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Determining the Threshold Concentration of Birch Pollen for Inducing Allergic Symptoms Using an Allergen Challenge Chamber

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Keywords
Birch pollen · Allergic rhinitis · Allergen challenge chamber · Threshold concentrations

Abstract
Introduction: To determine threshold concentrations of pollen inducing symptoms in seasonal allergic rhinitis patients has been a challenge for decades. Allergen challenge chambers (ACC) allow a controlled, reproducible experimental design to address this problem. Hitherto, ACCs were only run with high pollen concentrations. ***Methods:*** The Fraunhofer ACC was technically modified to deploy very low pollen concentrations. Then, adults with birch pollen-induced allergic rhinitis were challenged with varying birch pollen concentrations using a patient-blinded, sham challenge-controlled, part-randomized, titrate-to-effect clinical study setting. Mean increase in Total Nasal Symptom Score (TNSS) ≥0.55 compared to sham challenge was regarded as minimal clinically important difference (MCID). Further endpoints were nasal secretion weight, exhaled nitric oxide (FeNO), and inflammatory cells from nasal lavage. ***Results:*** Fifteen participants with mild to moderate allergic rhinitis participated in the experimental study part (mean age 45 years [22–64]; 7 females). Mean TNSS was: 1.08 at 0 pollen/m^3; 1.05 at 10 pollen/m^3; 1.2 at 50 pollen/m^3; 1.74 at 100 pollen/m^3; 1.61 at 200 pollen/m^3; 2.79 at 1,000 pollen/m^3. MCID of TNSS was observed at 100, 200, and 1,000 pollen/m^3. More than half of the study population showed a lack of response at 10, 50, and 200 pollen/m^3. Nasal secretion increased slightly with concentration. No clinically meaningful results could be derived from FeNO and inflammatory cells. ***Conclusions:*** The applied technical modification of the Fraunhofer ACC produced stable, low pollen concentrations. Based on mean TNSS data, the threshold concentration for inducing symptoms with birch pollen was 50–100 pollen/m^3.

Introduction

Determining threshold concentrations for various aeroallergens has been attempted many times in published literature [1–8]. In fact, first experiments have been reported since the 1960s [9]. However, obtaining

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comparable results prove difficult due to a plethora of methods, outcome measures and choices in study population. Basically, threshold concentrations can be assessed by (i) target organ specific allergen challenge (conjunctival, nasal or inhalative exposure), (ii) field study, and (iii) allergen chamber challenge – each of which has its own benefits and drawbacks [10].

To date, allergen challenge chambers (ACCs) have been used primarily for proof-of-concept studies with anti-allergic agents [11, 12]. Technically, the chambers are designed to maintain high pollen concentrations, which are meant to reliably induce symptoms in sensitized participants. Pollen concentrations are maintained at a constant level for hours to determine the maximum possible effect size of the investigated therapeutic intervention. At Fraunhofer ACC, pollen concentrations in the four-digit range are used in clinical trials [13]. Threshold concentrations, however, may be at the low three-digit or even two- or one-digit range. Stable metering and monitoring of pollen at such low concentrations present technical challenges.

With respect to determining threshold concentrations, the artificial conditions in the ACC are both the strength and weakness of this method. On the one hand, the control of environmental conditions such as humidity and room temperature is an advantage over field studies to keep these variables constant and thus to obtain reproducible results. On the other hand, this very artificially produced constancy might limit the transferability of the results to the natural environment [10]. Furthermore, other relevant environmental variables are missing such as atmospheric gases, e.g., nitrogen dioxide or ozone, and precipitation, which significantly influence pollen concentration and allergenicity [14–16]. In this study, we developed and validated a technical modification of the ACC to deploy low pollen concentrations and challenged patients with birch pollen-induced allergic rhinitis at varying concentrations of birch pollen, including a sham challenge, to assess the minimum effective concentration.

Materials and Methods

Study Design

This was a mono-centre, patient-blinded, sham challenge-controlled, part-randomized, titrate-to-effect clinical study in adult patients with birch pollen-induced allergic rhinitis (NCT05346718). The study was performed at Fraunhofer Institute for Toxicology and Experimental Medicine ITEM in Hannover, Germany. The objective was to determine the minimum effective concentration of birch pollen.

Ethics

The study was approved by the Local Ethics Committee of Hannover Medical School on 21 October 2021 (No. 10024_BO_S_2021) and was conducted in compliance with the Declaration of Helsinki (7th revision, Fortaleza, Brazil), International Council for Harmonisation Guideline for Good Clinical Practice (E6, Step 4, November 2016), and all national laws and regulations. Written informed consent was obtained from the participants for participation in the study and publication of their medical details.

