Original Article

Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure

S. R. Durham¹, H. S. Nelson², H. Nolte³, D. I. Bernstein⁴, P. S. Creticos⁵, Z. Li³ & J. S. Andersen⁶

¹Allergy and Clinical Immunology, Imperial College London, London, UK; ²National Jewish Health, Denver, CO; ³Merck Research Laboratories, White House Station, NJ; ⁴Division of Immunology, Allergy and Rheumatology, University of Cincinnati College of Medicine, Cincinnati, OH; ⁵Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; ⁶Global Clinical Development, ALK, Harsholm, Denmark

To cite this article: Durham SR, Nelson HS, Nolte H, Bernstein DI, Creticos PS, Li Z, Andersen JS. Magnitude of efficacy measurements in grass allergy immunotherapy trials is highly dependent on pollen exposure. Allergy 2014; 69: 617-623.

Keywords
allergen-specific immunotherapy; grass pollen counts; grass sublingual immunotherapy tablet.

Abstract

Background: The objective was to evaluate the association between grass pollen exposure, allergy symptoms and impact on measured treatment effect after grass sublingual immunotherapy (SLIT)-tablet treatment.

Methods: The association between grass pollen counts and total combined rhinoconjunctivitis symptom and medication score (TCS) was based on a post hoc analysis of data collected over six trials and seven grass pollen seasons across North America and Europe, including 2363 subjects treated with grass SLIT-tablet or placebo. Daily pollen counts were obtained from centralized pollen databases. The effect of treatment on the relationship between the TCS and pollen counts was investigated, and the relative difference between grass SLIT-tablet and placebo as a function of average grass pollen counts was modelled by linear regression.

Results: The magnitude of treatment effect based on TCS was greater with higher pollen exposure ($P < 0.001$). The relative treatment effect in terms of TCS for each trial was correlated with the average grass pollen exposure during the first period of the season, with predicted reduction in TCS = 12% + 0.35% × pollen count (slope significantly different from 0, $P = 0.003$; $R^2 = 0.66$). Corresponding correlations to the entire grass pollen season and to the peak season were equally good, whereas there was a poor correlation between difference in measured efficacy and pollen exposure during the last part of the season.

Conclusions: In seasonal allergy trials with grass SLIT-tablet, the observed treatment effect is highly dependent on pollen exposure with the magnitude being greater with higher pollen exposure. This is an important relationship to consider when interpreting individual clinical trial results.

The levels of rhinoconjunctivitis symptoms and the use of symptomatic medications are influenced by allergen exposure (1). However, the association between the assessment of the allergy immunotherapy (AIT; please refer to (2) for semantic framework) treatment effect and pollen exposure remains unclear. The year-to-year and in-season variability of pollen exposure makes the efficacy assessment of immunotherapy during natural pollen exposure highly variable, and the magnitude of the treatment effect may be affected by the efficacy of the treatment and actual pollen exposure.

Due to the fluctuations in pollen levels, symptoms may be intermittent during the pollen season, and even subjects with severe pollen allergy may experience days during the season with no or few symptoms. The current standard for the evaluation of efficacy in clinical trials of pollen immunotherapy involves assessment of symptoms and symptomatic medication use during natural seasonal exposure. It is therefore important to understand the impact of seasonal and regional variations on the assessment of treatment outcomes.

Traditionally, AIT trials have been conducted with small numbers of subjects recruited in one region. Over the last 10 years, several larger clinical trials of sublingual immunotherapy (SLIT) for seasonal grass pollen-induced rhinoconjunctivitis (comprising more than 100 subjects per treatment
Efficacy in AIT trials depends on pollen exposure

Durham et al.

and regions, is that a more precise estimate of the relationship between pollen exposure and observed treatment effect may be provided.

