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ABSTRACT

Background. Field clinical trials of pollen allergy are impacted by the impossibility to predict and to determine individual allergen exposure, due to many factors (e.g., pollen season, atmospheric variations, pollutants, lifestyles). Environmental exposure chambers (EEC), delivering a fixed amount of allergens in a controlled environmental setting, can overcome these limitations. EECs are currently already used in phase 2, 3, and even 4 trials. Unfortunately, few chambers exist in the world, and this makes it difficult to perform large, multicenter clinical trials. The new GA²LEN mobile exposure chamber is a step forward, since the mobility of the chamber makes it convenient for patients to participate in clinical testing.

Objective. This study was made to validate the reproducibility, sensitivity, and specificity of the results obtained in the new GA²LEN chamber.

Methods. Seventy-two adult patients (19–61 years), with allergic rhinitis with/without asthma due to grass pollen, were included in different clinical validation tests. Total Symptom Scores (TSS) and Total Nasal Symptom Scores (TNSS) were recorded at time zero (0), and every 10 minutes during exposures, along with nasal and respiratory parameters.

Results. Exposure tests confirmed the reproducibility between subsequent runs, the sensitivity ($p < 0.00001$ vs. patients exposed to placebo) and the specificity (very low score in nonallergic subjects) in the GA²LEN chamber. No adverse reactions were recorded during the tests.

Conclusions. The mobility of the GA²LEN chamber provides a new, potentially effective, and safe way of generating reliable data in allergy multicenter clinical trials.

Clinical implications: Environmental exposure chambers are increasingly used in allergy studies, but have some limitations. The use of a mobile exposure chamber can represent an advantage in clinical studies of allergen immunotherapy and pharmacotherapy.

Capsule summary: The novel GA²LEN chamber has been technically and clinically validated in grass-allergic patients to allow multicenter trials in allergy with a mobile environmental exposure chamber.

Key words: *Allergy trial, Environmental exposure chamber, validation, grasses, placebo, GA²LEN*

114 ABBREVIATIONS

115 ARIA: Allergic Rhinitis and its Impact on Asthma

116 AIT: Allergen Immunotherapy

117 EAACI: European Academy of Allergy and Clinical Immunology

118 EEC: Environmental Exposure Chamber

119 EMA: European Medicine Agency

120 FeNO: Fractional Exhaled Nitric Oxide

121 FEV1: Forced expiratory volume in 1 second

122 GA²LEN: Global Allergy and Asthma European Network

123 PEF: Peak Expiratory Flow

124 PNIF: Peak Nasal Inspiratory Flow

125 SAR: Seasonal allergic rhinitis

126 SEM: Standard Error of Mean

127 TNSS: Total Nasal Symptom Scores

128 TSS: Total Symptom Scores

129 VAS: Visual Analogue Score

130

INTRODUCTION

Field allergy clinical trials depend on natural pollen exposure and are therefore biased by a number of factors, the most important being the variability of the pollen seasons, the changing environmental conditions, and the uncertainty in determining the actual individual exposure.¹⁻³ This is especially true in multicenter trials, where pollen counts and environmental conditions can be very different from one site to another. These aspects are well summarized by Bousquet et al.⁴ In multicenter studies there is also a risk of excluding some patients from the final analysis due to the lack of relevant pollen exposure in one area.⁵ These problems can be overcome by the use of Environmental Exposure Chambers (EEC).^{2,6,7} Currently, however, EEC are accepted by European Medicine Agency (EMA) and the U.S. Food and Drug Administration only for phase 2 dose-ranging studies⁸ even though the use of EEC facilitates proper control and standardization of the quantitative allergen exposure. Further advantages include shortening the time frame of the dose-finding process, avoiding the confounding effects of rescue medications, and using smaller numbers of subjects than would be required in natural-exposure studies.⁶ EEC have been previously used for evaluating the efficacy of pharmacotherapies for seasonal allergic rhinitis,⁹⁻¹¹ both in phase 2 and still ongoing in phase 3 and even in phase 4 trials¹² and studies have also employed EEC in Allergen Immunotherapy (AIT) investigations.¹³⁻¹⁵ A recent position paper from the European Academy of Allergy and Clinical Immunology (EAACI) states that “EEC is likely to be a good option as an adjunct to natural exposure studies for phase 3 Randomized Controlled Trials,” also in AIT studies.¹⁶

There are, however, drawbacks of the existing stationary EECs. Very few such facilities exist around the world³ and this makes it very difficult to perform multicenter clinical trials.⁷ The reproducibility of allergen exposure and treatment outcomes between different EECs is also a potential problem.¹⁷ Furthermore, pollen in the stationary EECs are reventilated by a strong airflow, prohibiting the measurement of individual exposure or a placebo control within a run, and being potentially irritating for eyes and nose.

