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Birch-induced allergic rhinitis: Results of exposure during nasal allergen challenge, environmental chamber, and pollen season

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ABSTRACT

Background: Pollen variation can affect field study data quality. Nasal allergen challenge (NAC) is considered the gold standard for evaluating allergic rhinitis, while environmental exposure chambers (EECs) are mainly used in phase 2 drug development studies. We aimed to study birch-induced allergic rhinitis under 3 different conditions.

Methods: This study included 30 participants allergic to birch pollen, based on birch skin prick test, specific immunoglobulin E (IgE), and positive NAC. Participants were exposed to placebo twice, followed by 2 consecutive 4-h birch airborne exposures, repeated on 2 occasions to evaluate reproducibility and priming effect. Nasal response was defined as total corrected nasal symptom score (ΔTNSS) $\geq$ 5 during NAC and EEC. The primary end-point was to measure TNSS during the last 2 h of first allergen exposure. TNSS was also analyzed during natural exposure.

Results: The dose most commonly yielding positive TNSS during NAC was 175.2 ng/200 µL. Eighteen participants experienced ΔTNSS $\geq$5 during the last 2 h of the first exposure, whereas 21 had positive responses at all 4 exposures. Mean ΔTNSS was 1 with placebo versus 6 with birch. Exposures were reproducible, with no observed priming effect. Airborne Bet v 1 was 25 ng/m^3, while the pollen measurement was 279/m^3 during pollen season. TNSS reached 5 in 67.9% of participants during peak pollen season.

Conclusion: EEC outcomes were similar to those obtained with NAC and natural exposure, suggesting the usefulness of EEC in allergic rhinitis studies. The primary end-point was reached, as 60% of participants experienced nasal responses.

Keywords: Birch allergy, Allergic rhinitis, Nasal allergen challenge, Environmental exposure chamber, Birch pollen

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INTRODUCTION

To study allergic rhinitis under controlled conditions, the gold standard method is the nasal allergen challenge (NAC), in which a pre-titrated dose of allergen is intranasally delivered to both nostrils.[1] However, NAC is rarely used in clinical studies including large cohorts. Clinical field studies of pollen allergies are impacted by the impossibility of predicting and determining individual allergen exposure, due to co-founding factors, such as pollutants, climate variability, and lifestyles during the pollen season.[2] Therefore, environmental exposure chambers (EECs) have become important in clinical research, particularly for studies of allergic rhinitis.[3–12] EECs deliver a fixed concentration of allergen in an enclosed and tightly controlled environmental setting, which avoids the limitations of NAC and field studies. Therefore, EECs have been extensively used in phase 2–4 clinical studies for over 3 decades.[13] However EECs worldwide have different technical settings, making it difficult to perform large multicenter clinical trials.[14] For the performance of pivotal immunotherapy trials, regulatory authorities require harmonization of clinical assessments and documentation between clinical outcomes obtained using EECs versus environmental exposure.[15]

The EEC used in the present study has previously been validated with 3 different allergens, including birch, for evaluations of allergic conjunctivitis.[16] Previous studies have compared nasal allergen responses during NAC and EEC,[17,18] or EEC and natural exposure,[19] but scarce data exist regarding the analysis of allergen nasal outcomes during all 3 different types of exposure, in the same population. The present study was first designed to validate our EEC for studying allergic rhinitis to birch. Its secondary aim was to analyze nasal allergen responses using three different methods of exposure–NAC, EEC, and the natural birch pollen season–in the same study population. It was shown that 25 ng/m^3 of airborne Bet v 1 induced more than 60% of nasal responses during 2 consecutive 4-h exposures on 2 separate occasions among participants who demonstrated a positive NAC at inclusion.

METHODS

Study participants

The main inclusion criteria for study participation were age of 18–65 years; history of birch pollen-related moderate-to-severe persistent rhinitis requiring symptomatic medication for at least 2 consecutive birch pollen seasons based on the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines,[20] with no birch pollen-induced asthma; positive skin prick tests to birch allergen, with a wheal diameter of $\geq$5 mm compared to negative control; positive birch-specific Immunoglobulin E (IgE) $\geq$ 0.7 kU/L to *Betula verrucose* (Phadia ImmunoCap, Thermofisher®); and a positive nasal response during an individual NAC. Before allergen exposures, participants had to undergo a wash-out period of 14 days for topical corticosteroids and anti-leukotrienes, 7 days for systemic antihistamines, and 3 days for topical antihistamines. The main exclusion criteria were nasal polyposis, chronic sinusitis, nasal septum deviation, diagnosis of non-allergic rhinitis, and sensitization to indoor environmental allergens with obvious exposure.

Nasal assessments

The total nasal symptom score (TNSS) was used to evaluate nasal responses during NAC, EEC, and natural exposure. This score is the sum of scores for nasal congestion, sneezing, nasal itching, and rhinorrhea at each time-point, using a four-point scale (0–3), where 0 indicates no symptoms, 1 indicates mild symptoms that are easily tolerated, 2 indicates moderate awareness of symptoms that are bothersome but tolerable, and 3 indicates severe symptoms that are difficult to tolerate and interfere with activity.[21] TNSS was evaluated with a maximum score of 12 and considered positive if the scores differed by $\geq$ 5 points from baseline.

