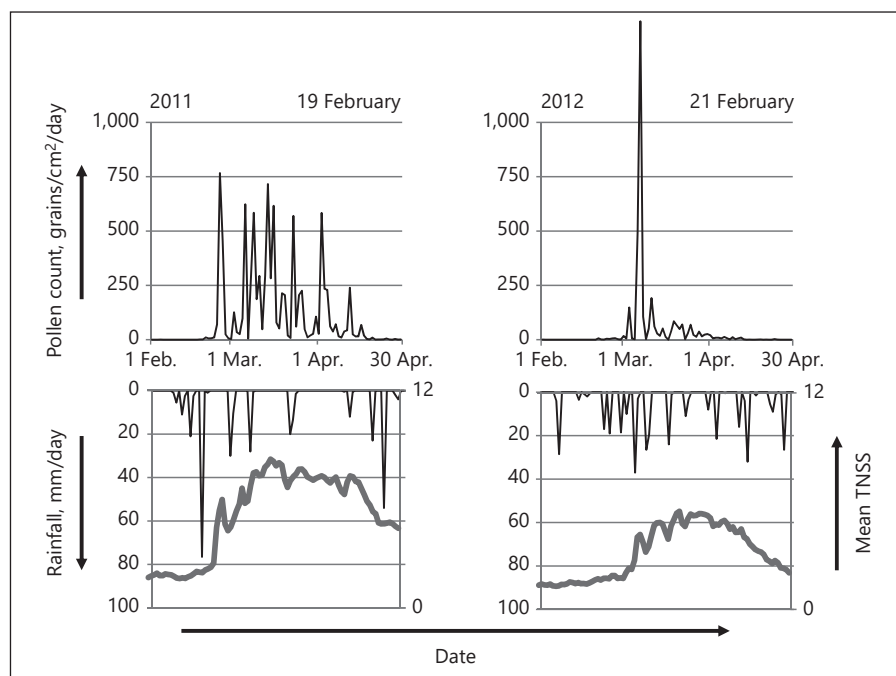
cedar-induced allergic rhinitis showing a mild, moderate, and severe total nasal symptoms score, respectively. The start date of the pollen dispersal season is shown at the top right corner of each figure.

Table 2. Baseline demographic profile of study subjects

	2007, 2008		2009		2010		2011, 2012	
	placebo (n = 37)	SLIT (n = 51)	placebo (n = 24)	SLIT (n = 28)	placebo (n = 29)	SLIT (n = 35)	placebo (n = 266)*	SLIT (n = 265)*
M/F ratio	8/29	17/34	7/17	12/16	9/20	12/23	101/164	103/163
Mean age, years	42.3	44.4	42	43	41	44	39.8	40.1
Range	19–70	16–73	18–64	21–59	22–62	23–63	14–64	12–63
Mean total IgE	258	198	178	152	282	135	224	227
Range	8.6–2,090	6.8–1,480	26.6–910	10.4–483	35.2–1,940	18.5–677	13–3,200	10–4,800
Mean JC-specific IgE	29	27	22	27	30	23	25	25
Range	1.5–100	0.8–100	2.7–85	1.6	3.1–100	2.4	3.5–100	3.6–100
Disease duration, years	>2	>2	15.8±9.0	16.1±9.8	15.3±8.8	16.1±9.4	16.6±8.3	15.7±9.1
Polysensitization (house dust mite), %	35	47	41	32	48	29	35	34

JC, Japanese cedar pollen. * 14 and 49 participants were excluded in 2011, 2012, respectively.

Fig. 2. Relationships between the seasonal dispersal of Japanese cedar pollen, rainfall, and nasal symptoms in 2011 and 2012. Upper figures show the pollen dispersal. Lower figures show precipitation (black lines) and mean total nasal symptom score (TNSS) of all participants (gray lines). The start date of the pollen dispersal season is shown at the top right corner of each figure.



Pollen dispersal is affected by the weather, especially by rainfall. Figure 2 shows the individual rainy days (rainfall of ≥ 1 mm/day), the corresponding pollen counts, and the TNSS during the 2011 and 2012 seasons. Although the amount of pollen dispersed decreased on wet days, the mean TNSS did not decrease dramatically on rainy days, even after the peak pollen dispersal. The mean TNSS was observed as a smooth arch-shaped continuum rather than as a series of peaks. The participants, therefore, experienced only slight relief from nasal symptoms on wet days throughout the study period.