To overcome these problems, GA²LEN has developed a novel mobile EEC, called GA²LEN chamber. The new mobile GA²LEN chamber has been technically and clinically validated according to the current needs and requirements as described in this paper.³

TECHNICAL VALIDATION

The GA²LEN chamber consists of two standard 24 feet high cube containers forming a compound of 7.45 m × 4.90 m × 2.86 m. One container accommodates an office and changing room (A), while the other (B) container houses the test chamber itself and the installations for air conditioning (Fig. 1). When set up, the containers are interlocked, forming an interconnected compound, allowing access to the test chamber through the changing room. Even though the GA²LEN chamber consists of two containers, it can be transported by a single truck equipped with a crane. To ensure the GA²LEN chamber can be operated at a maximum number of trial sites, minimal requirements are necessary for the onsite setup, which requires only external electricity (or a generator). The test chamber can accommodate up to nine patients plus a nurse or a doctor, providing chairs which are recommended for a seating time up to 12 hours, with adjustable footrests and surfaces that can be sanitized. Each chair has its own tablet holder. The chairs are at fixed positions, but can be removed for full cleaning (Fig. 2).

The airflow in the test chamber was designed to be as low as possible, meaning not recognizable, at each seating position to not create any adverse reactions due to the airflow (e.g., feeling of nasal obstruction or eye reddening), but still create sufficient air exchange to keep CO₂ and O₂ levels at comfortable levels. Nevertheless, the air condition installed allows the chamber temperature to be adjustable from 15°C to 35°C while relative humidity levels can be set from 30% to 80% (noncondensing). Gas levels—namely, oxygen, carbon dioxide, carbon monoxide, and ozone—are constantly monitored and recorded. The outside air is filtered using a multistage filter system, removing more than 99% of particles smaller than 1 µm (and all allergens) from the air.

Due to the air condition capability and the extensive insulation, experiments can be performed at outside temperatures from -10°C to 35°C, while outside relative humidity can be handled from 20% to 80%. The test chamber is monitored by cameras and microphones. This allows for the doctors and nurses to also monitor the patients remotely throughout the test procedure while being located in the office room of the GA²LEN chamber. If agreed to by signed consent, the visual and audio data are also recorded. These recordings can be used to validate symptom scores, such as, number of sneezes.

Particle Exposure

Due to the limited seating available in the exposure chamber, a fast turnaround time—meaning, the time from ending one test until being able to start another test—is necessary to enable several test runs within a day. The major issue contributing to the turnaround time is the necessity to run the cleaning protocol after each test which ensures equal conditions for each test run. In order to reduce the cleaning time, the GA²LEN chamber is not flushed with pollen, which would result in pollen

sticking to every surface in the room, including ceiling, walls, etc. In the GA²LEN chamber, pollen are only dispersed where the subjects are seated and the chamber airflow is designed so that no other surfaces can be reached by the pollen, except the subject chairs, tablet holders, the floor directly beneath the subjects, and the subjects themselves. Therefore, the subjects are required to wear clean-room apparel to minimize the number of pollen adhering to the patient when leaving the chamber. Wearing clean room apparel also prevents other aeroallergens from being introduced into the chamber. The floor is laid out as a double flooring, so pollen reaching the floor will fall through a metal grid, which is the upper floor. Pollen falling through the grid will then be either sucked through the exhaust air outlet, and then removed by filtering the air, or will stick to the lower surface. The airflow design in the chamber prevents the pollen from refloating. Not only does this approach allow for faster back-to-back testing, it also allows for individualized testing, especially for research purposes where there are smaller cohorts but multiple differently parametrized exposures. The GA²LEN chamber dispersal system is designed to have a dedicated particle dispersal unit (PDU) placed above each chair, with its nozzle positioned in a way that the particle concentration in the breathable air of a patient being seated underneath is constant in a distinct region. This region is thoroughly confined, so that the particles dispersed from one PDU are not able to be inhaled by a patient at another seat.

The height of the chair is adjusted when a patient is seated, so that the nostrils of the patient are at a defined level, called calibrated exposure height (CEH). The breathable air is defined as the 2 L of air surrounding the upper airway organs during each breath intake. Since the patient may move around (not moving the chair but moving the upper body), the breathable air is actually defined as an ellipse of 40 cm by 30 cm around the nose and mouth at CEH. The PDU takes particles from a blister and disperses these particles through a nozzle. A blister in this case is made of two sealed stripes with a single layer (height) particle line in between. The width of the line is controlled with narrow margins in the blistering process. Before dispersing, each particle is photographed and counted. Each image is stored for later quality assurance tasks. Due to previously individually performed calibration, the defined and required particle concentration in the breathable air of the patient can easily be controlled by the speed the blister is emptied. A PDU can generate a particle concentration from 100 particles per cubic meter breathable air up to 15,000 particles per cubic meter during a time frame of up to 8 hours.

