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Birch allergen challenges in allergic conjunctivitis using unitary tests and an exposure chamber

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Abstract

Background: Environmental exposure chambers (EECs) have been used extensively to study allergic rhinoconjunctivitis. Few studies have been published using EECs in conjunctivitis only, and none have used conjunctival allergen challenge as a selection criterion. The present study validated ALYATEC EEC in allergic conjunctivitis to birch allergens. Methods: Sixteen patients with a positive conjunctival allergen challenge (CAC) were ex-posed to 60 ng/m3 of Bet v 1 in an EEC on 2 consecutive days for a maximum of 4 hours. Re-producibility was tested among seven of the patients. A positive conjunctival response during the CAC and the EEC exposure was defined as a Total Ocular Symptom Score (TOSS) [?] 5. Results: Fifty percent of patients had a positive conjunctival response during the first expo-sure and 75% during the second. The mean time to a positive conjunctival response was 81.2±33.9 minutes and 101.6±57 (P>0.05) during the first and second exposure, respectively. No difference in the TOSS occurred between the two exposures. The time necessary to ob-tain a positive response during the CAC was significantly shorter than with the EEC. The es-timated quantity of Bet v 1 inducing a positive response was 0.07±0.03 ng (exposure 1), 0.07±0.07 ng (exposure 2), 980±784 ng (CAC). The frequency of conjunctival responses and quantity of Bet v 1 was reproducible in all six EEC exposures. Conclusions: Birch allergen exposures inducing early conjunctival responses were different than those identified with direct installation during CAC. EEC appears to be closer to natural exposure than CAC.

Birch allergen challenges in allergic conjunctivitis using individual tests and an exposure chamber

Short title: Allergen challenges in conjunctivitis to birch

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Abstract

Background: Environmental exposure chambers (EECs) have been used extensively to study allergic rhinoconjunctivitis. Few studies have been published using EECs in conjunctivitis only, and none have used conjunctival allergen challenge as a selection criterion. The present study validated ALYATEC EEC in allergic conjunctivitis to birch allergens.

Methods : Sixteen patients with a positive conjunctival allergen challenge (CAC) were exposed to 60 ng/m^3 of Bet v 1 in an EEC on 2 consecutive days for a maximum of 4 hours. Reproducibility was tested among seven of the patients. A positive conjunctival response during the CAC and the EEC exposure was defined as a Total Ocular Symptom Score (TOSS) [?] 5.

Results : Fifty percent of patients had a positive conjunctival response during the first exposure and 75% during the second. The mean time to a positive conjunctival response was 81.2+-33.9 minutes and 101.6+-57 (P>0.05) during the first and second exposure, respectively. No difference in the TOSS occurred between the two exposures. The time necessary to obtain a positive response during the CAC was significantly shorter than with the EEC. The estimated quantity of Bet v 1 inducing a positive response was 0.07+-0.03 ng (exposure 1), 0.07+-0.07 ng (exposure 2), 980+-784 ng (CAC). The frequency of conjunctival responses and quantity of Bet v 1 was reproducible in all six EEC exposures.

Conclusions : Birch allergen exposures inducing early conjunctival responses were different than those identified with direct instillation during CAC. EEC appears to be closer to natural exposure than CAC.

(245 words)

Key words : birch allergy, conjunctivitis, conjunctival allergen challenge, environmental exposure chamber

ABBREVIATIONS

Bet v 1 Betula verrucose major allergen

CAC Conjunctival allergen challenge

EAACI European Academy of Allergy and Clinical Immunology

EEC Environmental exposure chamber

Expo Exposure

FEV1 Forced expiratory volume in one second

GCP Good Clinical Practice

GINA Global Initiative for Asthma

ICH International Conference on Harmonization

IgE Immunoglobulin E

TOSS Total Ocular Symptom Score

TNSS Total Nasal Symptom Score

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Introduction

Allergic conjunctivitis occurs in atopic individuals exposed to specific antigens and manifests as an early reaction, within minutes or hours of exposure to allergens, and may or not be associated with allergic rhinitis.[1] IgE-mediated hypersensitivity inducing conjunctivitis is frequent, from 15-20% in general practice[2, 3] to 40% in the US population when examined in an ophthalmological survey.[4]