Study Population

Adults 18–65 years of age were eligible if they had (i) a history of birch pollen-induced allergic rhinitis, (ii) a positive skin prick test (≥3 mm) to birch (*Betula pendula*) and birch-specific IgE level ≥0.7 kU/L, (iii) no allergic symptoms defined by a Total Nasal Symptom score (TNSS) of 0 prior to entering the ACC at all exposures, (iv) mild to moderate rhinitis symptoms defined by a mean TNSS between 1 and 8 after the four-hour screening challenge with 1,000 pollen/m^3 to avoid participants who are extremely sensitive, and (v) no nasal abnormalities potentially blocking nasal airflow.

Patients were excluded if one or more of the following conditions applied: (i) taking anti-allergic or immunosuppressive drugs including corticosteroids; (ii) having asthma requiring step 2 treatment or higher according to the Global Initiative for Asthma guidelines [17] (daily use of inhaled steroids with or without long acting beta agonists); (iii) having a forced expiratory volume in 1 s <80% of the predicted normal value; (iv) having concomitant allergies to seasonal aeroallergens or to animal dander which were anticipated to be active during study participation; (v) having received specific immunotherapy for birch allergy within 5 years before participation. Women could not be pregnant or lactating.

Birch Pollen

A single batch of birch pollen was purchased from Pharmallerga, 37372 Lisov, Czech Republic. Pollen was produced, tested, and released according to ISO 9001 standards and stored under controlled conditions at 5 ± 3°C at the study site.

Allergen Challenge Chamber Setting

ACC experiments were done with the validated Fraunhofer ACC according to site specific standard operation procedures [13]. After each exposure day, the chamber was cleaned by damp wiping the floor and surfaces. In the ACC, all participants were under constant

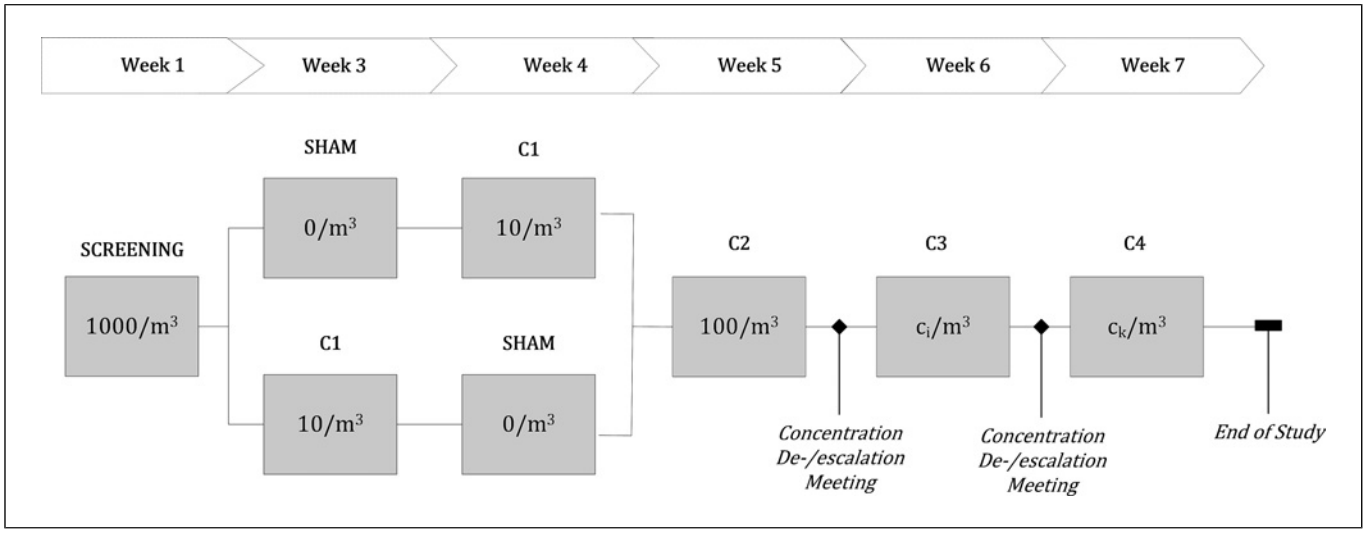


Fig. 1. Study design. C, concentration.

supervision by trained staff outside the chamber. Communication was possible through clear glass windows and via intercom system. Allergen challenges lasted for 4 h.

The chamber was operated with indoor air, which was filtered, conditioned, and then supplemented with a qualitatively and quantitatively determined allergen load. Condition of the air and pollen concentration were continuously monitored, ensuring a constant humidity (40 ± 10%), temperature (22 ± 2°C), airflow (1,500 ± 100 m^3/h), and pollen load (maximum variability of 12%) throughout the exposure day.