CLINICAL VALIDATION

Patients and methods.

All tests were performed in Berlin, Germany, outside the relevant pollen season, using Phleum pratense (Allergon AB, Ängelholm, Sweden) as grass species. Reproducibility tests were also performed after the chamber had been disassembled, transported to Barcelona, and back. Based on power analysis altogether 72 adult patients (19–61 years), males and females (40% and 60%, respectively), were included in the different validation tests. Inclusion criteria were history of mild to moderate allergic rhinitis (SAR) with/without asthma due to grass pollen for ≥ 2 years, skin prick test ≥ 3 mm and/or ImmunoCAP ≥ 2 , and FEV1 $\geq 70\%$ predicted value. Exclusion criteria included previous treatment with AIT, treatment with systemic corticosteroids within the last month, treatment with antihistamines or other drugs with antiallergic properties within the last 3 days, and suspicion of pregnancy. Five control patients were nonatopic. Patients were exposed during different lengths of time (90, 120, and 240 minutes) to two different pollen concentrations (4,000 and 8,000 pollen grains/m³) and placebo. Since 4,000 and 8,000 grains concentrations gave comparable results (Fig. 3), we report here only the outcomes of exposures to 4,000 grains/m³.

Scoring system. Patients recorded eye (itching, foreign body sensation, redness, watering), nose (itching, sneezing, running, blocked), and bronchial (wheezing, shortness of breath, cough) symptoms. For every symptom a score of 0 to 3 was applied (none, mild, moderate, and severe). Total Symptom Score (TSS) is the sum of all three scores eye/nose/lung (minimum 0, maximum 36). Total Nasal Symptom Score (TNSS) is the sum of the four nasal symptoms (minimum 0, maximum 12). Subjective parameters were recorded at time zero (0), and every 30 minutes during exposure. Visual Analogue Score (VAS), Peak Nasal Inspiratory Flow (PNIF), and Peak Expiratory Flow (PEF) were also recorded before entering the chamber, after acclimatization in chamber (10–30 minutes, time zero), and every 30 minutes during exposure.

Statistical methods

Primary analysis is based on area under the curve (AUC) of values based on per-protocol population (PP). The statistical model is based on an assumption of data having a symmetrical distribution (optimally a Gaussian distribution), and hence the log AUC values are expected to be better suited for the analysis than the arithmetic AUC values. This was confirmed by analysis. As some AUC values are zero, values of $\log(\text{AUC}+1)$ are used; an approximate back transform from a $\log(\text{AUC}+1)$ value y is given by $x = (\exp(y)-1)/120$, where x corresponds to a slightly weighted average of TNSS over the 13 time points (time 0–120). Aggregated levels of values are analyzed as the values of the $\log\text{AUC}_{120}$, calculated by the traditional trapezoidal formula. The active effects are estimated and tested using a mixed ANCOVA model where covariates include age, gender,

baseline score, number of the run, and subjects. All analyses are programmed using SAS version 9.3.

Ethical conduct of the study. The study was approved by the ethical committee of the University Hospital Charité, Berlin, and all patients provided written informed consent according to the Helsinki declaration.

RESULTS

Reproducibility. The reproducibility of symptoms evoked in the EEC is one of the crucial points for the clinical validation.³ Eighteen allergic subjects were exposed two times in the mobile chambers to 4,000 pollen grains/m³ for 90 minutes, at intervals of 1 to 5 weeks between visits. VAS and PNIF were recorded every 30 minutes. Both VAS (average maximum score first visit = 2.34, second visit = 2.52) and PNIF (average maximum score first visit = 136 L/min, second visit = 151 L/min) results were comparable between the two exposures (Fig. 4). There was no change in pollen distribution and exposure observed after disassembling, moving, and reassembling the chamber.