Figure 3 shows the relationship between the integrated pollen count and the percentage of patients with moderate-severe symptoms. Two symptom phases (an acute exacerbation period and a near-plateau period) were evident from this analysis, and the transition appeared to occur when the integrated pollen count reached approximately 500 grains/cm². Thus, for further analysis, we divided the cedar pollen dispersal season into an “early period,” defined as day 1 of the cedar pollen season to the day when the pollen count reached the transition period, and a “late period,” lasting from that day until the end of the observation period (March 31). Figure 4 shows the relationship between the percentage of patients with moderate-severe symptoms and the integrated pollen counts in the early period of each season. The correlation coefficients were similarly high in each year.

The number of days in the early and late periods of each pollen dispersal season and the corresponding mean TNSS in the placebo and SLIT groups are shown in Table 3. The TNSS in the early periods were not markedly different between the SLIT group and placebo group in each year; however, the scores in the late periods were significantly lower in the SLIT group than in the placebo group for each year except 2007.

Discussion

The dispersal of cedar pollen is followed by Japanese cypress (*Chamaecyparis obtusa*) pollen dispersal, starting at the end of March and peaking in the middle of April [16]. Approximately 70% of patients with cedar-induced allergic rhinitis are also allergic to Japanese cypress pollen because the two pollens share a common antigen [17, 18]. To avoid the influences in nasal symptoms caused by the Japanese cypress pollen, we set the end of the observation period as March 31.

In Japan, pollen counts are typically measured using the gravimetric method with a Durham sampler [14]; in contrast, Western countries predominantly use the infinite volumetric method with a Burkard sampler [19]. Compared with the Burkard sampler, the Durham sampler is relatively inexpensive and technically easy to use;