Immunotherapy should be initiated 2–4 months prior to the season, to afford protection prior to exposure to the causative allergen (3). Thus, rather than treating symptoms, the primary objective of immunotherapy is in the prevention of symptoms from occurring. When comparing the magnitude of treatment effects of pharmacotherapy agents and immunotherapy in clinical trials, it is important to keep some methodological differences in mind. In contrast to immunotherapy trials, traditional therapeutic trials conducted with antihistamines and nasal steroids include a baseline lead-in period during the pollen season and before randomization to allow for inclusion of subjects with sufficiently high symptom levels, followed by a shorter study treatment period of typically 14 days. This type of trial design allows for the inclusion of high symptom patients providing, in particular, an initially ‘greater signal’, which would be expected to make it more sensitive to demonstrate a treatment effect. In contrast, the start of symptom recording in immunotherapy trials is triggered by pollen counts regardless of symptom levels, and the length of the recording extends throughout the allergy season which may be highly variable, including more days with low or no pollen exposure (Fig. 1). In addition, in traditional trials of pharmacotherapy for rhinoconjunctivitis, the use of additional symptomatic medications is not permitted, while this use is allowed in immunotherapy trials. With regard to the analysis, in the pharmacotherapy trials, the endpoints are analysed as the mean change from baseline, while in the immunotherapy trials the endpoints are analysed as the observed differences in the means over the GPS. Thus, given the significant design differences, this methodological approach would tend to diminish the measured effect of immunotherapy.

Further, immunotherapy trials are often conducted over several years and measured treatment effect may vary between years due to differences in seasonal pollen levels. On a subject level, the variability may be considerable because the pollen exposure for all participants is estimated based on pollen counts obtained from regional pollen stations that may be situated several kilometres from the allergy clinics, the workplace or school and associated daily activities of the participants (4, 5).

Besides variability in exposure, patient variability in the manifestation of the allergic disease itself is important. Different patients may experience different symptoms both from the nose and from the eyes, making it necessary to include symptoms in the symptom score that may not all be present in all patients (e.g. runny nose and blocked nose).

The influence of pollen exposure on treatment effect has previously been reported in one 5-year study (6). The primary objective of this pooled analysis, of six large, randomized, placebo-controlled trials with the same grass SLIT-tablet formulation, was to get a more precise estimate of the association between grass pollen levels, rhinoconjunctivitis symptoms and the measured treatment effect using multiple cohorts of pollen-allergic subjects across diverse geographical regions such as North America and Europe.

Methods

Data from six previously published randomized, placebo-controlled grass SLIT-tablet trials (6–14) conducted in Europe and North America were used to evaluate the association between rhinoconjunctivitis symptoms and pollen exposure and its impact on the measured efficacy. Four trials included adults aged 18–65 years, and two trials included children aged from 5 years to 16 or 17 years. Overviews of the trials have been published previously by Nelson et al. (15, 16). In all trials, subjects with a clinical history of grass pollen-induced allergic rhinoconjunctivitis requiring treatment during the grass pollen season and a positive skin prick test and serum specific IgE to *Phleum pratense* were included. Subjects with clinically relevant allergies potentially overlapping the grass pollen season were excluded.

The grass SLIT-tablet was supplied as fast-dissolving, neutral-tasting oral lyophilisates for sublingual application. The active ingredient was *Phleum pratense* grass pollen extract in strength of 75 000 SQ-T/2800 BAU.1 Placebo was indistinguishable from the active tablet in appearance but contained no *Phleum pratense* grass pollen extract. All subjects were randomized to grass SLIT-tablet or placebo outside the grass pollen season and received preseasonal treatment ranging from 8 to 35 weeks. Total treatment duration ranged from 18 to 29 weeks (in 5 one-season trials) or up to 3 years (in one trial with 3 years of treatment and 2 years of follow-up).

The North American trials included more than 100 sites in Canada, North East, North West, Mid-Atlantic and South East of United States. The European trials included from 15 to 55 sites in Austria, Belgium, Denmark, Germany, Italy, Netherlands, Norway, Spain, Sweden and the United Kingdom. Grass pollen counts were provided by European Aeroallergen Network Pollen Database for the European sites and from Aerobiology Research Laboratories for the North American sites derived from daily grass pollen counts reported from regional agencies or investigative sites. In Canada and in the United States, pollen collections were carried out using continuous, volumetric spore trap, intermittent suction-trap or rotation-impaction samplers, and in Europe, continuous, volumetric samplers were used. The grass pollen counts reflected to the best possible extent the exposure in the area of the trial site. It was anticipated that most subject’s home or work was in the geographical vicinity of the

---

1standardized quality tablet units (SQ-T) and biological activity units (BAU) are quantitative measures of biological activity; that is, the potency of allergen extracts. One grass SLIT-tablet contains 75 000 SQ-T of *Phleum pratense* grass pollen extract (measure of total biological potency using ALK in-house reference), equivalent to 2800 BAU (measure of total biological potency, defined by the FDA).
investigator’s office and therefore was exposed to the grass pollen levels recorded.