Peak nasal inspiratory flow (PNIF) was measured with the participant in a sitting position, pre-exposure, post-exposure, and every 30 min during exposures, as well as before and during NAC. PNIF was recorded in triplicate at each time-point. Measurements were obtained using an in-check peak flow meter (Mediflux®, Croissy, France) connected to face mask. Participants were previously trained. We analyzed the mean of 3 measurements, and a

change was considered significant if there was at least a 40% drop from baseline nasal flow.[22]

The use of a visual analogue scale (VAS) for rhinitis is a semiquantitative subjective means of evaluating nasal symptoms. On a scale of 0–100 mm, results are considered moderately positive if symptoms are rated as ≥23 mm.[23] Participants self-evaluated their severity of rhinitis by positioning the cursor of the scale on the device pad.

Bronchial assessment

To promote safety, spirometry was performed during allergen challenges (Spiro Bank II, MIR®, France). Forced expiratory volume in 1 s (FEV_1) was assessed every 30 min, with supplementary assessments if asthma symptoms developed. A 20% drop in FEV_1 was defined as an early asthma response (EAR) during a 4-h exposure, and a 15% drop in FEV_1 was defined as a late asthma response (LAR), from 3 h after EAR up to 24 h in home measurements.[24,25] The same protocol has already been used in this EEC for validation in asthmatic patients to cat and house dust mite.[25]

Allergen exposures

Nasal allergen challenge (NAC)

This procedure was performed following the updated European Academy of Allergy and Clinical Immunology (EAACI) guidelines,[1] and comprised the direct intranasal application of 50 µL, with 2 puffs per nostril: 1 in the inferior meatus and 1 on the direction of the middle turbinate. Diluted birch standardized allergen (Allergopharma®) was dispensed using a pump-aerosol spray, by increasing doses until ΔTNSS reached 5. First, a placebo dose was administered, then a maximum of 5 increasing doses were applied at an interval of 10 min until achieving a nasal response. TNSS was assessed every 10 min, after each increasing dose of allergen.

Environmental exposure chamber (EEC)

This EEC is 65 m^2 with 20 seats, and enables the provision of a homogenous distribution of allergens for each participant. This facility was conceived as a clean room, in which the airflow system, environmental conditions, and airborne particles are continuously monitored. Communication between participants inside the EEC and with medical supervisors was enabled using wireless telecommunication.

The same batch of birch allergen extract (Allergopharma®) was used for all exposures. Participants entered the EEC once the plateau of airborne allergen was reached. Before each allergen session, participants received saline nasal lavage. All exposures lasted up to 4 h. Homogeneous allergen distribution was ensured using particle counters, as previously described.[16,25] After each exposure, the Bet v 1 concentration was determined using ELISA (Indoor Biotechnologies®, Charlottesville, VA, USA) by collecting allergen on glass fiber filters located next to the participants' chairs.

Phenotypes of rhinitis

Four rhinitis phenotypes have been defined.[26,27] Early-phase responders (EPRs) were those who experienced at least a 50% drop in TNSS by 7 h post-exposure, and returned to baseline with no second increase in symptoms afterward. Protracted early-phase responders (pEPRs) were defined as those who exhibited no 50% drop in TNSS, as well as no increase in symptoms up to hour 12 post-exposure. Late-phase responders (LPR) exhibited a 50% drop in TNSS by hour 7 post-exposure, followed by stable symptoms without recovery of 2 points in TNSS. Finally, dual responders (DRs) were those who experienced at least a 50% decrease in TNSS by hour 7 post-exposure, with an at least 2-h period of decline in TNSS severity, followed by a plateau of symptoms.

Natural exposure: Peak pollen period

The definition of the birch pollen season—including its peak period, start, and end, and the correlation of patient-reported symptom loads for birch pollen-induced allergic rhinitis—was elaborated by the EAACI. The start of the peak pollen period corresponds to first day of 3 consecutive days, each with ≥100 pollen/m^3, and the end is the last day of at least 3 consecutive days, each having ≥100 pollen/m^3.[28,29] In Eastern France, the birch pollen season starts at the end of March and peaks between early and mid-April. During the peak period, TNSS was evaluated twice a day, in the morning and evening, and considered positive if the score was ≥5.

Study design

This single-center, single-blind, placebo-controlled study was designed to validate use of a EEC for studying allergic rhinitis to birch pollen. We also performed a second analysis of nasal allergen responses during NAC, EEC exposure, and natural exposure in 30 adults allergic to birch.

The primary end-point was defined as the frequency of nasal response assessed by the TNSS during the last 2 h of the 4-h EEC exposure (H2–H4), and was met when at least 60% of participants reached ΔTNSS ≥5 at first allergen exposure (Visit 5), as compared to placebo (Visit 3). The secondary end-points were to assess TNSS over 4 h of exposure (H0–H4), as well as nasal obstruction using PNIF[22] and VAS rhinitis.[23] Finally, we measured the TNSS under three different conditions: NAC, EEC, and natural exposure.