To achieve a temporal constant, very low pollen concentration in the ACC, a combination of a microfeeder and a downstream depletion nozzle was used (in-house developments by Fraunhofer ITEM). The microfeeder was equipped with a rotating disk with several holes through which pollen were dosed from the reservoir into a suction tube (operated via a Venturi nozzle driven by oil-free compressed air). The hole size and number as well as speed of rotation determined the amount of pollen. This primary dosing principle allows pollen concentrations down to a few hundred pollen/m^3. To further reduce pollen concentration with high temporal stability, an adjustable partial flow of the primary pollen aerosol was extracted via a filter to remove the pollen (depletion nozzle). A second partial flow was directed through a particle number counter (development of Fraunhofer ITEM). This concentration was used as a feedback signal to control the speed of the primary dosing unit. The remaining pollen flow passing the depletion nozzle was distributed to the four inlets at the ceiling of the ACC (Fig. 1g in Pfaar et al. [11]). To validate interday variability of the desired pollen concentrations, preliminary tests were carried out in the ACC without test participants by 5 replicate runs for each target concentration using two Rotorod systems (Rotorod, Multidata, Minneapolis, MN, USA). In parallel, temporal stability was tested with a laser particle spectrometer with high sampling volume flow (Lasair Pro 5100, Particle Measuring Systems, Boulder, CO, USA). Data for interday variability (online suppl. Fig. S1A–C) and temporal stability (online suppl. Fig. S1D) at different concentrations are presented in the online supplementary material at https://doi.org/10.1159/000545509. Four-hour mean actual concentrations (± coefficient of variance) of the target concentrations were:

- 10 pollen/m^3: 11.3 ± 12.1%
- 30 pollen/m^3: 35.4 ± 5.4%
- 100 pollen/m^3: 124.7 ± 12.0%

Without the supply of pollen, the particle concentration in the size regime relevant for pollen (~20 μm) was 0/m^3, as the air in this size range (without the presence of test participants) was completely particle-free due to the filtering of the supply air.

To determine the pollen concentration within the ACC on the days of patient exposure, two approaches were run: (i) over the entire exposure period pollen samples were collected using the two Rotorod sampling systems described above for subsequent microscopic pollen analysis. (ii) At the beginning of the challenges, particle concentration was measured with the above-mentioned laser particle spectrometer with high sampling volume flow.

During exposure, direct monitoring of the low pollen concentrations in the allergen chamber was not possible due to the high input of foreign particles into the room by test participants [13].

Exposure Sequence

To assess eligibility, participants were exposed to 1,000 pollen/m^3 for 4 h in a screening challenge. Overall, five exposures were planned in the experimental study part (Fig. 1). It started with a sham challenge and 10 pollen/m^3 in a crossover, randomized fashion to control expectancy bias, followed by 100 pollen/m^3. After that, the concentration was escalated or de-escalated based on mean difference in TNSS between sham challenge and the latest concentration. After the 100 pollen/m^3 challenge, two concentration de-/escalation meetings were planned. The screening exposure was conducted 14 days prior to the start of the 5 experimental study exposures which were each separated by 7 days based on prior findings on repeatability and absence of priming of TNSS [18, 19].

Study Endpoints

Nasal symptoms were evaluated as a patient-reported outcome prior to and every 20 min during allergen challenge using the TNSS. The TNSS is an ordinal scale with four items: nasal congestion, rhinorrhoea, nasal itching, and sneezing. Each item is scored according to symptom severity with 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The item scores are summed up to calculate the TNSS of each assessment time. The TNSS ranges between 0 and 12 points. In previous studies, group mean TNSS showed a distinct kinetic pattern: increasing in the first 2 h, then stabilizing, and slightly declining towards the end of ACC [13, 20]. For statistical analysis, TNSS data from the stabilization phase (140–240 min) were used.

Nasal secretion was assessed using pre-weighed handkerchief sachets. Each participant had to use one sachet per hour to collect nasal discharge. After the challenge, the handkerchief sachets were weighed again. The weight difference resembled the amount of nasal secretion produced during the challenge.

Exhaled nitric oxide (FeNO) as a marker for airway inflammation was measured before, directly after and 1 day after each allergen challenge, except for the screening challenge. Neutrophils and eosinophils were measured in nasal lavage. It was collected before, 2 h after, and 1 day after each allergen challenge, except for the screening challenge.

Spirometry was assessed at screening. For safety monitoring of lung function, peak expiratory flow (PEF) was measured with a hand-held mechanical PEF device once before, hourly during, and two-hourly after exposure until bedtime and once the next morning at each exposure day.