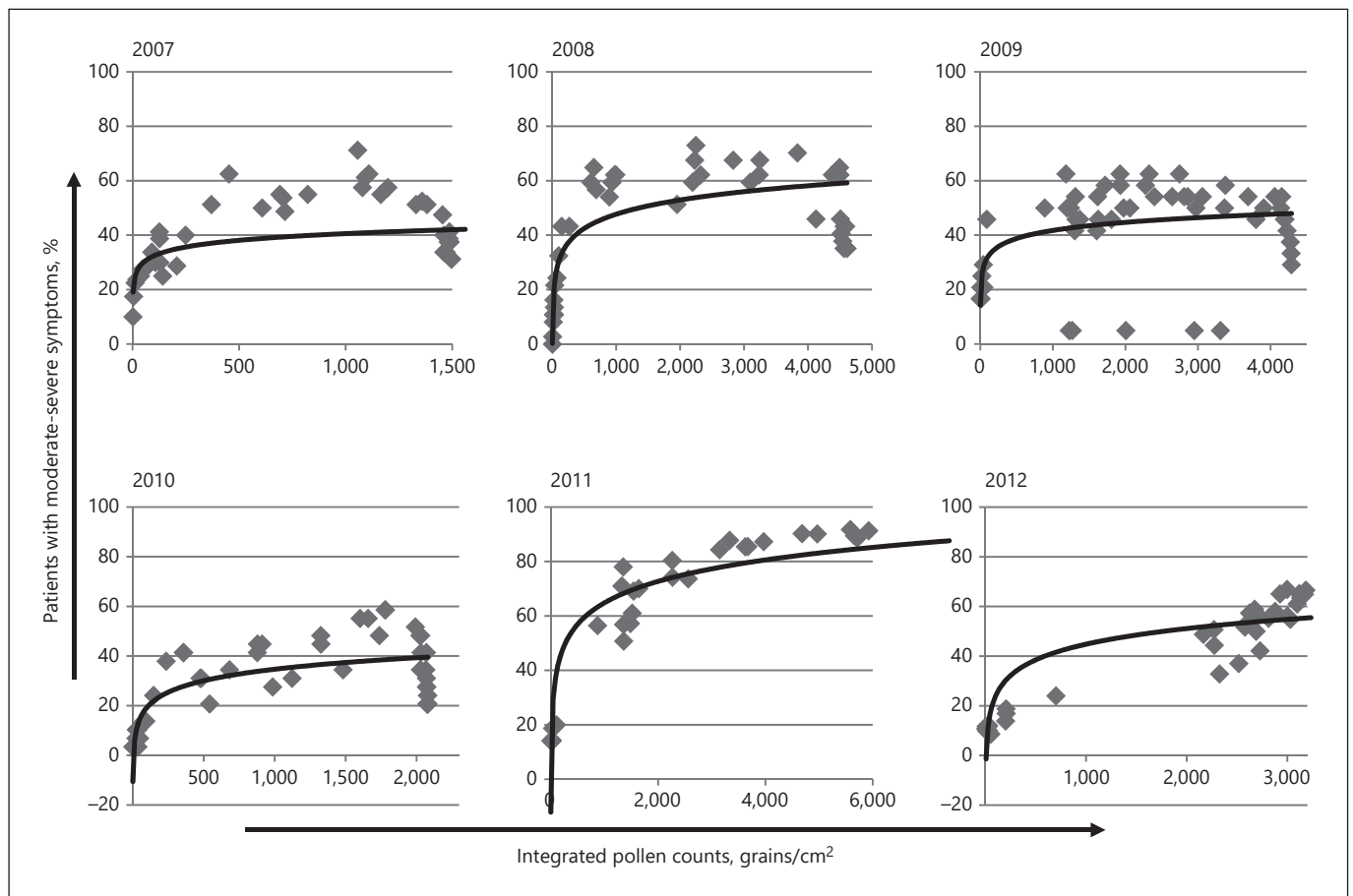


Fig. 3. Relationship between the integrated pollen counts of Japanese cedar and the percentage of patients in the placebo group with moderate to severe total nasal symptom score. Each point represents the measurement on a single day in the dispersal season, and the black curves represent a regression curve.

however, it is affected by wind power and direction, and it is an unrealistic method for hourly quantification of scattered pollen. A previous study noted a significant correlation in the conifer pollen count, such as cedar pollen, between Durham and Burkard samplers [15].

In this study, the total amount of scattered pollen differed widely from year to year, resulting in an approximately 6-fold difference between the highest and lowest amount of pollen dispersed in the 6 years studied. The dispersal pattern was observed as a series of peaks rather than as a smooth arch-shaped continuum, and the peak was not always observed at the same time. The symptom fluctuation due to differences in daily pollen scattering shown in Figure 1 suggests that knowing pollen counts during the season is very useful for predicting the degree of patients' symptoms. The daily pollen counts also varied greatly from year to year. In 2009 and 2012, pollen dispersal peaked at

the beginning of the season and remained low until the end of March. In contrast, several dispersal peaks were detected in 2011, whereas peaks were unclear in 2010.

Pollen concentrations are thought to be associated with several meteorological conditions, including the overall trend towards increased temperature; indeed, the concentrations of some types of pollen have significantly increased as a consequence of global warming [20]. In the present study, pollen dispersal was affected by the weather, and pollen counts were drastically reduced on rainy days. This result is consistent with a previous study suggesting that pollen concentration trends were inversely associated with changes in relative humidity and rainfall trends [20]. Despite our observation that dispersed pollen amounts decreased on rainy days, this was not accompanied by a marked amelioration of nasal symptoms. Taylor et al. [21] found that pollen can rupture at high humidity,