Comparison between placebo and active run. A total of 101 subjects took part in a placebo and active run. Seventy-seven subjects were exposed to 4,000 grass pollen grains/m³ for 120 minutes. Active grass allergic subjects reached a mean maximum TSS of 5.2 ($\pm$ standard error of mean [SEM] 0.4) and TNSS 3.8 ($\pm$ SEM 0.2) at 70 minutes. By comparison, 24 placebo subjects reached a mean maximum TSS of 2.6 ($\pm$ SEM 0.4) at 70 minutes and TNSS 1.7 ($\pm$ SEM 0.3) at 70 minutes (Fig. 5 for TNSS). The differences between TSS and TNSS levels recorded after exposure for 120 minutes to 4,000 grains/m³ of grass and placebo were highly significant ($p < 0.00001$). Significant differences were also observed in PNIF values and in nasal secretion between grass-exposed and placebo-exposed subjects ($p < 0.0001$).

No symptoms in nonatopic subjects. The specificity of the EEC exposure was demonstrated in a test where five nonatopic subjects were exposed for 120 minutes to 4,000 grass grains/m³ and compared to 36 allergic patients. Subjects recorded the TNSS, the Ocular Score, and the Bronchial Score every 10 minutes, according to the previously described scoring system (minimum 0, maximum 36). None of the nonatopic patients reached a TSS value of 1 (Fig. 6).

No effect of priming. Six patients were exposed for 120 minutes to 4,000 grass pollen grains/m³ on five consecutive days. TSS was recorded every 10 minutes and VAS every 30 minutes. No difference was detected in either outcome between day 1 and day 5 (Fig. 7).

Plateau reached. Seventeen patients were subjected to a longer exposure, up to 240 minutes. A plateau of TSS and TNSS was reached after 70 minutes with a peak at 90 minutes and no further increase at up to 240 minutes. Average peak values at 90 minutes were 5.4 (min–max 1–14) for TSS and 4.1 (min–max 1–8) for TNSS (Fig. 8).

Safety. All the exposure tests were safe and well tolerated. No clinically significant adverse reactions were observed during the EEC exposure, and no rescue medication had to be provided. No late reactions were reported after 24 hours.

DISCUSSION

Clinical tests performed in the new GA²LEN mobile chamber showed high sensitivity, specificity, and reproducibility. Allergic patients showed a highly significant response compared to patients exposed to placebo, while nonallergic patients showed no response in terms of TNSS. VAS values observed in our validation study indicate that patients included in this study could be classified as having “mild to moderate rhinitis,” according to the ARIA Guidelines,¹⁸ as stated by Bousquet et al.¹⁹ This is important since the difference to placebo is in general statistically less pronounced than in severely affected patients.²⁰ TNSS values observed in our study are comparable with those reported by Hohlfield et al.²¹ with the same scoring system and at the same pollen concentration. A minimum placebo response was observed in the placebo patients. This has been observed before³ and it is well known that the placebo effect is particularly relevant in allergy trials.^{22,23} To avoid subjective biases, we assessed the reproducibility in the GA²LEN chamber with subjective and objective parameters, VAS and PNIF, in two subsequent visits.²⁴ VAS has been found to correlate with the disease severity and to detect with high sensitivity the variations of symptoms in allergic rhinitis.^{25,26} PNIF measurement is a very effective tool for assessing the severity of congestion in allergic rhinitis,^{27,28} and nasal flow has also been evaluated by Krug et al. for the validation of the Fraunhofer chamber.²⁴ In our study both VAS and PNIF showed comparable results between the two subsequent exposures. Similar results have been observed in the past with stationary chambers. Hohlfield et al. exposed 60 grass pollen-allergic patients to 4,000 grains/m³ in the Fraunhofer ECC on two occasions out of the pollen season, with 2-week intervals. TNSS was highly reproducible between the two exposures.²¹ Interestingly, VAS values reported by Hohlfield et al. at the end of a 4-hour exposure are comparable with the VAS scores observed in our study, and were also highly reproducible. Krug et al., still in the Fraunhofer ECC, found comparable results between five consecutive exposures evaluating TNSS, nasal flow rate, nasal secretion, FEV1, and peak expiratory flow, with a grass pollen concentration of 8,000 grains/m³ for 4 hours. In this study a significant but not clinically relevant reduction was observed in the mean FEV1, indicating a priming effect.²¹ All these results confirm that symptoms elicited in consecutive exposures in the GA²LEN mobile chamber have the same reproducibility of symptoms provoked in the classic stationary EECs. In contrast to previous reports,^{3,24,29} we did not observe any priming effect with objective endpoints in six patients challenged on five consecutive days. This can be due either to the small number of studied patients, the shorter exposure, or lower pollen grains exposure compared to the Krug study¹⁷ (2 vs. 4 hours; 4,000 vs. 8,000 pollen grains/m³) or to the lack of a seasonal exposure, which has been deemed relevant in previous publications.^{6,29}