Sample Size

The sample size was based on feasibility due to the maximum ACC capacity of 18 individuals. The required standard deviation of the group mean difference was calculated from own unpublished data with paired samples challenged with escalating concentrations of birch pollen, including a sham exposure. With a fixed sample size of 18, a standard deviation of 1.26 of mean group difference between 0 and 1,000 pollen/m^3 (mean = 1.04), and a two-tailed type I error probability of 5%, a power of 91% could be achieved. The power was calculated with G*Power 3.1.9.4.

Statistics

Statistical analysis was performed using GraphPad Prism 10.1.2. Missing and incomplete data were not replaced, and no imputation was performed. The minimal clinically important difference (MCID) of mean TNSS between challenges was 0.55, as suggested in a meta-analysis by Barnes et al. [21]. Descriptive statistics are provided for TNSS, nasal secretion and FeNO (n, arithmetic mean, median, range). To assist concentration escalation/de-escalation decisions during the study, paired *t* tests comparing mean TNSS of sham challenge with latest verum challenge were used descriptively, without correction for multiple testing. After study completion, Dunnett's multiple comparison's test was performed, comparing sham challenge with all verum challenges, including the 1,000 pollen/m^3 screening exposure. The following hypotheses were tested at a two-sided 5% significance level:

- H0: There is no difference between sham challenge and verum challenge.
- H1: There is a difference between sham challenge and verum challenge.

To assess the concentration-response relationship, a Chi-squared test for trend was calculated.

Results

Participants and Demographics

The study was conducted between 1 September 2022 and 11 November 2022. After screening challenge, 19 participants were eligible. Eventually, only 15 participants