In France, the prevalence of seasonal allergic rhinoconjunctivitis (SAR) was found to be 13% among 10-year-old children and approximately 20% among adults.[5] Birch is one of the most frequent sources of allergens that induce rhinoconjunctivitis.[6]In the general French adult population, the prevalence of birch sensitization was 4.7%.[7] A relationship between pollen counts and the occurrence of symptoms was shown recently.[8] The frequency of the ocular response to natural exposure to birch pollen in sensitized patients was linear until birch daily average concentrations reached a plateau of 110 grains/m^3, with a cutoff of ocular symptoms at 70 grains/m^3 at the beginning of the season.[9]

In 1990, Abelson *et al* demonstrated a correlation between skin sensitization (grass pollen, ragweed pollen, and cat allergen) and positivity to conjunctival allergen challenge (CAC). This tool confirms allergen involvement in the diagnosis of allergic conjunctivitis, allowing precise selection of the patients in clinical studies. The CAC model is the only clinically validated method recognized by the Food and Drug Administration (FDA) for testing the efficacy of eye anti-allergic molecules.[10] The practical aspects were described in a position paper of the European Academy of Allergy and Clinical Immunology (EAACI) Task Force.[11]

Environmental exposure chambers (EECs) have been in development since 1985 to study new therapeutics for allergic pathologies, including conjunctivitis.[12, 13] EECs have the advantage of achieving reproducible and safe exposure with controlled levels of allergen for several hours in several subjects simultaneously by avoiding confounding factors during exposure.[14, 15]The ALYATEC EEC has been validated in mite and cat-induced asthma.[16, 17] These studies demonstrated that the allergen exposures are standardized with an inter-test coefficient of variation of less than 30%.

To validate the ALYATEC EEC with birch pollen, we exposed patients affected by seasonal allergic conjunctivitis caused by birch pollen to airborne birch allergen. We investigated the amount of Bet v 1 and the time necessary to induce a conjunctival response in at least 50% of patients. We also evaluated the reproducibility of the exposures.

Material and methods

Patients

Patients aged 18 to 65 years were selected for eligibility based on having a history of >2 years of moderate allergic conjunctivitis during birch pollen season.[1, 11]Allergic sensitizations were documented by a positive skin prick test to birch allergen with a wheal diameter [?] 6 mm compared to negative control and positive birch-specific IgE (> 0.10 kU/l). The main inclusion criterion was a positive conjunctival response during an individual conjunctival allergen challenge.[10, 11] The study was performed outside the pollen season in France. A 7-day washout period was required for topical or systemic anti-histamines or other ophthalmic treatment. Exclusion criteria were evaluated prior to inclusion and were as follows: patients experiencing a single ocular symptom in the previous week; patients who received long-acting corticosteroids within the past 4 weeks; ocular laser treatment within the past 3 months; ocular surgery within the last 6 months; abnormality or clinically significant ocular disorder, including symptoms of allergic conjunctivitis; ongoing immunotherapy to any allergen or, within the last 5 years, to birch allergen.

Interventions

This was an open, single-center study designed to determine the concentration of airborne Bet v 1 inducing an allergic conjunctivitis response in patients allergic to birch pollen during allergen exposures to birch pollen extracts in the EEC. During the first screening visit, the patient gave informed written consent and underwent the following procedures and assessments: medical history review, skin prick testing to birch pollen

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allergen (ALK-*Abello(r)*), and a blood draw for specific IgE to birch (*Betula verrucose,* Phadia ImmunoCap, Thermofisher(r)). The second screening visit was for an individual conjunctival allergen challenge (CAC) to birch allergen. All responders in the CAC were included in the present study.

The CAC was performed according to the updated EAACI guidelines.[1, 11] This procedure consisted of the instillation of 20 µl of diluted birch allergen extract (100 IR lyophilized extract, Stallergenes Greer®) in the inferior-external quadrant of the bulbar conjunctiva in incremented dilutions at 10-min intervals: 3, 6, 12, 25, 50, and 100 IR/ml.[11] The clinical response was assessed by the Total Ocular Symptom Score (TOSS) with the same cumulated positivity criteria for the CAC and EEC exposure. If the TOSS was <5 at 10 minutes after each instillation, the test was considered negative. The next concentrated dose was then instilled until a positive response was reached.