The first part of the study consisted of a screening visit to obtain informed written consent to participate, and to conduct a medical history review, skin prick testing, and a blood draw for birch IgE. On the second visit, patients underwent NAC to birch allergen, and only those with positive NAC were selected to participate in the rest of the study. Participants who met the inclusion and exclusion criteria underwent 6 EEC exposures. First, participants attended two 4-h consecutive placebo EEC visits (Visits 3 and 4). After 7 days, participants attended two 4-h consecutive EEC allergen exposures (Visits 5 and 6), which were then repeated two additional times at 14 days intervals (Visits 7 and 8), to assess reproducibility and priming effect (Fig. 1). Exposures were conducted outside of the 2020 tree pollen season in France. The second part of the study involved field evaluations of allergic rhinitis signs and symptoms, and rescue medication use, during the peak birch pollen period. Participants completed the TNSS by the end of March–beginning of April 2021 (with some SARS-CoV-2 pandemic restrictions).

This study was approved by an independent ethics committee, and was conducted in accordance with Good Clinical Practice (GCP) standards, using the guidance documents and practices offered by the International Conference on Harmonization (ICH) and European directive 2001/20/CE. The study was registered at ClinicalTrials.gov under number NCT04583202.

Statistical methods

Statistical analysis was performed using SAS 9.4 software® (SAS Institute, Cary, NC). Statistical analyses were conducted according to the Statistical Analysis Plan (SAP) version 3.0 July 16, 2021. Since the intention-to-treat (ITT) and per protocol (PP) populations were similar, efficacy analyses were performed on the ITT population. Quantitative variables were described using the usual parameters (total number of data points, missing data, mean, median, minimum, maximum), as well as classical dispersion parameters (standard deviation and interquartile range for each group, period, and time). There were no reliable data regarding the reproducibility of symptoms in participants who developed a nasal reaction in the EEC. The number of participants required was determined pragmatically. We decided to include a total of 30 participants, with an expected nasal reaction proportion of 60%, and a two-sided 95% confidence interval ranging from 41 to 77%. Missing data were not replaced. Continuous variables were described as the number of

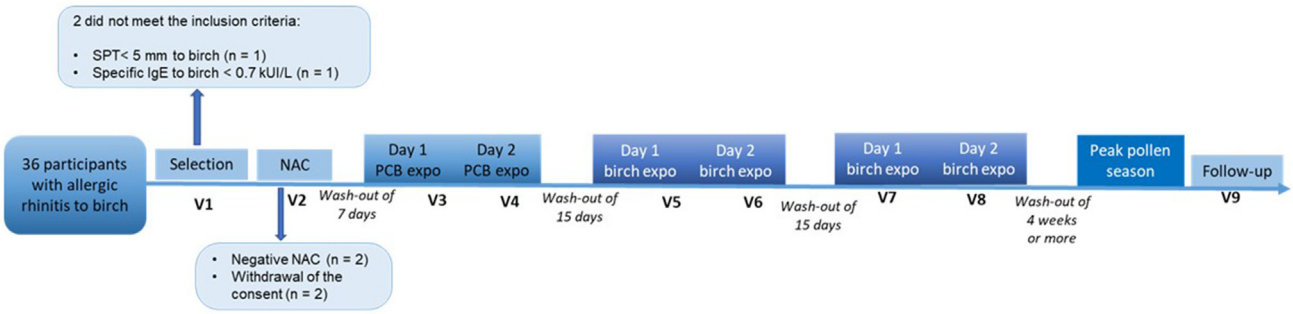

Fig. 1 Flow diagram and study design. Abbreviations: PCB, placebo; expo, exposure; NAC, nasal allergen challenge; SPT, skin prick test

observed data points, mean and standard deviation of normally distributed values, or median (interquartile range) for non-normally distributed values. Categorical variables were described as the number of participants and percentage in each category. For inferential statistics, *P* values of $< .05$ were considered significant. The priming effect was evaluated by the ratio of the scores for the presence of a nasal response at V5 and V6. The same calculation was performed between V7 and V8 for confirmation. Odds ratios and their two-sided 95% confidence intervals were estimated using a logistic model for repeated measures.

RESULTS

Study participants and nasal responses

Among 36 screened participants, 30 met inclusion eligibility, including positive NAC. The included participants were 50% male, and had a mean age of 33 years. They were mostly polyallergic with high sensitization to birch pollen, with no patient-reported or physician-diagnosed asthma (Table 1).

Nasal allergen challenge (NAC)

All participants had a positive nasal response at inclusion. The dose that most frequently induced nasal responses during NAC was 175.2 ng of Bet v 1 (dose D) for 2 puffs per nostril, corresponding to a total volume of 200 µL (Table 1). The mean ΔTNSS was 5.8 (±1.9), with a median of 6.0 (5.0; 8.0), and confidence interval of [5.01; 6.66]. PNIF revealed nasal obstruction in 63.3% of participants, with a mean decrease of 40.3% (±16.1) for dose D compared to baseline. The PNIF mirrored TNSS evolution. We found no correlation between the TNSS and PNIF values obtained with the NAC compared to the EEC.