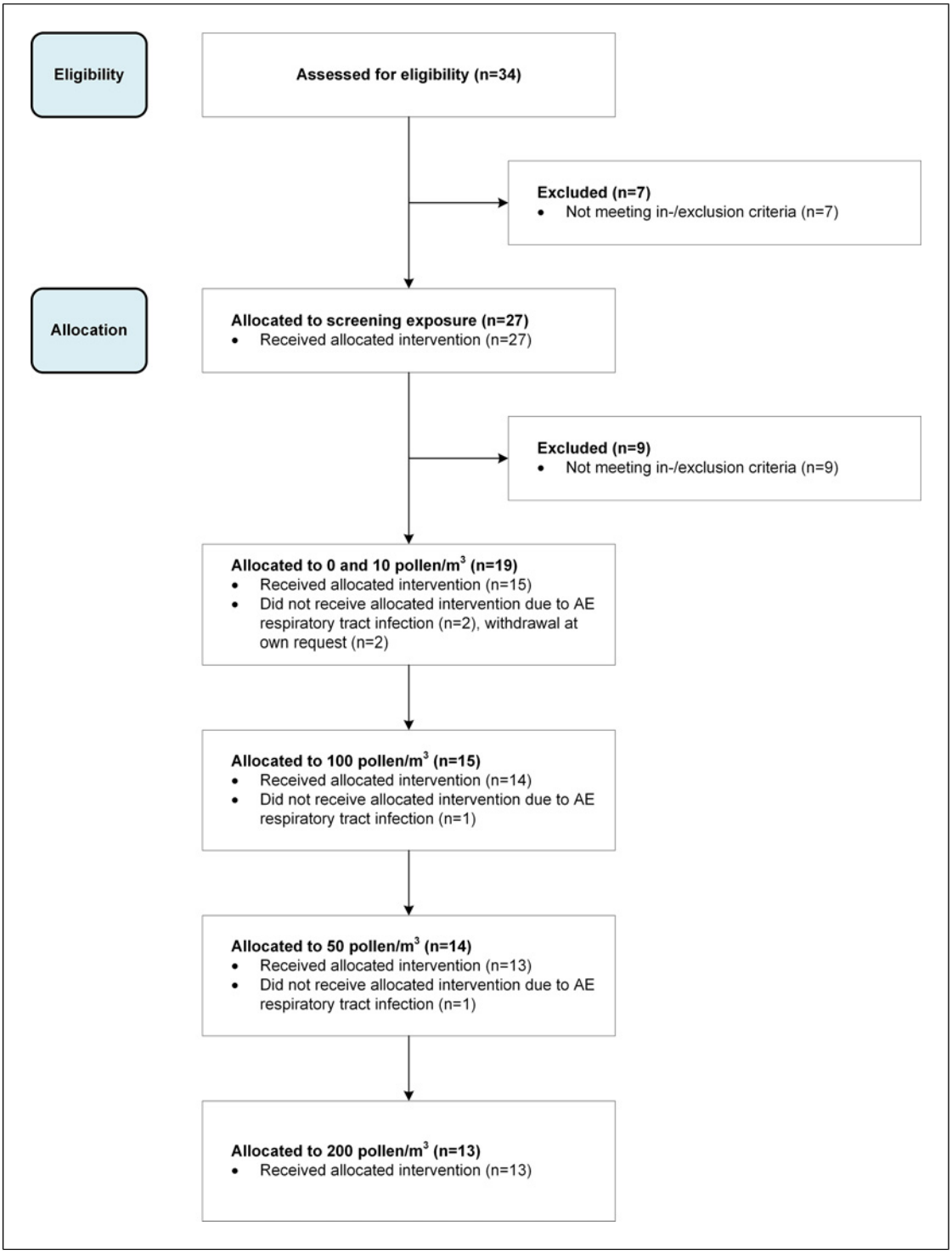


Fig. 2. Consort flow diagram. AE, adverse event.

received the first intervention due to four withdrawals (Fig. 2). Genders were approximately equally distributed (Table 1). All participants had normal lung function at screening with forced expiratory volume in the first second of >80% of predicted normal (Table 1). No participant had an asthma history.

Table 1. Demographics of the participants who received first intervention

Participant number	Gender	Age, years	FEV_1, % pred.	Specific IgE: birch, kU/L	Mean TNSS 140–240 min Screening exposure	Missed exposures, pollen/m^3
1	Male	64	92.7	25.8	5.3	–
2	Male	54	83.4	4.1	4.0	–
3	Female	58	105.9	11.1	1.5	–
4	Male	23	108.1	7.2	3.3	–
5	Female	40	112.2	12.4	2.8	–
6	Male	60	98.7	11.6	2.0	–
7	Male	55	115.5	7.6	3.7	–
8	Female	22	110.0	1.8	1.8	–
9	Female	43	105.6	4.4	1.2	50, 200
10	Male	57	85.2	13.2	1.8	–
11	Female	26	98.0	34.3	2.2	–
12	Female	30	107.2	4.3	2.8	–
13	Male	64	120.6	1.6	2.0	–
14	Female	56	102.0	6.7	3.5	50, 100, 200
15	Male	28	95.0	5.4	4.0	–
Mean±SD (range) or number	7 females 8 males	45.3±15.8 (22–64)	102.7±10.2 (83.4–120.6)	10.1±9.0 (1.6–34.3)	2.8±1.2 (1.2–5.3)	13 per protocol

Data given as means ± standard deviation (SD) and range in brackets or number. FEV1, forced expiratory volume in 1 s; % pred., percent of predicted normal; kU/L, kilounits/liter; TNSS, Total Nasal Symptom Score; SD, standard deviation.

Sample Size

During the intervention, 2 participants dropped out at various times due to respiratory tract infections (Fig. 2). Eventually, 13 participants completed the study per protocol, reducing statistical power from 91% to 78%.

Allergen Challenge Sequence

The final sequence of allergen concentrations was 0, 10, 100, 50, and 200 pollen/m^3. Since the 100 pollen/m^3 challenge already produced the desired increase of ≥0.55 TNSS points, a concentration de-escalation to 50 pollen/m^3 was decided, followed by a doubling to 200 pollen/m^3 to see if the increasing trend of 100 pollen/m^3 would continue.

Total Nasal Symptom Score

Considering the time-response relationship, the expected crude pattern of a steep increase phase followed by a stabilization phase was seen in all challenges (Fig. 3a). In the three-digit concentration range, mean TNSS of 140–240 min was lowest at 0 pollen/m^3 and highest at 100 pollen/m^3 (Table 2). At 0 pollen/m^3, mean TNSS was 1.08 (ranging from 0.0 to 2.8), with only one-third of participants scoring 0 points throughout. This indicates a profound nocebo effect similarly seen in previous sham challenges at Fraunhofer ITEM [13, 20]. Mean difference between sham challenge and verum challenges increased with pollen concentration, except for a minor reduction at 200 pollen/m^3. MCID of 0.55 TNSS points compared to sham challenge was observed at 100, 200, and 1,000 pollen/m^3 (group means). Two-sided *t* tests descriptively comparing sham challenge with verum challenges gave *p* values <5% at 100 pollen/m^3 and 1,000 pollen/m^3. However, in Dunnett's multiple comparisons test an adjusted *p* value <5% was found only at 1,000 pollen/m^3.

Looking at individual data, an inconsistent rating behaviour or seeming lack of response was frequently observed: At 10, 50, and 200 pollen/m^3 most participants documented no change or less symptoms compared to 0 pollen/m^3 (Fig. 3b).