Step 1 of the study consisted of two consecutive EEC exposures to the same birch pollen extract (Expo 1 and Expo 2). The primary endpoint of the study was met when 50% of the patients were positive. The main judgment criteria were the amount of Bet v 1 inducing a positive conjunctival response after EEC exposure. In addition, we compared the intensity of the clinical response induced in patients exposed to birch pollens using the TOSS and the mean time to reach a positive ocular response (i.e., TOSS [?] 5). In step 2, we studied the reproducibility of the allergen exposure. Patients who responded to Expo 1 and Expo 2 were enrolled in step 2 and exposed two additional times on 2 consecutive days (from Expo 3 to Expo 6). Each double EEC exposure test was separated by 7 days.

Clinical assessments

The TOSS was used during both the CAC and EEC exposures. This score was first described[10] as the sum of four conjunctival symptom scores: itching, redness, chemosis, and tearing (range: 0-13). It was evaluated after instillation of the allergen on one side, with the other eye serving as a negative control after instillation of the physiological serum. A slit lamp examination was used to score redness and chemosis only. Itching was assessed by the patient using a 5-point severity scale from 0 (none) to 4 (very severe: incapacitating itch with irresistible urge to rub), with 1=mild (intermittent tickling sensation), 2=moderate (continual awareness but without the desire to rub), and 3=severe (continual awareness with the desire to rub). For ocular redness, ratings were collected for the nasal and temporal area of each eye and averaged by the study physician using a 4-point severity score (0=absent, 1=mild, 2=moderate, 3=severe). Tearing was also rated by the physician using a 4-point severity score (0=absent; 1=mild, eyes feel slightly watery; 2=moderate, blows nose occasionally; and 3=severe, tears rolling down cheeks). Chemosis was rated by the study physician as follows: 0=absent, 1=mild (detectable with slit lamp, conjunctiva separated from sclera), 2=moderate (visually evident, raised conjunctiva, especially at the limbal area), and 3=severe (ballooning of conjunctiva). The patient left the EEC when the mean TOSS of both eyes was [?] 5.

Safety monitoring of pulmonary function was performed by clinical survey. The Total Nasal Symptom Score (TNSS) and portable spirometry were performed every 20 minutes during exposure. Early asthma response was defined as a drop in the forced expiratory volume in 1 second (FEV_1) of 20%. Patients with this asthma response during allergen exposures were discharged. At the end of an exposure, all patients were treated as needed with topical antihistamines, eye drops, and oral second generation H1-antihistamines according to the persistence and severity of the conjunctival or rhinitis symptoms. When an early asthmatic response occurred, patients remained under supervision for 6 hours. Thereafter, they were discharged with a rescue therapy kit containing oral antihistamines, topical mast cell stabilizers, and short-acting beta 2 agonist inhaler.

This study was approved by an independent ethics committee and was conducted according to 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 NCT04641130.

Environmental exposure chamber

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The ALYATEC EEC is a new generation EEC located in Strasbourg, France. Its capacity and allergen exposure conditions are described elsewhere.[16, 17] In this study, patients were exposed to the same batch of lyophilized birch allergen GMP extract (100 IR;*Stallergenes Greer* (r)), diluted in saline serum, as the one used for the CAC. Before the patients entered the EEC, the exposure was initiated in order to reach a plateau of airborne birch allergen and then maintained for a maximum of 4 hours. A homogeneous allergen concentration was ensured by using particle counters and online measurements of the temperature, relative humidity, and air exchange as described previously.[16, 17] The birch allergen was collected on five glass fiber filters located next to the patients' chairs during exposure to determine the Bet v 1 concentration using ELISA (Indoor Biotechnologies(r), Charlottesville, VA, USA), after each allergen exposure. The concentration of the Bet v 1 airborne exposure was estimated to be 60 ng/m^3. During EEC allergen exposure, the conjunctival response was assessed every 10 minutes during the first hour and then every 20 minutes.