The duration of the grass pollen season was defined with boundaries of three consecutive days with grass pollen count ≥10 grains/m³. The peak season was defined as the 15 consecutive days within the grass pollen season with the highest 15-day moving average pollen count (see Fig. 1).

Shortly before and during the grass pollen season, subjects filled in daily diaries with rhinoconjunctivitis symptoms [four nasal symptoms and two ocular symptoms scored on a 0–3 scale (3 = severe symptoms)] and symptomatic medications use. The primary outcomes were the average rhinoconjunctivitis daily symptom score (DSS) and the average rhinoconjunctivitis daily medication scores (DMS) within the grass pollen season, calculated for each subject as the average of the observed total daily scores throughout the grass pollen season. The total combined rhinoconjunctivitis score (TCS) was calculated as the sum of the rhinoconjunctivitis DSS and DMS. The relative differences [(placebo-active)/placebo] were used for comparisons.

Statistics

The effect of SLIT-tablet treatment on the relationship between the daily TCS and daily grass pollen counts was modelled by a generalized additive model with an interaction between treatment and a smoothing spline with five degrees of freedom as a function of daily grass pollen counts. The 95% pointwise prediction intervals were calculated as 1.96 times the pointwise standard errors. The relative difference between grass SLIT-tablet and placebo per trial (or season within a trial) as a function of the average grass pollen counts over the first 20 days, the last 20 days, the 15-day peak and the entire grass pollen season was modelled with a robust linear regression (lmRob; TIBCO Spotfire S+ 8.1 for Windows® TIBCO Software Inc., Palo Alto, CA, USA).

Results

The trial designs, eligibility criteria, endpoints and methodology of the measurement tools were similar across the trials, while the subject demographics differed with respect to age and thus years with allergy (see Table 1).

Study population

The total number of subjects included from the six trials was 2363; 1198 subjects were treated with grass SLIT-tablet and 1165 with placebo. 923 (39%) of subjects were from North America. Between 72% and 89% of subjects had sensitizations

Table 1 Overview of key demographic parameters for each trial in the pooled analysis

| Trial  | GT-02 | GT-07 | GT-08 | GT-12 | P05238 | P05239 |
|--------|-------|-------|-------|-------|--------|--------|
| Number of subjects | 580†  | 114   | 634   | 253   | 438    | 344    |
| Age (mean) | 35 years | 36 years | 34 years | 10 years | 36 years | 12 years |
| Sex (% male) | 62   | 68   | 59   | 66   | 50    | 65     |
| Years with grass allergy | 20 years | 20 years | 16 years | 3.5 years | 21 years | 6.5 years |
| Subjects with asthma (%) | 10   | 100  | 20   | 41   | 24    | 26     |
| Polysensitized subjects (%) | 74   | 81   | 72   | 82   | 85    | 89     |
| Serum level of specific IgE (Phleum pratense) | 27 kU/l | –    | 50 kU/l | 53 kU/l | 17 kU/l | 33 kU/l |

For the GT-07 trial, specific IgE was not analysed at inclusion.

† The GT-02 trial included a total of 855 subjects treated with three different active doses or placebo. Only subjects treated with 2800 BAU/75 000 SQ-T or placebo are included in this analysis.

Figure 1 Example of allergy immunotherapy trial course with a random pollen season.
in addition to grass, commonly to trees, weeds, house dust mites, dogs and cats. The pooled data set comprised 157,799 daily diary records over the 2003–2009 grass pollen seasons.

Pollen seasons

The grass pollen counts varied from year to year and from day to day and season duration from year to year (Table 2). The grass pollen counts per day ranged from 0 to 1686 grains/m\(^3\), with average daily counts within each trial varying from 32 to 57 grains/m\(^3\).

Correlations between pollen counts, symptoms and symptomatic medications use

Figure 2 depicts the relationship between TCS in the SLIT-tablet and placebo-treated subjects and the daily grass pollen counts during the defined grass pollen season. The figure shows that the TCSs for both groups are dependent on the grass pollen counts, that is, at increased pollen counts, the scores increase. The same relationship was found for the separate symptoms and symptomatic medication scores (not shown).