EEC outcomes

TNSS

No participant reported positive nasal responses upon exposure to placebo (V3–V4). At V5, 18 participants (60%) had ΔTNSS ≥ 5 during the last 2 h of first allergen exposure (V5), whereas 21 (70%) had positive responses during all 4 exposures (Table 2). TNSS differed by 5 between both first allergen and placebo exposures, which was significantly different from 0 (paired Student's t-test $P < .001$) (Table 3). The mean corrected TNSS was 1 with placebo, compared to 6 with allergen. (Fig. 2, TNSS box-plot). The ΔTNSS area under the curve (AUC) values were 15.6 and 33.9 with 4-h placebo exposures. With allergen exposures, AUC was reproducible, with values of 887.1, 883.3, 933.9, and 842.8 at V5, V6, V7, and V8, respectively.

The median times to onset of rhinitis response were not calculated for visits V3 and V4 since no patient reached TNSS ≥ 5. At visit V5, the median time to onset of rhinitis response was 79.6 min (mean, 70 min). The priming effect was evaluated based on the odds ratio of the nasal reactions at V5 and V6. The same evaluation was performed between V7 and V8 for confirmation. The study did not reveal a priming effect, with *P* values of .28 between V5 and V6, and 0.86 between V7 and V8 (Table 4).

Rhinitis VAS

The rhinitis VAS results were similar to the TNSS evolution. For placebo exposures, the values remained less than 23 mm over time, with mean values of 1.9 and 1.5 mm. For allergen visits, the mean rhinitis VAS rapidly increased at around 30 min (Fig. 3c). Then the mean values decreased within 1 h, without returning to baseline values, following the trend of TNSS (Fig. 3a and c).

PNIF

During birch exposures, we observed a rapid decrease of PNIF during the first hour, followed by a more stable phase. The rapid decrease was by 35% at V5, and by 40% at V7 (Fig. 4). No subject experienced a PNIF decrease of $>10\%$ during placebo exposure. PNIF results mirrored the TNSS and rhinitis VAS results (Fig. 3b).

Phenotypes of rhinitis

Among the participants with nasal responses during V5, 56.67% had a dual response. These patients developed nasal symptoms, which then returned to baseline (showing an approximately 3-h window without nasal symptoms), followed by a second increase of nasal symptoms. Among them,

	N (ND)	30 (0)
Age, years	Mean (±SD)	33.07 (±10.2)
	Median (Q1; Q3)	33 (24; 38)
	(Min; Max)	(19; 60)
Sex	Male	15/30 (50%)
Ethnicity	Afro American	1/30 (3.3%)
	Caucasian	24/30 (80%)
	African descendant	3/30 (10%)
	Japanese	2/30 (6.6%)
Height, cm	Median (Q1; Q3)	171.5 (163; 179)
Weight, kg	Median (Q1; Q3)	69 (61; 80)
BMI, kg/m^2	Median (Q1; Q3)	23.7 (20.7; 25.6)
Birch prick test, mm	Median (Q1; Q3)	8.5 (7.50; 9.5)
Sensitization, 10 aeroallergens prick tests	Pauci-sensitized[a]	2/30 (6.6%)
	Poly-sensitized[b]	28/30 (93.3%)
	Pollen food allergy syndrome	3/30 (10%)
	Skin prick test to apple	19/30 (57%)
Birch specific IgE, kU/L	Median (Q1; Q3)	10.6 (5.8; 28.6)
PNIF male, L/min	Median (Q1; Q3)	130 (103.3; 223.3)
PNIF female, L/min	Median (Q1; Q3)	113.3 (90.0; 153.3)
FEV 1, L/min	Median (Q1; Q3)	3.8 (2.9; 4.3)
FEV 1/FVC, %	Median (Q1; Q3)	81.3 (78.4; 88.2)
NAC Bet v 1 dose	**NAC Δ TNSS**	
Dose A = 1.40 ng, 200 µL	Median (Q1; Q3)	0.0 (0.0; 1.0)
	95% confidence interval	[0.2; 1.3]
Dose B = 7.01 ng, 200 µL	Median (Q1; Q3)	1.0 (1.0; 3.0)
	95% confidence interval	[1.0; 2.7]
Dose C = 35.04 ng, 200 µL	Median (Q1; Q3)	2.0 (1.0; 3.0)
	95% confidence interval	[1.7; 2.9]
Dose D = 175.20 ng, 200 µL	Median (Q1; Q3)	6.0 (5.0; 7.5)
	95% confidence interval	[5.01; 6.6]
Dose E = 876 ng, 200 µL	Median (Q1; Q3)	6.0 (5.0; 8.0)
	95% confidence interval	[4.11; 9.5]
NAC Placebo	Median (Q1; Q3)	0.0 (0.0; 0.0)

Table 1. Study participants' demographic and baseline characteristics. *N population size; ND normal distribution, NAC Nasal Allergen Challenge, TNSS Total Nasal Symptom Score.* [a]*Pauci-sensitized: 2-3 allergens.* [b]*Poly-sensitized: >3 allergens*

Exposures	Nasal response	Results H2–H4	Results H0–H4
Birch allergen (V5)	Negative	12/30 (40.0%)	9/30 (30.0%)
	Positive	18/30 (60.0%)	21/30 (70.0%)
Placebo (V3)	Negative	30/30 (100.0%)	30/30 (100.0%)

Table 2. EEC primary outcome. *The frequency of nasal responses with placebo (V3) versus birch allergen (V5), were statistically significant ($p < .0001$). During the H2–H4 time period, no participants reported nasal response with placebo (V3), while 18 participants (60%) experienced a rhinitis response to allergen (V5). Similar results were found for the H0–H4 time period, with rhinitis responses in 21 patients (70%) exposed to allergen versus 0 with placebo*

19.05% had an EPR, 42.86% an LPR, and 9.50% a pEPR.