One of the key questions regarding EEC is whether symptoms evoked in the chambers are comparable to those observed during seasonal real life exposure.^{3,24,30} Two studies addressed this particular point. Jacobs et al. found a strong correlation between nasal and ocular symptoms reported during the natural ragweed exposure and those evoked in an EEC.³¹ By contrast, Hohlfield et al. found no correlation between the TNSS evoked at the end of a 4-hour grass pollen exposure in the EEC and symptoms registered during the pollen season.²¹ However, a good correlation was found with the TNSS registered 24 hours after the challenge. The authors concluded that TNSS after 24 hours better reflect the late-phase reactions occurring during natural pollen exposure.¹⁹ The correlation between the symptoms in the chamber and natural allergen exposure suggests that EEC can be used as a surrogate for the natural allergy season exposure in clinical trials.¹⁷ Field pollen allergy clinical trials suffer from some biases, the most important being the variability of the real allergen exposure of every study site and every single subject included in the studies.^{3,7} Seasonal and local variations (humidity, temperature, local specific pollutants, different concentrations of major allergens in the pollen) can have a big influence on the pollen counts and symptoms. Furthermore, the real exposure heavily depends on the lifestyle of the subjects (indoor/outdoor) and the indoor environment exposures.^{1,3} Another problem is the difficulty of correctly defining the pollen season, with its peak and duration being very different from one year to another and differing by locations.⁷ Furthermore, the classic inclusion criteria in clinical trials (skin tests, allergen-specific IgE, clinical history) are not able to distinguish patients with different levels of symptoms, leading to the inclusion of patients with very low symptom scores, another possible bias cause.^{30,31} Screening in an EEC, as done before,⁶ could overcome this issue by guaranteeing minimal symptoms with exposure according to entry criteria.^{1,2,17} For these reasons, while EMA already accepts the use of provocation tests (nasal, bronchial, conjunctival, or exposure chambers) for phase 2 and dose-finding studies in AIT,⁸ the already mentioned EAACI position paper on clinical outcomes in allergen immunotherapy considers the EEC also a promising vehicle for phase 3 clinical trials in AIT.¹⁶ Actually, a number of controlled phase 3 and 4 studies are ongoing with antiallergic drugs using exposure chambers.¹² Compared to the traditional provocation tests, the EEC allows the testing of many patients at the same time in a more natural way and, above all, records all of the elicited organ symptoms, as happens in the natural exposure. Furthermore, there are also important financial issues. The high costs of drug development are mainly based on the costs of the clinical trials and shorter trials are needed. The novel EEC technology has a potential to perform trials more efficiently than field trials as less patients need to be included under standardized conditions. Unfortunately, very few such chambers today exist in the world, and comparability studies of individual EEC facilities are lacking due to the great geographic distances

between individual pollen chambers and the specific technical features of each EEC facility.^{16,17}

These issues make the required multicenter clinical studies very difficult to perform. Thus, the mobile GA²LEN chamber represents a unique and novel solution to explore the biology of diseases of the airways and potential therapeutic interventions utilizing multiple clinical trial sites to assure diversity of populations necessary to better define study endpoints.

FIGURE LEGENDS**Figure 1.**

The GA²LEN Mobile Exposure Chamber.

Figure 2.

The GA²LEN Mobile Exposure Chamber: interior layout. Numbers refer to patients seats.

Figure 3.

Comparison of response to 4,000 and 8,000 grass pollen grains/m³ exposure in allergic subjects.

TNSS = Total Nasal Symptom Score; n = 16 patients.

Figure 4.

Reproducibility of EEC challenges in subsequent exposures. Eighteen allergic subjects were exposed two times to 4,000 pollen grains/m³ for 90 minutes, at intervals of 1 to 5 weeks between visits. A: VAS Scores; B: PNIF Scores. VAS = Visual Analogic Score; PNIF = Peak Nasal Inspiratory Flow.

Figure 5.

Comparison of response to grass pollen and placebo exposure in allergic subjects. TSS = Total Symptom Score; n of patients: grass pollen 55, Placebo 30; exposure: 4,000 grass pollen grains/m³. ($p < 0.0001$).

Figure 6.

Comparison of response to grass pollen exposure in allergic subjects and nonallergic controls. TSS = Total Symptom Score; n° of patients: 36 allergic subjects, 5 nonallergic controls; exposure: 4,000 grass pollen grains/m³.

Figure 7.

No evidence of priming effect in five exposures in consecutive days. TSS = Total Symptom Score; n = 6 patients; exposure: 4000 grass pollen grains/m³.

Figure 8.

No symptom increase after prolonged exposure. TNSS = Total Nasal Symptom Score; n = 17 patients; exposure: 4,000 grass pollen grains/m³.

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