Descriptively, a positive trend in the concentration-response relationship was observed when regarding the

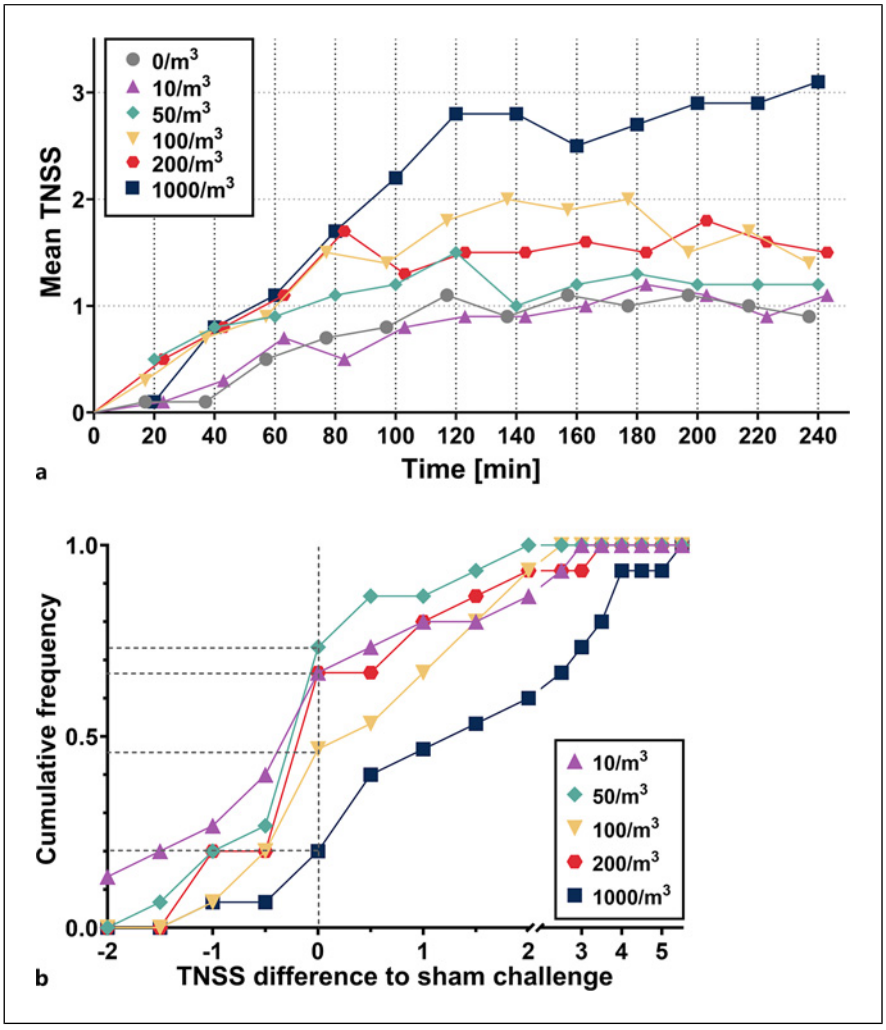


Fig. 3. TNSS means per time point (**a**) and cumulative frequency distribution of TNSS difference between verum and sham challenges (**b**). In **b**, a negative TNSS difference between verum and sham challenge indicates more allergic symptoms at 0/m^3 than at the respective verum concentration (inconsistent rating). TNSS, Total Nasal Symptom Score.

Table 2. Summary statistics of TNSS, nasal secretion and exhaled nitric oxide

Concentration	*N*	TNSS		Nasal secretion, g		FeNO (ppb)		
						Pre	1 day post	*p* value
		mean±SD	median (IQR)	mean±SD	median (IQR)	mean±SD	mean±SD	
0 pollen/m^3	15	1.08±0.94	1.00 (0.00–1.80)	0.11±0.11	0.08 (0.01–0.13)	23.17±12.96	28.40±16.24	0.04
10 pollen/m^3	15	1.05±1.02	0.80 (0.00–2.00)	0.12±0.11	0.09 (0.01–0.20)	25.10±16.28	27.37±15.05	0.03
50 pollen/m^3	13	1.20±1.02	1.00 (0.35–2.00)	0.14±0.15	0.08 (0.00–0.26)	24.31±14.81	27.27±15.72	0.01
100 pollen/m^3	14	1.74±0.94	1.75 (1.10–2.35)	0.17±0.15	0.12 (0.06–0.30)	25.21±14.12	26.75±13.72	0.15
200 pollen/m^3	13	1.61±1.19	1.20 (0.65–2.85)	0.19±0.20	0.12 (0.00–0.29)	25.38±13.55	25.50±12.31	0.92
1,000 pollen/m^3	15	2.79±1.15	2.80 (1.80–3.70)	0.60±1.08	0.24 (0.11–0.54)	N/A	N/A	N/A

proportion of participants with a mean TNSS ≥0.55 of each verum challenge (Fig. 4). However, a Chi-squared test for trend excluding the 1,000 pollen/m^3 challenge gave no statistically significant result for the low dose range (X^2 = 1.209, p = 0.27).