Natural exposure: peak pollen period

Patients who exhibited a positive nasal response during EEC were included in the second part of the study during the peak birch pollen season. Here, rhinitis severity was similar to that measured in the EEC (eFig. 1). The concentration of *Betula* was found to be 279 pollen grains/m^3 during the peak pollination (eFig. 1). The time spent outdoors during high pollination days was 0–4 h for over 50% of participants, and 4–8 h for about 20%. Participants also spent a maximum of 40% of their time indoors, firstly due to coronavirus pandemic restrictions (curfew after 6 p.m., and the wearing of face masks outside), and secondly due to worsening of symptoms during the first high pollination days (eFig. 2). However, 19 of 28 participants (67.9%) experienced a positive nasal response, with a mean TNSS of 5.8 in participants with or without antihistamine intake. Overall, the mean TNSS was 4.1 (±2.4) among responders with and without antihistamine intake. This did not significantly differ from the TNSS values obtained during the EEC visits, for either Visit 5 ($P = .07$) or Visit 7 ($P = .06$) (eFig. 3) (eTable 1).

Bronchial allergen responses

Among participants, 53.3% experienced an EAR and 16.6% a LAR in the EEC. The mean time to experience an EAR was 77 min (range 30–240 min).

	n	Statistics	Results
Delta corrected TNSS [V5–V3]	30	(paired) Student's t-test	<0.001 (S)
Delta [Birch (V5) – Placebo (V3)]	30	Mean (±SD)	5.0 (±3.14)
		Median (Q1; Q3)	5.5 (3.0; 7.0)
		(Min; Max)	(−1.0; 12.0)
Placebo exposure (V3)	30	Mean (±SD)	1.0 (±1.5)
		Median (Q1; Q3)	1.0 (0.0; 2.0)
		(Min; Max)	(−2.0; 4.0)
Birch exposure (V5)	30	Mean (±SD)	6.0 (±2.5)
		Median (Q1; Q3)	6.0 (4.0; 7.0)
		(Min; Max)	(1.0; 11.0)
Birch exposure (V7)	27	Mean (±SD)	6.15 (±2.7)
		Median (Q1; Q3)	6.0 (4.0; 9.0)
		(Min; Max)	(0.0; 11.0)

Table 3. Reproducibility of Nasal Responses in the EEC at Visit 5 (V5) vs Visit 7 (V7). *The mean corrected TNSS at Visit 3 (V3; placebo) was 1, while the mean at V5 (birch allergen) was 6. The delta between V5 and V3 is 5, which is significantly different from 0. These results were confirmed at V7 (birch allergen)*

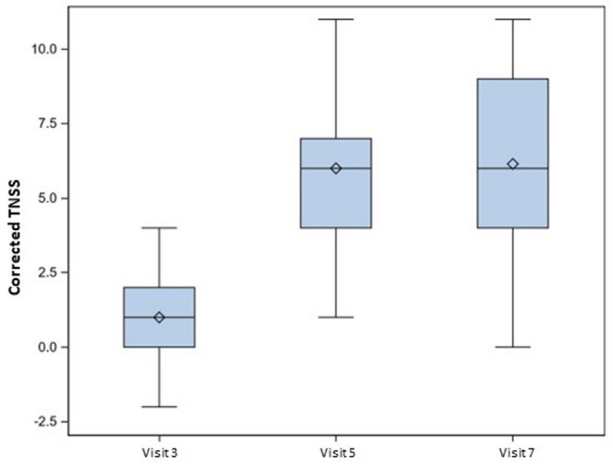

Fig. 2 Reproducibility of total corrected nasal symptom score (TNSS) between the first allergen visits versus placebo. Allergen exposure was reproducible, with a mean ΔTNSS of 5 for the primary outcome (Visit 5; birch allergen exposure) and a score of 6 for Visit 7 (allergen exposure), as compared to Visit 3 (placebo)

A dual asthmatic response occurred more frequently among patients with an early nasal response within 1 h (mean ΔTNSS = 5.7 ± 1.1).

DISCUSSION

In this study, among participants with positive responses during NAC at inclusion, over 60% exhibited allergic rhinitis symptoms elicited by 25 ng/m^3 of airborne Bet v 1 in the EEC. The same percentage of participants experienced symptoms during natural exposure. Under the three testing circumstances (NAC, EEC, and natural exposure), nasal responses were assessed using validated tools, including TNSS, VAS, and PNIF.