Correlations between pollen exposure and measured treatment effect

Figure 2 also depicts the distribution (histogram) of the daily grass pollen counts within the defined grass pollen seasons included in the pooled analysis. Notably, less than 50% of the days during the pollen seasons had grass pollen counts above 30 grains/m\(^3\). From the figure, it is also evident that the magnitude of the treatment effect (i.e. the difference between active and placebo) increases with higher pollen counts. Similar relationships were found for the DSS and DMS (\(P < 0.001\)).

This graph further indicates that the dependency of efficacy measurements on pollen exposure is steeper for daily pollen counts below 90–100 grains/m\(^3\) than for higher counts.

Overall, the relative treatment effect on the TCS for each trial (or each year of a trial) was correlated with the average grass pollen exposure in the beginning of the season. The predicted percentage reduction in TCS = 12\% + 0.35\% \times pollen count (see Fig. 3), implying that for each increase in average daily exposure of 10 grains/m\(^3\) during the first 20 days of the season, the relative difference in TCS is

| Trial code | Region | Year | Pollen counts (grains/m\(^3\)) | Length of pollen season (days) |
|-----------|--------|------|-------------------------------|-------------------------------|
| GT-02     | EU + CA| 2003 | Mean 32 | Median 21 | Min–Max 0–772 | Mean 59 | Median 54 | Min–Max 8–92 |
| GT-07     | DK + SE| 2004 | Mean 34 | Median 17 | Min–Max 0–285 | Mean 53 | Median 52 | Min–Max 52–60 |
| GT-08 (y1)| EU     | 2005 | Mean 55 | Median 30 | Min–Max 0–992 | Mean 58 | Median 59 | Min–Max 16–86 |
| GT-08 (y2)| EU     | 2006 | Mean 57 | Median 33 | Min–Max 0–1686 | Mean 57 | Median 50 | Min–Max 30–116 |
| GT-08 (y3)| EU     | 2007 | Mean 42 | Median 22 | Min–Max 0–513 | Mean 73 | Median 70 | Min–Max 44–117 |
| GT-08 (y4)| EU     | 2008 | Mean 49 | Median 30 | Min–Max 0–613 | Mean 72 | Median 69 | Min–Max 21–110 |
| GT-08 (y5)| EU     | 2009 | Mean 36 | Median 21 | Min–Max 0–303 | Mean 83 | Median 78 | Min–Max 39–116 |
| GT-12     | DE     | 2007 | Mean 32 | Median 16 | Min–Max 0–683 | Mean 81 | Median 81 | Min–Max 42–126 |
| P05238    | US + CA| 2009 | Mean 38 | Median 20 | Min–Max 0–772 | Mean 59 | Median 57 | Min–Max 7–162 |
| P05239    | US + CA| 2009 | Mean 38 | Median 23 | Min–Max 0–612 | Mean 67 | Median 65 | Min–Max 7–162 |

EU, Europe; CA, Canada; DK, Denmark; SE, Sweden; DE, Germany; US, United States.

References: GT-02 (6); GT-07 (7); GT-08 years 1–5 (5); 11–13; GT-12 (8); P05238 (10); P05239 (9).
expected to increase by 3.5% (absolute number). A test for the slope showed that it was significantly different from 0 ($P = 0.003; R^2 = 0.66$).

The corresponding correlations with the grass pollen exposure during the entire grass pollen season and during the peak pollen season were equally good [$R^2 = 0.67$ (entire); $R^2 = 0.67$ (peak)], whereas pollen exposure during the last part of the season was a poor predictor of treatment effect [$R^2 = 0.25$ (last 20 days)].

Discussion

In this analysis, it is demonstrated that combined rhinoconjunctivitis symptoms and symptomatic medications use in grass-allergic subjects is closely associated with the level of grass pollen exposure. Importantly, the magnitude of the measured AIT treatment effect compared to placebo measured in large placebo-controlled trials is also dependent on the pollen counts and in particular the early seasonal exposure.