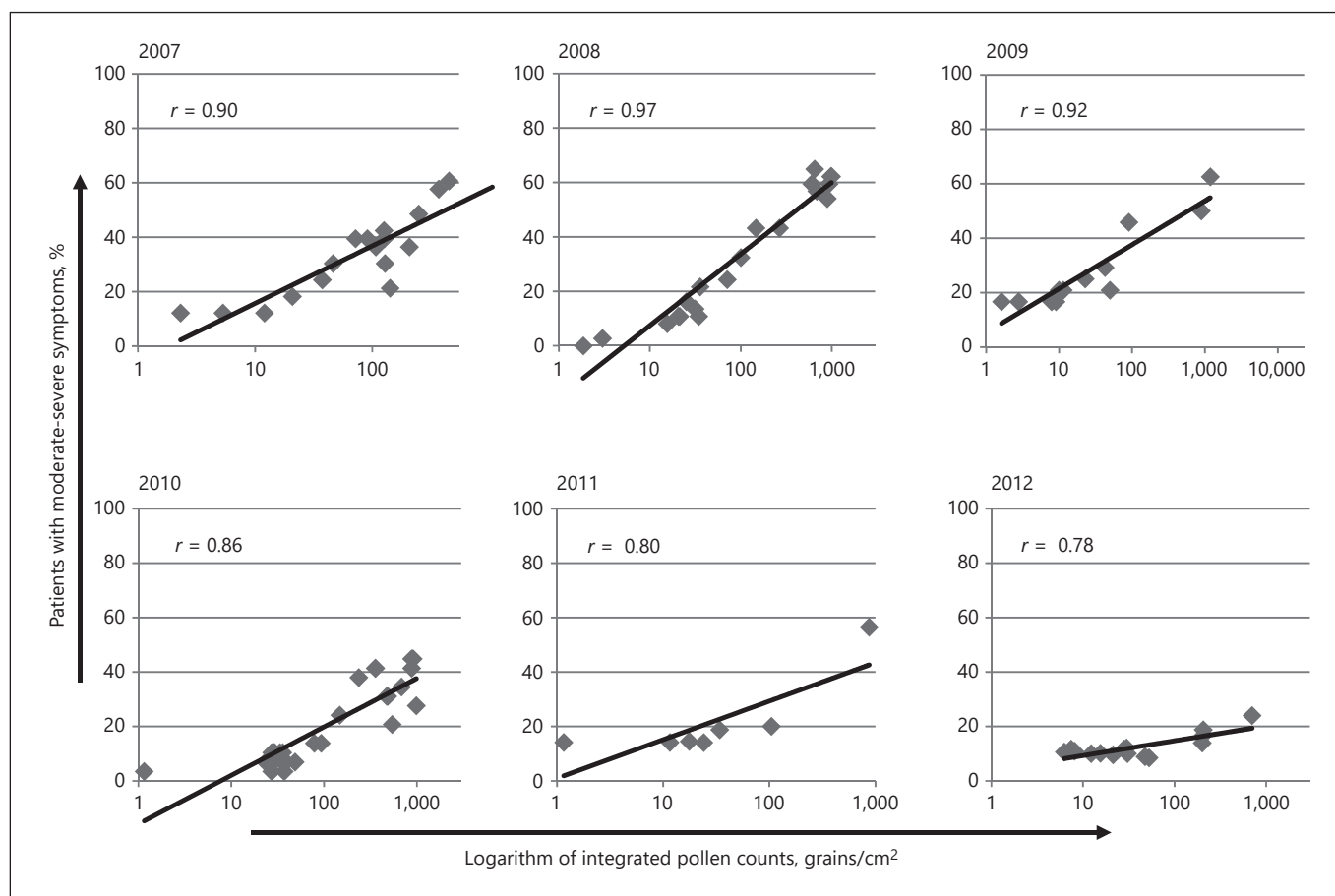


Fig. 4. Relationship between the logarithm of integrated pollen counts of Japanese cedar and the percentage of patients with moderate to severe total nasal symptom scores during the early period. Each point represents the measurement on a single day in the early period of the dispersal season. Pearson's correlation coefficients are shown for each year (all $p < 0.01$).

thus producing particulate aerosols that contain pollen allergens. In addition, it is reasonable to assume that changes in the weather can affect chemical aerosols and Asian sand dust, both of which could enhance rhinoconjunctivitis symptoms in pollen-allergic individuals [22, 23]. Further studies on meteorological parameters and air pollutants are needed to test and validate these possibilities.

In this study, we detected a logarithmic function-like form of relationship between the integrated pollen count and symptom severity throughout the dispersal season, although the mean TNSS varied greatly between seasons, even on days with similar pollen count in the same season. This finding suggests that symptoms were affected by the time at which pollen exposure occurred within the dispersal season. Symptoms of rhinitis rapidly worsened between the start of pollen dispersion and the day when

the integrated pollen count reached approximately 500 grains/cm² ("the early period") in all years studied. A strong correlation was observed between the integrated pollen count and symptom scores in the period in both the placebo and SLIT groups. However, the symptom scores remained high from the transition day onward ("the late period"), with little decrease in the symptom scores clearly observed in the period, even on rainy days.

Overall, the relationship between integrated pollen counts and symptom scores was similar in the placebo and SLIT group, and a significant correlation was observed only in the early period. The SLIT group showed no clear improvement in symptoms compared with the placebo group in the early period in any year studied. In the late period, however, the symptom scores were significantly lower in the SLIT group than in the placebo group, albeit

Table 3. Mean total nasal symptom score (TNSS) and duration of early and late periods in each year of the study