EEC exposures were reproducible in terms of nasal response frequency and severity. Participants showed no response with placebo, thus excluding eventual non-specific rhinitis. PNIF results showed an artificial drop of 10% in some participants, probably related to the device's self-assessment. Artificial drops can be minimized by selecting a threshold of a 40% decrease of PNIF,[22] which was confirmed at Visit 7. Nasal congestion was correlated with TNSS during Visit 7, but not during V5. The mean VAS rhinitis scores observed during these two visits reflected that the participants had mild-to-moderate rhinitis.[20]

The mean and maximum TNSS values obtained in the EEC were similar to those obtained during the peak pollen period, suggesting that EEC could replace field studies or be used as a complementary method in phase 2 or 3 investigations of birch allergic rhinitis. Notably, in 2020–2021, it was particularly difficult to perform clinical studies due to low concentrations of airborne birch pollen, and restrictions due to the coronavirus pandemic. Several authors[30–32] have demonstrated a clinical correlation between natural exposure to different allergens (ragweed, grass, and birch pollens, as well as cat allergens) and controlled exposure in an EEC, reinforcing the role and of EECs in the development of new therapeutics in the allergy field. However, Hohlfeld et al[31] did not find a correlation between TNSS values obtained in an EEC compared to during pollen season. They evaluated the specificity, sensitivity, and reproducibility of clinical end-points following exposures in an ECC and under natural conditions, in 60 adult patients with allergic rhinitis to grass pollen and 60 healthy participants. They found a good reproducibility of TNSS in the EEC (intraclass correlation coefficient = 0.86) and good sensitivity/specificity (AUC = 0.99) of all measures. Symptoms of seasonal allergen exposure also had good sensitivity/specificity but were far less reproducible. On the other hand, nasal flow had good sensitivity/specificity but its reproducibility was limited. In EEC trials, it is important to consider the method of clinical response assessment. Indeed, with a 4-h exposure to pollen, the severity of rhinoconjunctivitis symptoms increased during the first 2 h, followed by a 2-h stabilization allergen plateau. The efficacy of anti-allergic medications is often evaluated based on the changes in symptoms obtained during this plateau.[9]

Comparison	Odds ratio (n = 30)	P value	
V5 vs V6	0.7058 [0.3753; 1.3272]	.280	(NS)
V7 vs V8	1.0495 [0.6234; 1.7666]	.856	(NS)

Table 4. Priming effect. *The priming effect was evaluated based on the odds ratio for the nasal response at V5 and V6, then at V7 and 8. Odds ratios were estimated using a repeated measures logistic model. The study did not reveal a priming effect (P value of .28 and .86 respectively)*