This is an important relationship that must be considered when interpreting individual clinical trial results and in particular when attempting to compare efficacy estimates from different AIT trials in meta-analyses. Even in trials with the same product, the efficacy estimate may differ significantly. As shown here, this may largely be explained by seasonal variations in grass pollen counts. Other sources of variability include differences in populations (subjects with/without asthma, moderate vs severe allergic disease), differences in preseasonal treatment (or duration of treatment) and physical/chemical properties of the pollen exposure.

One phase III grass SLIT-tablet trial was omitted from the pooled analysis as post hoc findings suggested that the included trials. The line is the fitted linear regression with 95% confidence intervals. —Y1–Y5 represent the five seasons included in the GT-08 trial.

Symptoms and medication use reported in the trial were not primarily reflective of the effects of grass pollen exposure. It is conceivable that subjects in the trial were suffering symptoms due to some other unidentified cause or that some subjects understood poorly the standards by which they were to score their symptoms (17). However, a sensitivity analysis was performed including this trial and the qualitative conclusions remained unchanged.

One strength of this pooled analysis is that pollen counts were obtained from numerous collection sites and compiled in centralized databases. Although different sampling devices have been used, it is anticipated that they produce roughly equivalent results with larger particles such as grass pollen (18, 19) and that the pollen data obtained represent the best available data on outdoor pollen levels. However, it is still debateable as to which extent the pollen counts obtained reflect the actual daily exposure for the individual subject. Furthermore, high humidity, moisture and barometric pressure may cause pollen to rupture into very small grains that are easily carried by the wind and easily inhaled, causing symptoms (20). These fine pollen particles in the air may not be detected by the pollen monitoring stations. Nevertheless, pollen counts are presently the only exposure estimate available for large multisite trials in seasonal allergy and on average correlate well with airborne allergen levels and with clinical symptoms (21–23). Although individual monitoring of pollen exposure is possible from a technical point of view, it is complex and not feasible in large trials or over longer periods.

It has long been known that symptoms of allergy are dependent on exposure to the causative allergen, but the level of exposure required to elicit symptoms has been debated. In
all trials included here, the boundaries for onset and stop of the grass pollen season have been set as 10 grains/m³. A study from Australia showed that with increasing pollen counts from above 6 grains/m³, there was a linear increase in grass pollen-induced asthma emergency department presentations in children (24). Similarly, a cohort study from USA showed that grass pollen exposures of ≥2 grains/m³ were associated with wheeze, night symptoms and persistent cough among sensitized children (4–12 years of age) (25). The present data set supports that grass pollen counts of 10 grains/m³ is sufficient to induce symptoms.

Recently, it was published that the relationship between pollen exposure and allergy symptoms is not linear. In a trial with 109 grass pollen-allergic volunteers providing symptom data each day for 4 months, it was shown that in the beginning of the season (i.e. on days with pollen levels below 10 grains/m³), the relationships between pollens and nasal and ocular symptoms were globally linear and odds ratios were high, consistent with no or very low threshold (26). However, the effect of grass pollen on nasal and ocular symptoms reached a saturation point of 80–90 grains/m³, and beyond this, an increase in pollen counts did not imply significantly increased symptom levels. This observation resembles what we have found in our pooled data set.

In the current analysis, we report the average grass pollen exposure during the first 20 days of the season to be a good predictor for the efficacy outcome in terms of the combined symptom and medication score for trials of the grass SLIT-tablet. This is of value for interpretations and comparisons of clinical data as it is fast and easily obtainable from pollen collection sites. The relationship was equally good during the peak season and the entire season, whereas the last part of the season was a poor predictor of efficacy outcome. This is somewhat in contradiction to the theory of priming established by Connell (27), where the clinical consequences of a given pollen load are thought to increase as the season progresses. Potential explanations may relate to patient adaptation to symptoms, differences in pollen allergenicity in the early vs the late season or fewer peaks with very high counts observed in the last part of the season. Co-sensitizations did not impact the relationship.

On the other hand, the data reported here confirm what was previously shown in a Dutch study where grass pollen-allergic subjects had more symptoms during the first part of the season than during the last part of the season for the same pollen levels (28). This effect could not be explained by any of the confounding factors including self-reported medication use, co-sensitization to birch pollen and a clinically relevant house dust mite allergy.