Nasal Secretion

Mean nasal secretion weight increased with pollen concentration (Table 2). Two-sided t tests descriptively comparing sham challenge with verum challenges gave no p values <5%.

Exhaled Nitric Oxide

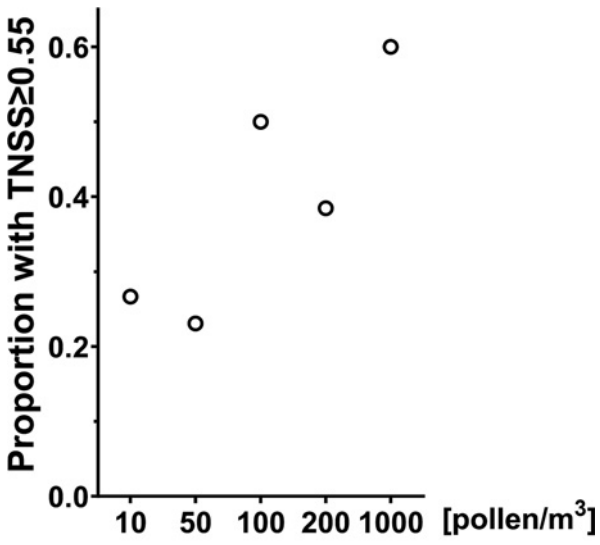
Pre-challenge FeNO values lay close together in all challenges, ranging between 23.2 and 25.4 ppb. Post-challenge values were increased in all challenges compared to pre-challenge values (Table 2). The increase was statistically significant at 0, 10, and 50 pollen/m^3.

Neutrophils and Eosinophils in Nasal Lavage

In many pre-challenge samples, no cells were detectable, or cell content was below 50,000 cells. Median neutrophil percentages were >99% with median eosinophil percentages below 1%. No relevant changes in counts of neutrophils and eosinophils were measured following allergen challenges.

Lung Function

PEF during and after pollen exposure remained stable with a mean maximum decline of 10.5 ± 5.8%. Three participants experienced an asymptomatic maximum PEF decline of >20% (22.7%, 21.9%, 21.1%) at one of the six exposure days irrespective of pollen concentration.



Concentration	Mean TNSS ≥0.55	Mean TNSS ≤0.55	∑
10 pollen/m^3	4	11	15
50 pollen/m^3	3	10	13
100 pollen/m^3	7	7	14
200 pollen/m^3	5	8	13
1000 pollen/m^3	9	6	15

Fig. 4. Proportions of positive allergic responses (mean TNSS ≥0.55) per pollen concentration. TNSS, Total Nasal Symptom Score.

Discussion

In our study, the threshold concentration of birch pollen for inducing symptoms in seasonal allergic rhinitis patients was between 50 and 100 pollen/m^3 based on TNSS results. A crude positive concentration-response relationship with minor deviations was observed. These findings were not supported by objective outcome measures (nasal secretion, FeNO, inflammatory cells).

For birch pollen, threshold concentrations have been reported from field studies, ranging between 10 and 100 pollen/m^3 [4, 6–8, 22]. Various outcome measures were used in those investigations: symptom scores, symptom diaries, physical examinations, and numbers of medical consultations. A systematic review analysing five studies found a pooled threshold of 45 pollen/m^3 [1]. Altogether, the findings of our study appear to be in line with literature. However, due to the different outcome measures used, comparisons may only be made with caution.

A case-by-case analysis of our data showed an inconsistent rating behaviour and lack of response in more than half of the study population at 10, 50, and 200 pollen/m^3. Those participants experienced no symptoms in verum challenges or more symptoms in the sham challenge than in verum challenges. The latter phenomenon is clinically implausible. This indicates that in the low dose range, most participants could not discriminate between applied concentrations. For some of the participants, the threshold may thus lay at concentrations larger than 200 pollen/m^3, probably closer to 1,000 pollen/m^3.

Unexpectedly, 200 pollen/m^3 evoked lower responses than 100 pollen/m^3. A technical failure of the ACC was

ruled out. Normal variability might be an explanation, considering the small sample size. Also, markable responses seem to be more likely when the concentration is changed on the logarithmic scale (10, 100, 1,000 pollen/m^3) rather than on the linear scale.