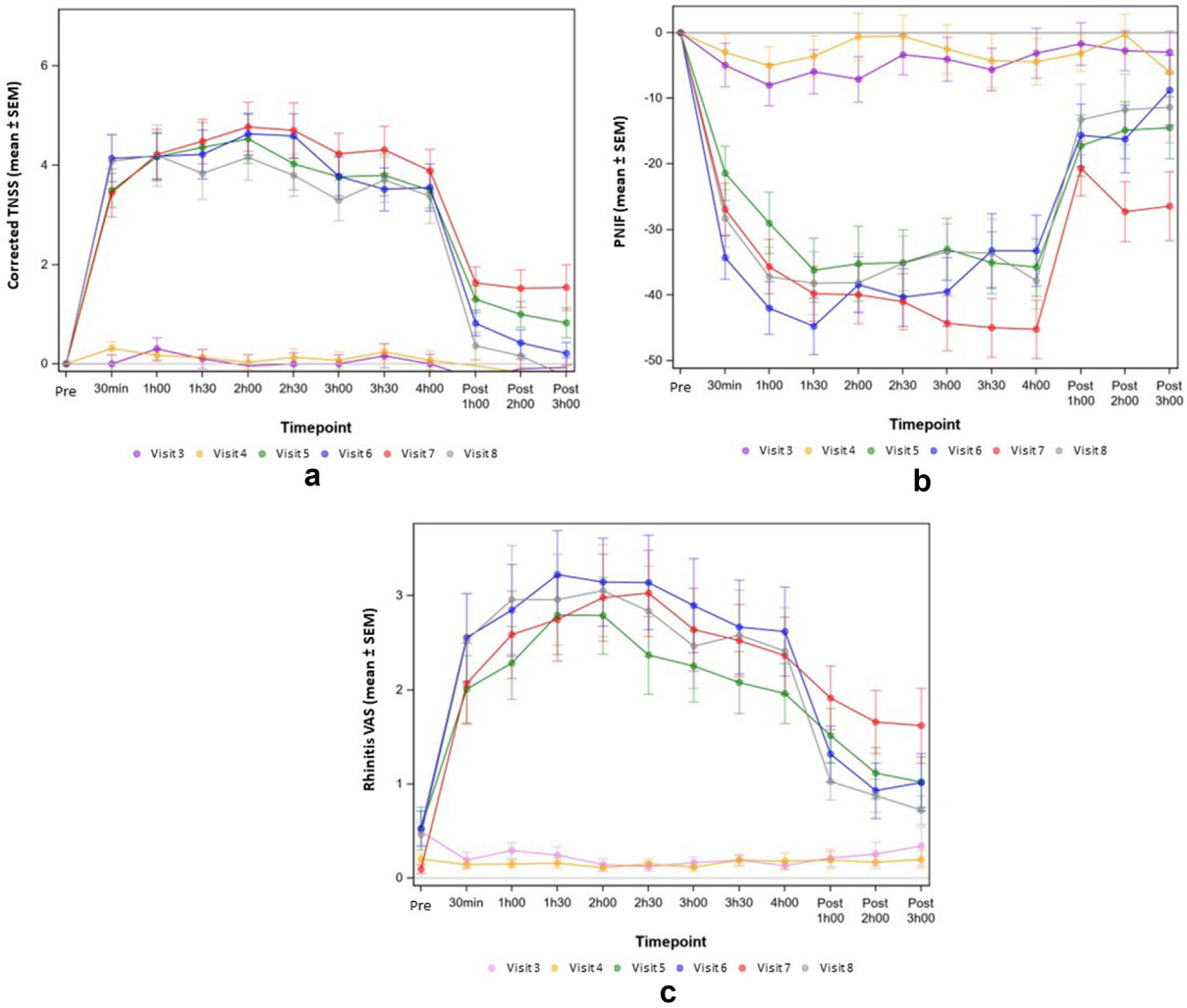

Fig. 3 a. Kinetics of TNSS among positive responders in the EEC. TNSS and corrected TNSS were visually comparable. During placebo, corrected TNSS was stable and close to 0. During allergen exposures, TNSS increased in the first hour, and remained stable until the end of the exposure, then rapidly decreased post-exposures. b. Kinetics of the decrease of PNIF in the EEC. During placebo, there was no decrease in PNIF. During allergen exposures, PNIF decreased quickly in the first hour, and remained stable until the end of the exposure. There was a good correlation between AUC TNSS and PNIF (r = 0.6). c. Kinetics of rhinitis VAS at each EEC exposure. The VAS evolution follows the trends of TNSS. During placebo (V3 and V4), VAS remained close to 0. For allergen exposure V5 to V8, the mean rhinitis VAS rapidly increased to around 3 and rapidly decreased post-exposure

Another recent study was designed to compare the EEC and NAC methods, and revealed strong correlations between clinical responses and the immunological responses, such as changes in IL-5 and IL-13. Additionally, gene expression changes in local tissues correlated with the clinical and immunologic responses. NAC and EEC were not correlated with each other regarding symptom magnitude.[18] We obtained the same results in terms of symptom magnitude.

The dose of Bet v 1 inducing nasal responses during NAC was dramatically higher than those measured in the EEC or field (eTable 1). This suggests that the EEC is closer to natural exposure, as compared to NAC. Overall, all of these studies that have compared different methods of inducing allergic rhinitis responses and allergen exposure levels support the usefulness of EEC in drug development investigation, including allergen immunotherapy.[33,34]

The priming effect was first described with ragweed pollen by Connell.[35] It is currently accepted that the pathophysiology of allergic rhinitis involves an immediate and late response. The delayed response is associated with cellular

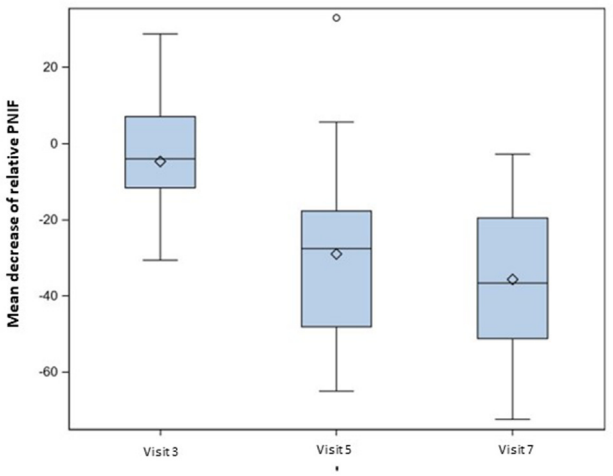

Fig. 4 Decrease of peak nasal inspiratory flow (PNIF) at Visit 3 (placebo), Visit 5 (V5; birch allergen), and Visit 7 (V7; birch allergen). The mean decrease of relative PNIF was around 35% at V5 and 40% at V7, as compared to the baseline