Quantity of Bet v 1 (Q) inducing a conjunctival response

As the allergen affected the ocular surface, we used the tear film renewal rate (TRR) to estimate the amount of extract applied during EEC exposure. The TRR was calculated to be 154x10^{6}mm^3/min by Beaudouin et al.[18] Thus, the quantity (Q) of extract applied was calculated in nanograms as follows: Q = C x Time x TRR, where C is the concentration of airborne Bet v 1 in ng/m^3 and Time is the time required to induce the conjunctival response in minutes.

Statistical analysis

All statistical analyses were performed using SAS$^{(r)}$guide Enterprise software (SAS Institute, Cary, NC, USA). Missing data were not replaced. Continuous variables were described as the number of observed data, mean and standard deviation of normally distributed values, or median (interquartile range). Categorical variables were described as the patients' size and percentage in each category. For inferential statistics, P-values < 0.05 were considered significant. Tests were two-tailed. Reproducibility was tested using a Pearson correlation between exposure days.

Results

Patients

Among 23 screened participants, 16 met the inclusion criteria and performed two consecutive EEC exposures. Eight patients who responded at Expo 2 were included in step 2 (Figure 1). Patient characteristics are given in Table 1. Roughly, sensitization to birch pollen was evidenced by the skin prick test wheal diameter of 7.4 +- 1.6 mm and specific IgE value of 61.3 +- 109.3 kUI/L for the total study population. Co-sensitizations were frequent, as only one patient was mono-sensitized to birch pollen. Approximately 90% of patients had concomitant rhinitis, and 43% presented with asthma according to GINA 1 classification.

Conjunctival outcomes during CAC and EEC exposures (step 1)

Among the 16 patients included in step 1, 12 presented a conjunctival response during allergen exposure in the EEC during Expo 1 or Expo 2. In the EEC, the mean TOSS was 5.7 +- 0.8 in Expo 1 and 5.5 +- 0.6 in Expo 2, whereas the mean TOSS during the CAC was 6.2 +- 1.1. No correlation was observed between the TOSS in the EEC versus CAC (r= 0.05). The maximal TOSS was 9 for both types of exposure (CAC and EEC). The mean time needed to obtain a positive conjunctival challenge was not significantly different between Expo 1 (81.2 +- 33.9 min) and Expo 2 (101.6 +- 57 min). During the CAC, positivity was obtained in 36 +- 15 min. The estimated quantity of Bet v 1 inducing a conjunctival response was 980 ng in the CAC and 0.07 +- 0.03 ng during Expo 1 and 0.07 +- 0.07 ng during Expo 2. This level was significantly lower with the EEC than the CAC (Table 2).

Reproducibility of EEC exposures (step 2)

The eight patients included in step 2 had identical characteristics to the whole cohort from step 1 (Table 1). One patient dropped out of the study before the last exposure and was not analyzed here. This patient left the EEC before reaching a positive TOSS due to an early asthma response. Among the seven remaining patients,

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all except one exhibited a positive conjunctival response to the entire course of six designed exposures (Expo 1 to Expo 6; Table 3). The clinical response was identical throughout the six exposures in terms of TOSS. Moreover, the time necessary to reach TOSS [?] 5 was <2 hours for all exposures. The amount of Bet v 1 inducing a positive conjunctival response was similar in all six exposures. Reproducibility was studied regarding the time and quantity of allergen necessary for a positive challenge. Time exposition was highly reproducible (Table 3) with a Pearson correlation coefficient of 0.78 ($p<0.05$) between Expo 1 and Expo 4. As for the quantity of allergen inducing a positive response, reproducibility was also assessed between Expo 1 and Expo 4 with a Pearson correlation coefficient 0.81 ($p= 0.028$).

Kinetics of ocular symptoms after exposure in the EEC

Time of onset and intensity of each symptom in the seven patients following step 2 are reported in Figure 2. During all six exposures, redness was the first symptom to appear, with a mean time of 16 +- 6.8 min, reaching maximum intensity in 55 +- 20.2 minutes. Tearing and itching appeared second, with a mean of 25 +- 3.4 min and 35 +- 16.9 min, respectively. Thus, reproducibility of redness, tearing, and itching occurred in 100% of individuals completing all six exposures. Chemosis was observed in Expo 4, 5, and 6 in six patients with a mean time of 28 +- 16.3 min after the patient entered the EEC. The maximum TOSS was 9 and occurred in three patients. No severe conjunctivitis was induced during exposure. All observed ocular reactions were considered mild and were controlled with a topical rescue treatment.