Regarding technical methods, one study from 1974 was found where paediatric patients were challenged in an "Environmental Control Unit" with ragweed pollen [23]. Exposure lasted for a couple of minutes, then inhaled pollen dose was extrapolated from room pollen concentration, tidal volume, and challenge duration. This method allowed for the application of very low pollen doses but lacked a continuous exposure with low pollen concentration comparable to real life exposure.

In our approach, the four-hour ACC experiment lasted long enough to induce a stable allergic response. Also, it allowed to control environmental factors (humidity, temperature) and ensured that all participants were exposed to the same pollen concentration. With this setting, repetition of the experiment is possible and could be used, for example, to compare allergenicity of different plant pollen.

Some limitations are worth noting: (i) no applied pollen concentration could be repeated due to limited feasibility; thus the reproducibility of results was not tested. The investigation of allergen threshold concentrations in patients using this approach is not only a technically complex and costly procedure but also very time consuming for the patients included. (ii) Variability in the allergenicity of different pollen batches might produce other results (more or less response) in a repetition of the experiment. This, however, is a general limitation when using whole pollen preparations and not unique to the ACC approach. (iii) The repetitive and lengthy nature of the experiment might have had a negative impact on participants' attention and motivation. In the beginning of the study, expectancy to experience symptoms at some point may have been high, possibly leading to an increased nocebo effect. Although we did not evaluate these psychological factors objectively, they are likely to have played a role to some degree. (iv) Over time the sample size decreased, reducing the power to detect differences between sham and verum challenges. (v) Finally, the inclusion criteria for TNSS in screening exposure could limit the generalisability of our results.

In conclusion, the applied technical modification of the Fraunhofer ACC successfully produced stable, low pollen concentrations that allow for the investigation of allergic symptom thresholds. Based on mean TNSS data, the threshold concentration of birch pollen for inducing symptoms in our cohort was between 50 and 100 pollen/m^3. Especially in the light of lesser-known allergens or those which will gain more importance in the future, e.g., because of climate change ecosystem alterations, this method might become of importance for the evaluation of allergenic effects of airborne allergens for the population.

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Statement of Ethics

The study was approved by the Local Ethics Committee of Hannover Medical School on 21 October 2021 (No. 10024_BO_S_2021) and was conducted in compliance with the Declaration of Helsinki (7th revision, Fortaleza, Brazil), International Council for Harmonisation Guideline for Good Clinical Practice (E6, Step 4, November 2016), and all national laws and regulations. Written informed consent was obtained from the participants for participation in the study and publication of their medical details.

Conflict of Interest Statement

Nadja Struß, Katharina Schwarz, Horst Windt, Wolfgang Straff, and Conny Höflich have no conflict of interest to disclose. Philipp Badorrek reports personal fees from LETI Pharma GmbH, which are outside the submitted work. Jens M. Hohlfeld reports grants to his institution from AltamiraPharma GmbH, Astellas Pharma GmbH, AstraZeneca AB, Bayer AG, Beiersdorf AG, Boehringer Ingelheim Pharma GmbH & Co. KG, CSL Behring GmbH, Desitin Arzneimittel GmbH, F. Hoffmann-La Roche AG, Genentech, Inc., GlaxoSmithKline GmbH & Co. KG, Janssen Pharmaceutica NV, M&P Pharma AG, Novartis AG, Sanofi-Aventis Deutschland GmbH, UCB Pharma GmbH, and personal fees from Boehringer Ingelheim Pharma GmbH & Co. KG, CSL Behring GmbH, Merck & Co, Inc., Nocion Therapeutics, Inc., HAL Allergy Group, Novartis AG, and Roche, all of which are outside the submitted work.

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Author Contributions

Nadja Struß: conceptualization, methodology, formal analysis, investigation, writing original draft, review and editing, and visualisation. Philipp Badorrek: conceptualization, methodology, investigation, resources, supervision, project administration, funding acquisition, and writing – review and editing. Katharina Schwarz: conceptualization, methodology, validation, formal analysis, investigation, resources, supervision, and writing – review and editing. Horst Windt: conceptualization, methodology, validation, and investigation. Wolfgang Straff: conceptualization, methodology, project administration, and resources. Conny Höflich: conceptualization, supervision, methodology, project administration, resources, and writing – review and editing. Jens M. Hohlfeld: conceptualization, methodology, formal analysis, supervision, project administration, funding acquisition, and writing – review and editing. All authors read, revised, and approved the submitted manuscript.

Data Availability Statement

All data analysed during this study are included in this article. Further enquiries can be directed to the corresponding author (J.M.H.).

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