**Conclusion**

In seasonal allergy trials with grass SLIT-tablet, the observed treatment effect is highly dependent on pollen exposure with the magnitude being higher with higher pollen exposure. The grass pollen exposure during the first part of the pollen season is the better predictor for the magnitude of the treatment effect. The dependency of the treatment effect on pollen exposure is an important relationship that must be considered when interpreting individual clinical trial results and comparing across trials and seasons.

**Acknowledgments**

The included trials were sponsored by ALK or Merck and Co. Medical writing services for this manuscript (drafting, editing and submission) were provided by Bente Riis (employee of ALK).

**Author contributions**

All authors made substantial contributions to conception and design of, or acquisition of, data or analysis and interpretation of data. All authors revised the manuscript critically for important intellectual content. All authors gave final approval of the version to be published. SRD, DIB, PSC and HSN were investigators in one or more of the trials included in the analysis; HN was responsible for trial conduct of the US trials; JSA and ZL provided the statistical analyses. SDR and JSA are guarantors of the paper.

**Conflicts of interest**

SRD has received via Imperial College grants, lecture fees and consulting fees from ALK and has been consultant for Merck, GSK, Boehringer Ingelheim, Stallergenes and Circassia; DIB has received grant and consulting fee from Merck; PSC has received grants from Merck, Greer, Circassia and Stallergenes and consulting fees from Merck, Greer, Circassia, AHRQ and UpToDate; HSN has received consulting fees from Merck, Pearl Therapeutics and Shionogi, and grant support from NIH, Lincoln Diagnostics, Rigel and Circassia; HN and ZL are employed by Merck; JSA is employed by ALK.

**References**

1. Canonica GW, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R et al. Sub-lingual immunotherapy: world allergy organization position paper 2009. *World Allergy Organ J* 2009;2:233–281.
2. Calderon MA, Casale T, Cox L, Akdis CA, Burks AW, Nelson HS et al. Allergen immunotherapy: a new semantic framework from the European Academy of Allergy and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRAC-TALL consensus report. *Allergy* 2013;68:825–828.
3. Calderon MA, Simons FE, Malling HJ, Lockey RF, Moingeon P, Demoly P. Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile. *Allergy* 2012;67:302–311.
4. Behrendt H, Becker WM. Localization, release and bioavailability of pollen allergens: the influence of environmental factors. *Curr Opin Immunol* 2001;13:709–715.
5. Reid CE, Gamble JL. Aeroallergens, allergic disease, and climate change: impacts and adaptation. *EcoHealth* 2009;6:458–470.
6. Durham SR, Emminger W, Kapp A, De Monchy JG, Rak S, Scadding GK et al.
Efficacy in AIT trials depends on pollen grains and atmospheric particles: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens. *Clin Exp Allergy* 1999;29:633–641.

22. Johnsen CR, Weeke ER, Nielsen J, Jensen J, Mosbech H, Frolund L et al. Aeroallergen analyses and their clinical relevance. II. Sampling by high-volume airsampler with immunochemical quantification versus Burkard pollen trap sampling with morphologic quantification. *Allergy* 1992;47:510–516.

23. Spieksma FT, Nikkels BH, Dijkman JH. Seasonal appearance of grass pollen allergen in natural, submicronic aerosol of various size fractions. Relationship with airborne grass pollen concentration. *Clin Exp Allergy* 1995;25:234–239.

24. Erbas B, Akram M, Dharmage SC, Tham R, Dennekamp M, Newbigin E et al. The role of seasonal grass pollen on childhood asthma emergency department presentations. *Clin Exp Allergy* 2012;42:799–805.

25. DellaValle CT, Triche EW, Leaderer BP, Bell ML. Effects of ambient pollen concentrations on frequency and severity of asthma symptoms among asthmatic children. *Epidemiology* 2012;23:55–63.

26. Caillaud DM, Martin S, Segala C, Besançon JP, Clot B, Thibaudon M. Nonlinear short-term effects of airborne Poaceae levels on hay fever symptoms. *J Allergy Clin Immunol* 2012;128:818–825.

27. Connell JT. Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. *J Allergy* 1969;43:33–44.

28. de Weger LA, Beerthuizen T, Gast-Strookman JM, van der Pias DT, Terreehorst I, Heemstra PS et al. Difference in symptom severity between early and late grass pollen season in patients with seasonal allergic rhinitis. *Clin Transl Allergy* 2011;1:18.