Nine patients (60% of the patients included) had a positive nasal response on the first two exposures (Expo 1 and Expo 2), confirmed by a positive TNSS [?] 6 with a mean TNSS of 8.17 +- 1.47. This mean score was reached in 61 min. Five out of 16 patients developed an early asthma response during allergen exposure, with a mean decrease in FEV_1 of 21.9% in 65 min on average (min 30 minutes; max 100 min). All early asthma reactions were treated by inhaled short-acting beta-2 agonist. Among patients presenting an early asthmatic response in the EEC, only one had a late asthmatic response, which was treated by oral corticosteroids and inhaled beta 2 agonist. No prolonged observation period was needed. No severe asthma reactions were observed during the study. No patients used the emergency kit provided during the test.

Discussion

Different clinical studies have assessed the effect of allergen exposure in EECs on rhinoconjunctivitis, but very few have focused on allergic conjunctivitis. The time course of allergic signs and symptoms differed between the CAC and EEC sessions. In a previous study evaluating 13 patients with a history of ragweed allergy who underwent CAC and EEC exposure, the response time was different but the intensity of the maximal response was similar.[19] In the present study, when comparing ocular symptoms of patients exposed to birch pollen in the EEC compared to the reference CAC, we achieved the primary endpoint of 50% positivity in 16 patients during the first exposure. The following day, 75% of patients had a positive response. Furthermore, the airborne concentration of birch pollen inducing the response was very low, reaching a mean 60 ng/m^3 of airborne Bet v 1 in the ALYATEC EEC.

The main inclusion criteria were a positive CAC, which is considered the gold standard for objectively evaluating conjunctival reactivity to a specific allergen at the mucosal surface. We chose a TOSS [?] 5 during the CAC as the threshold for a positive conjunctival response according to European guidelines.[11] This threshold has been demonstrated to allow a specificity and sensitivity of 100% and 90%, respectively, in mite allergic conjunctivitis.[20] We used the same clinical positivity criteria for EEC exposure. After EEC exposure, the mean TOSS was not significantly different after CAC and EEC exposure. The maximal TOSS was 9 in both the CAC and EEC. In contrast, the time necessary to obtain a positive response was significantly longer in the EEC than the CAC. To the best of our knowledge, studies have not reported time between natural exposure and the occurrence of ocular symptoms. Patients do not describe significant conjunctivitis symptoms in day-to-day life within 30 minutes after being exposed to birch pollen. Consequently, the duration to obtain a significant clinical response to birch pollen in the EEC appeared to be closer to natural exposure than after a CAC. Moreover, the quantity of birch allergen inducing a positive conjunctival response was dramatically different between these two exposures. During CAC, positive responses were obtained with

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a mean cumulative dose of 980 ng of Bet v 1, whereas it was calculated to be 0.07 ng with the EEC. According to the HIALINE study,[21] the amount of Bet v 1 per pollen grain can vary from 3.2 to 32 pg. Therefore, 980 ng of Bet v 1 corresponds to approximately 30,000 to 300,000 pollen grains. In the EEC, the amount of Bet v 1 inducing a positive response corresponds to 2 to 21 pollen grains. The literature assumed that patients allergic to pollen had symptoms as soon as the pollen grain threshold reached 22 to 30 grains/m^3 for grass pollens[22] and 70 grains/m^3 for birch pollen.[9] Even though the manner of exposure is different, the results of natural and EEC exposure are similar, whereas challenge of the ocular surface through CAC exposes the individual to a much greater amount of allergen. Moreover, exposure in the CAC is performed through diluted allergen in physiological serum instilled onto the ocular surface, whereas in EECs the allergens are nebulized in the air, which is a modality closer to naturel exposure. This triangular comparison enhances the clinical significance of the EEC challenge.