infiltration, which involves eosinophils and lymphocytes as important components and entails nasal mucosa hypersensitivity, increasing the response following exposure to allergens (eg, priming).[35,36] This concept has been adopted in EEC studies. EEC-induced priming effects can be quantified in the onset and duration of action protocols to assess, for example, 24-h coverage of a once-daily antihistamine. A previous study examined the effect of antihistamines in patients with grass pollen allergy, 2 h after the start of each 6-h EEC session. The results of this study revealed no difference in the clinical efficacy of treatment when administered during or out of the pollen season.[4] Recently, Ellis et al demonstrated an important priming effect in a study of birch allergic rhinitis.[32] However, another recent trial did not confirm the priming effect of either grass or birch pollen, among persons with allergic rhinitis and mild asthma.[37] Studies of allergic conjunctivitis have suggested that the priming could be a manifestation of a late reaction recorded within 24 h.[16,38] Additionally, a grass rhinitis study,[31] found no correlation between the TNSS evoked at the end of a 4-h grass pollen exposure in the EEC and symptoms registered during the pollen season. However, they observed a good correlation with the TNSS registered 24 h after the challenge. The authors concluded that TNSS after 24 h better reflects the late-phase reactions occurring during natural exposure. Therefore, in the present study, we carefully monitored the time to onset of the rhinitis response, and of spontaneous recovery after birch allergen exposures. More than 50% of participants exhibited a late-phase reaction, allowing us to study different rhinitis phenotypes.

The priming effect was not observed in our present study, with fewer birch allergen exposures at a 14-day interval. The same results were reported in the previous trial, with no evidence of a priming effect with 5 exposures on consecutive days to 4000 grass pollen grains/m^3, and no symptom increase after prolonged exposure.[39] When priming effects are observed, they do not necessarily influence the overall symptoms, but rather contribute to more rapid symptoms on day 2.[33]

An approach to studying asthma is lacking or not detailed in EEC-based studies of rhino-conjunctivitis. It is well known that birch allergy mainly induces rhino-conjunctivitis. However, it has been reported that 42% of birch-sensitized patients also have asthma.[40] In our analysis, we observed discordance between participant-reported symptoms and physician-diagnosed asthma, suggesting that birch-related asthma is underestimated among patients with allergic rhinitis. Birch allergen induced a dual bronchial response in over 50% of asthmatic patients. This result is comparable to previous findings in mite asthmatic patients, suggesting that birch pollen could be as asthmogenic as mite allergen.[40]

A previous study was designed to validate the same EEC for studies of conjunctivitis with 60 ng/m^3 airborne birch allergen,[16] and the main inclusion criterion was a unitary conjunctival allergen challenge. The primary outcome showed conjunctival responses in over 50% of participants based on the total ocular symptoms score (TOSS) during the first day of EEC exposure, followed by 70% on the second day. In the present study designed to examine rhinitis, we did not obtain a statistically significant ocular response in either the EEC (data not shown) or during natural exposure.

The limitations of this study include its small sample size. Importantly, EEC studies enable the attainment of data with small sample sizes. Additionally, the field results were impacted by missing data on the natural exposure, as well as unusual

conditions for time spent outdoors due to pandemic restrictions. In some patients, rhinitis scores could have been impacted by antihistamines intake as compared to EEC exposures, where antihistamines were prohibited (eFig. 3).

This EEC is a validated facility for studying allergic rhino-conjunctivitis related birch pollen, under controlled conditions, and at allergen levels significantly lower than those involved in unitary allergen challenges. The severity of nasal symptoms in the EEC is similar to those experienced during the pollen season. In the present study, we demonstrated that the symptoms evoked in the EEC were comparable with those observed during individual NAC and seasonal real-life exposure. This suggests that EECs could be used as a surrogate for natural allergy season exposure and NAC in allergic rhinitis clinical trials.

Abbreviations

DRs, Dual responders; EAACI, European Academy of Allergy and Clinical Immunology; EAR, Early asthma response; EEC, Environmental exposure chamber; EPRs, Early-phase responders; FEV1, Forced expiratory volume in 1 s; IgE, Immunoglobulin E; LAR, Late asthma response; LPR, Late-phase responders; NAC, Nasal allergen challenge; pEPRs, Protracted early-phase responders; PNIF, Peak nasal inspiratory flow; TNSS, Total nasal symptom scores; TOSS, Total ocular symptoms score; VAS, Visual analogue scale.

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Ethics approval

This study was approved by an independent ethics committee (references: EC Ouest V: 19/002-2; Registration number: 2018-A03107-48), and was conducted in accordance with Good Clinical Practice (GCP) standards, using the guidance documents and practices offered by the International Conference on Harmonization (ICH) and European directive 2001/20/CE. The study was registered at ClinicalTrials.gov under number NCT04583202, and included protective measures against SARS-COV-2 according to the French health regulatory administration. Participants signed informed consent.

Authors contributions

All authors reviewed the manuscript and consent for publication. AG conceived and designed the study, provided supervision, interpreted the data and drafted the manuscript; FD provided supervision and interpreted the data; MB provided supervision and interpreted the data. DN conceived and designed the study, interpreted the data. FdB conceived and designed the study and interpreted data.
Authors' consent for publication in WAO.

Declaration of competing interest

Alina Gherasim, Frank Dietsch, and Marine Beck are ALYATEC employees. Nathalie Domis is an ALYATEC co-founder and employee. FdB is the medical expert, co-founder, and shareholder of ALYATEC. Frederic de Blay reports grants from ASTRAZENECA, CHIESI, GSK, REGENERON, NOVARTIS, and STALLERGENES GREER, and personal fees from ALK ABELLO, ASTRAZENECA, CHIESI, MENARINI, NOVARTIS, and STALLERGENES GREER.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2023.100801.

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