Year	Placebo		Active SLIT		<i>p</i> value (placebo vs. active SLIT)	
	early period	late period	early period	late period	early period	late period
2007	(<i>n</i> = 37)		(<i>n</i> = 51)			
Days	23	31	23	31		
TNSS (mean)	3.04	3.58	2.91	3.36	0.62	0.14
2008	(<i>n</i> = 37)		(<i>n</i> = 51)			
Days	14	28	14	28		
TNSS (mean)	2.43	4.43	2.24	3.76	0.53	<0.01
2009	(<i>n</i> = 24)		(<i>n</i> = 28)			
Days	11	44	11	44		
TNSS (mean)	2.54	4.20	2.09	3.08	0.17	<0.01
2010	(<i>n</i> = 29)		(<i>n</i> = 35)			
Days	28	24	28	24		
TNSS (mean)	2.27	3.45	2.34	2.92	0.71	<0.01
2011	(<i>n</i> = 256)		(<i>n</i> = 261)			
Days	16	25	16	25		
TNSS (mean)	4.03	7.33	3.65	6.26	0.48	<0.01
2012	(<i>n</i> = 241)		(<i>n</i> = 241)			
Days	16	24	16	24		
TNSS (mean)	2.02	4.67	1.88	3.47	0.42	<0.01

TNSS, total nasal symptom score. *p* values assessed by the 2-tailed Student's *t* test.

with large variations, for every year except 2007. SLIT did not improve nasal symptoms in the 2007 season as previously reported [9], probably because the dose of cedar pollen extract (1.5–4.2 µg of Cry j 1 per week in the first study) was considered too low to have a beneficial effect compared with other formulations of SLIT [24]. Nevertheless, in the second year of the study, an improvement in nasal symptoms in the late period similar to other studies was observed. The TNSS in the peak symptom period was significantly lower in the SLIT group than in the placebo group: by 18 and 30% in the 2011 and 2012 seasons, respectively [10]. This result is clinically meaningful because a symptom improvement effect of more than 20% compared with the placebo is considered clinically relevant efficacy [25]. These results suggest that an increase in the antigen amount in the active drug or continuation of more than 2 years is desirable for enough effect manifestation.

Previous studies to expose subjects with cedar-induced allergic rhinitis to pollen in a pollen challenge test chamber outside of the cedar pollen season showed that nasal symptoms increased immediately after pollen exposure and plateaued within 2 h [4–6]. In these studies, symptoms such as nasal obstruction, rhinorrhea and sneezing continued in all subjects for 3–4 days after leaving the

chamber, even though they were no longer exposed to pollen. The persistent nasal mucosal inflammation following a type 1 allergic reaction was presumed to contribute to the prolonged nasal symptoms. In the present study of natural pollen exposure, we considered that worsening allergic inflammation in the nasal mucosa induced persistent nasal symptoms in the late period.

The lack of SLIT efficacy in the early period may provide clues about its mechanism of action. Cedar pollen-specific IgE levels did not decrease with SLIT treatment for at least 18 months [10, 12, 26]. Allergen-specific immunotherapy does not improve allergic rhinitis induced by other allergens, demonstrating that SLIT does not inhibit the mast cell responses, which play important roles in allergic rhinitis, particularly in the early phase [27]. Although pollen-specific IgG4 antibodies were induced by SLIT, the levels did not correlate with the suppressive effects of SLIT on nasal symptoms [10, 28]. Furthermore, these studies did not determine the potential interference of IgG4 in binding cedar pollen and pollen-specific IgE [29]. We previously showed that SLIT did not eliminate Th2 cells but instead inhibited the seasonal increase in cedar pollen-specific Th2 effector cells, which normally enhance allergic inflammation at the start of the dispersal

season [11, 26, 30]. Although SLIT inhibited this enhancement, a longer period of suppression might be required to reduce the inflammation [11]. Early intervention with conventional drugs, such as antihistamines and nasal steroids, may be able to improve the symptoms in the early period and contribute to further relief of symptoms.

Our study showed that the amount of cedar pollen dispersed daily affected the severity of symptoms only in the early period of dispersal and that continued exposure to pollen maintains the symptom severity. Although SLIT was not particularly effective during the early period, it significantly improved nasal symptoms in the late period of exposure. However, in our studies, patients were administered SLIT for a maximum of 18 months, and longer treatment periods or an increase in the amount of antigen in SLIT might significantly improve symptoms, even during the early period of pollen dispersal. Further studies should examine the effect of SLIT administered for at least 3 years at different points in the pollen dispersal seasons.

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Statement of Ethics

All participants gave written, witnessed, informed consent. The study was approved by the Ethics Committee of Chiba University (approval No. G201204).

Disclosure Statement

Shinya Kaneko is employee of Torii Pharmaceutical Co. Ltd., Tokyo, Japan. Other authors have no relevant conflicts of interest to declare.

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Author Contributions

T.S., S.Y., D.S., and Y.O. contributed to the study design; T.S., T.I., Y.M., and S.K. collected the clinical data; T.S. and Y.O. were involved in writing the manuscript.

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