The positive conjunctival response in three-fourths of patients during the second exposure in the EEC suggests that a priming effect occurs. However, we did not observe a difference in the severity of the TOSS between Expo 1 and Expo 2. Jacobs et al.[22] suggested that no priming effect exists when exposures are performed on 2 consecutive days and that the conjunctivitis reaction that occurred on the second day may be a simple manifestation of a late phase reaction captured within a 24-hour period after exposure.[23]Prior studies suggested that two, or even three, priming visits may be required to obtain high levels of symptoms.[24-25]However, the priming effect leads to rapid onset of symptoms and signs rather than a greater allergic response.[26]

We observed reproducibility of ocular response frequency during all exposures. Reproducibility was assumed when challenging the ocular surface in the EEC for the time and the quantity of allergen inducing a clinical reaction.

Symptoms and signs induced in the EEC were comparable to those induced by CAC. Ocular redness was the first sign to appear in the EEC and lasted until the end of exposure. Its reproducibility was consistent across six allergen exposures. Our findings were in line with Jacobs et al.,[22] who investigated phenotypes of allergic conjunctivitis. Other clinical symptoms of conjunctivitis, such as tearing and itching, occur rapidly. The kinetics of the appearance of the three main signs and symptoms is the same as in real life. Chemosis has also been associated with allergic conjunctivitis. When mild or moderate, chemosis requires slit-lamp examination, which was used in both CAC and EEC exposure. Chemosis was not observed in our patients who submitted to CAC but was mild during EEC sessions. We observed good reproducibility of the kinetics of conjunctival symptoms: ocular redness, tearing, and ocular itching followed by chemosis. The latter can be considered a sign of severity, occurring when conjunctivitis was clearly present. The mild intensity of the chemosis when it was observed enhances the safety aspect of EEC exposure. The absence of loco-regional symptoms, such as rhinitis, is another argument that reinforces the safety of the technique. Nevertheless, in five patients, we observed mild asthma symptoms during EEC exposure. This makes it possible to enroll mild asthma patients with allergic conjunctivitis when investigating ocular allergy. A control of spirometry parameters before, during, and after EEC challenge remains necessary.

The limitation of this study is the small number of patients. However, we could demonstrate the clinical validity and good reproducibility of the method. The estimation of the amount of allergen deposition on the ocular surface could be discussed, but the calculation took into account the different physiological factors involved in the pathophysiology of conjunctivitis.

Conclusion

We demonstrated that exposure to 60 ng/m^3 of Bet v 1 in the ALYATEC EEC induces conjunctival responses in more than 50% of patients with birch allergic conjunctivitis. Birch allergen exposures inducing early conjunctival responses were different than those identified with direct instillation during CAC, demonstrating that EECs more closely mimic a natural exposure during high pollinating days.

(2482 words)

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AUTHORS CONTRIBUTIONS

All authors reviewed the manuscript. GA provided supervision, interpreted the data, and drafted the manuscript. dBF conceived and designed the study, and interpreted the data. DN conceived and designed the study, and interpreted the data. NB provided clinical study supervision and interpreted the data. JLF provided supervision and reviewed the manuscript. SX provided the statistical analysis.

CONFLICTS OF INTEREST

GA and NB are ALYATEC employees. DN is cofounder and ALYATEC employee. dBF is medical expert, cofounder, and shareholder of ALYATEC. dBF reports grants from STALLERGENES GREER, grants from CHIESI, and personal fees from ALK ABELLO, MUNDIPHARMA,

NOVARTIS, and REGENERON and is a member of the Board at STALLERGENES GREER, NOVARTIS, ALK ABELLO, MUNDIPHARMA, MEDAPHARMA, BOEHRINGER, ASTRAZENECA, and CALOR. JLF: none

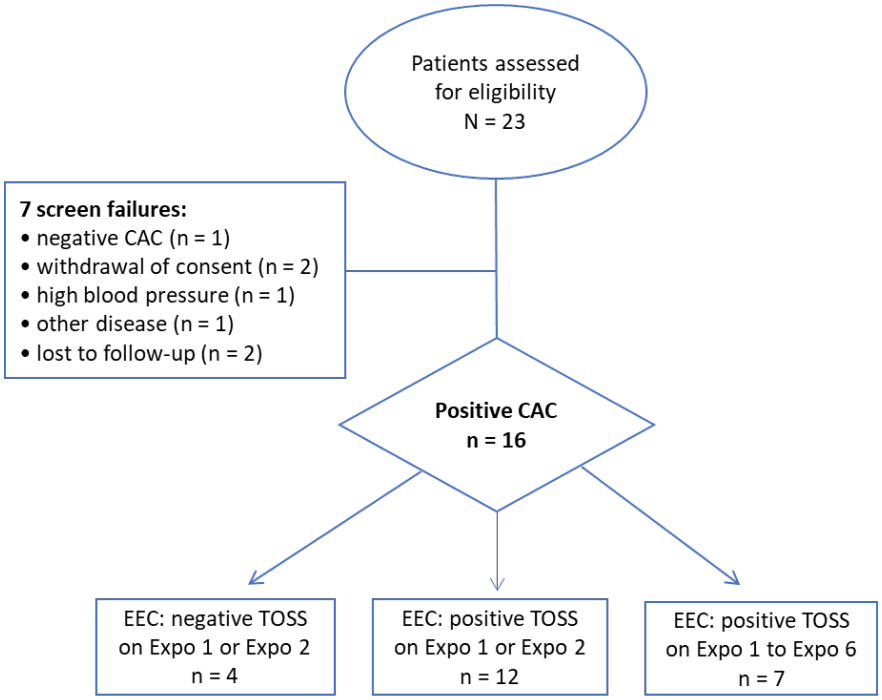

Figure 1. Flow diagram of patient inclusion

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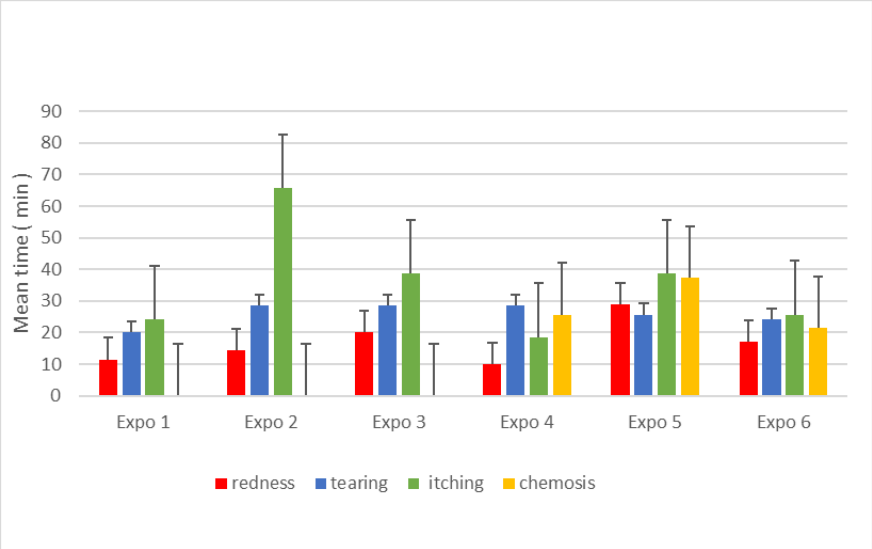

Figure 2a. Mean time to TOSS occurrence after beginning EEC challenge (n=7).

Time in minutes; Error bars are presented as standard deviation (SD)

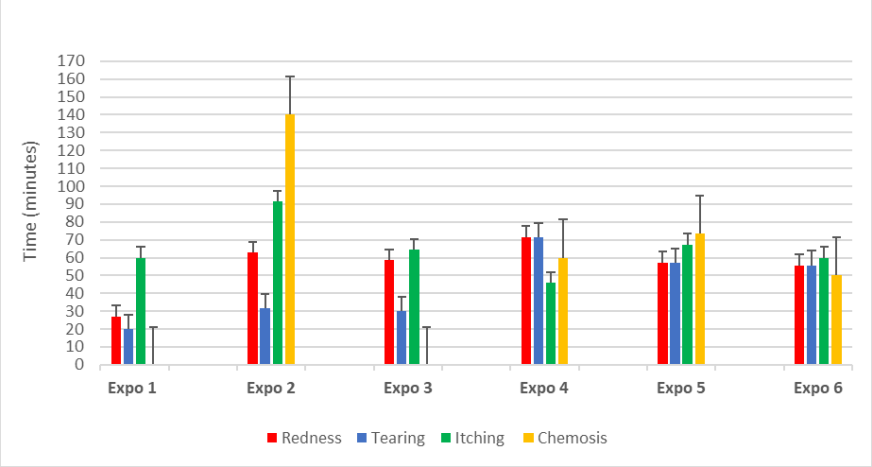

Figure 2b. Time to reach maximal intensity of TOSS during exposures (Expos) in the EEC (n=7). In Expo 2, only one patient experienced mild chemosis.

Time in minutes; Error bars are presented as standard deviation (SD)

	STEP 1	STEP 2
Number of patients	16	8
Age, years	26.4 ± 6.8	26.6 ± 7.5
Gender (male)	10 (62.5%)	5 (62.5%)
Skin prick test to birch, mm	7.4 ± 1.6	7.7 ± 1.6
Birch specific IgE, kU/I	61.3 ± 109.3	54.6 ± 114.1
Allergic comorbidities Positive skin prick test	1/16 (6.2%) +	0/16 (0%)

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	15/16 (93.7%) ++	16/16 (100%)
Asthmatic patients	9/16 (57%)	8/16 (50%)
Mean predictive FEV1, %	103.7 ± 9.6	101.63 (7.1)
CAC Provocative dose of Bet v 1, ng	507.5 ± 392.2	385 ± 349
CAC cumulative dose of Bet v 1, ng	980.0 ± 784.5	735.0 ± 698.0 §

Table 1. Study population

Data are given as mean ± standard deviation or n (%). +Mono-sensitization to birch; ++ Poly-sensitization; SS CAC cumulative dose for 8 patients

Positivity threshold TOSS [?] 5	CAC N=16	EEC Day 1 N=8 +	EEC Day 2 N=12 ++
TOSS units	6.2 ± 1.1	5.7 ± 0.8 n.s.	5.5 ± 0.6 n.s.
TOSS [Min; Max]	[0; 9]	[0; 7]	[0; 7]
Time until positivity, min	35 ± 15.06	81.2 ± 33.9 p<0.05	101.6 ± 57.3 p<0.05
Cumulative dose of birch allergen exposure, ng	980 ± 784.5	0.07 ± 0.03 p<0.001	0.07 ± 0.07 p<0.001

Table 2. Positive conjunctival responses after CAC and EEC.

Data are given as mean ± standard deviation unless otherwise noted. Step 1 (EEC versus CAC) n=16. +Exposure 1: 8 out of 16 patients; ++ exposure 2: 12 out of 16 patients had a positive response in the EEC. EEC = environmental exposure chamber; CAC = conjunctival antigen challenge; TOSS = Total Ocular Symptom Score; n.s. = not significant.

Positivity threshold TOSS [?] 5	Expo 1	Expo 2	Expo 3	Expo 4	Expo 5	Expo 6
TOSS Scoring units	5.7 ± 0.8	5.5 ± 0.6	5.8 ± 1.2	5.7 ± 1.1	6.8 ± 1.5	6.3 ± 1.9
TOSS Scoring Units [Min; Max]	[0; 7]	[0; 7]	[0; 8]	[0; 8]	[0; 9]	[0; 9]
Time until positivity, min	68.3 ± 25.6	91.4 ± 62.03*	77.1 ± 34.9	82.8 ± 31.4 *	72.8 ± 28.1	65.7 ± 21.4
Cumulative dose of birch allergen exposure, ng	0.06 ± 0.02	0.08 ± 0.06**	0.07 ± 0.03	0.08 ± 0.03**	0.07 ± 0.03	0.06 ± 0.02

Table 3. Reproducibility of results with EEC exposure in seven patients (step 2)

Data are given as mean ± standard deviation unless otherwise noted. EEC = environmental exposure chamber; Expo = exposure; TOSS = Total Ocular Symptom Score. * P = 0.045, **P =

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