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Clinical Validation of a house dust mite environmental challenge chamber model

Short title: Validation of mite allergen challenge

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Author contribution:

PZ, PL, RZ designed the study and drafted the manuscript, PZ, PL, CK, RS, RZ participated in the acquisition, analysis and interpretation of the data, PZ, PL, CK, RS, RZ, FH, OP and SV participated in the revision of the paper and gave final approval for submission.

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Capsule summary:

In this dust mite allergen challenge chamber model a reproducible correlation between allergen concentration and nasal symptoms has been demonstrated. Retest reproducibility of nasal symptoms measured by TNSS and decreased nasal flow quantified by RMM at three different occasions was demonstrated. Inter-chamber variability of nasal symptoms and nasal flow between two different chamber rooms (VCC 2 and VCC3) was determined to be 2.5% (SD 0.2%) and 3.3% (SD of 1.2 and 2.3) respectively. Chamber exposure in this model was safe in mite allergic subjects with controlled asthma.

Unstructured abstract:

Mite allergy causes significant socioeconomic burden. Environmental allergen exposure can be difficult to assess, and topical challenge offers limited value. In our investigation we evaluated a challenge chamber system in terms of correlation between mite allergen concentration and rhinitis symptoms, retest reproducibility as well as inter chamber variability. Data was obtained from adult mite allergic subjects from 4 different studies. Total nasal symptom score (TNSS) data was analysed for dose finding. TNSS and active anterior rhinomanometry (RMM) data was analyzed for retest reproducibility and inter chamber variability. Allergen dose delivered and allergenic profile were specified, for dose quantification particle counting and weighing were correlated. In 85 subjects dose response was defined with five challenges of increasing allergen concentration and six challenges of stable allergen concentration. A clear dose response was shown with a mean TNSS of 6.6 at 0.3g mite material. For evaluation of retest reproducibility TNSS and RMM of 158 placebo-treated subjects were compared. Curves were matching with a mean plateau TNSS of 6.7 and a mean flow decrease of 34%. For definition of inter-chamber variability 28 subjects were challenged in two chamber systems. A mean TNSS without "nasal blockage" of 3.9 in VCC2 and of 4.0 in VCC3 with a SD of 0.2% each and a mean reduction in nasal flow sum of 36.3% in VCC2 and 39.6% in VCC3 with a SD of 1.2 and 2.3, respectively, was determined.. No bronchial adverse events were observed. Thus, a clear and reproducible dose response in terms of mite allergen concentration was demonstrated. Similar symptoms scores were elicited between studies and between chambers. We conclude that the mite challenge chamber model offers a safe and reproducible option for investigation of mite allergic subjects.

Key words: Vienna challenge chamber (VCC), house dust mite allergens, allergic rhinitis, validation

Abbreviations used:

ELISA enzyme linked immunosorbent assay

FEV1 forced expiratory volume in 1 second

HDM house dust mite

NSS nasal symptom score without nasal discharge

OD: optical density

RMM active anterior rhinomanometry

SD standard deviation

SLIT sublingual immunotherapy

SPT skin prick test

TNSS Total Nasal Symptom Score

VCC Vienna Challenge Chamber

To the editor:

House dust mites are one of the most prevalent indoor allergen sources, and dust mite allergy imposes a considerable socioeconomic burden. It is associated not only with atopic dermatitis, but also with persistent allergic rhinitis and allergic asthma¹. Even today exposure to airborne house dust mite (HDM) can be difficult to assess². Allergen exposure chambers are clinical facilities allowing exposure of subjects to a controlled allergenic environment^{3,4}. They have contributed to some extent to our understanding of the pathophysiology of respiratory allergic diseases and the pharmacological properties of new therapies⁵. Some challenge chambers work with a mite model, but not all are using crude material. Lueer et al.⁶ recently described the use of extracted mite material in their pilot study. They showed reproducible induction of allergic rhinitis symptoms in 18 patients with mite allergy, but without providing information on the allergenic profile of the material used or demonstrating a clear dose response.

The aims of our investigation were to, (1) determine a reproducible correlation between HDM allergen concentration and Total Nasal Symptom Score (TNSS) in adult mite allergic subjects, (2) to demonstrate retest reproducibility at different time points, and (3) interchamber variability when exposing the same subjects to dust mite allergens in two different chamber systems (VCC2 and VCC3). VCC2 hosts up to 12 subjects, in VCC3 up to 20 subjects can be seated. In both systems allergenic particles are dispensed by outlets in the ceiling and exhausted at floor level without recirculation. For further technical description of the systems see methods section and **table E1** in this article's **Online Repository**.

Data were obtained exclusively from (n) placebo- or untreated volunteers of the clinical studies described below.

Trial 1 (carried out in VCC3) (n=85) was an allergen dose finding study with 6 hour challenges. Trial 2 (carried out in VCC3) (n=39) was a double blind placebo-controlled cross over study of desloratadine vs. placebo for validation purpose with 6 hour challenges. Trial 3 (carried out in VCC3) (n= 34) investigated the efficacy of a house dust mite SLIT tablet vs. placebo^{E2} in 6 hour challenges (ClinicalTrials.gov identifier: NCT01644617). Trial 4 (carried out in VCC2 & 3) (n=28) was a double-blind, placebo-controlled evaluation of a subcutaneous mite allergen immunotherapy (data unpublished). Subjects were challenged for 2.5 hours. Subjects were selected according to the respective trial protocol (short trial description in this article's **Online Repository**) (table E2).

Pre-weighed mite material was dispensed in the chamber and particles were counted every five minutes. Large particles (25µm and above) were used, as published studies have demonstrated that adequate clinical responses without late-phase reactions can only be achieved with particles larger than 10µm⁶. Smaller particles would have direct access to smaller airways causing bronchoconstriction as well as bronchial late phase reactions. In VCC2, *Dermatophagoides pteronyssinus* fecal material containing mainly Der p1 with a predetermined ratio of 20:1 of Der p 1 to Der p 2 (Allergon AB, Sweden) was used. The composition of the material for VCC3 consisted of whole cultures of *D. pteronyssinus* and *D. farinae* (Allergon AB, Sweden) and pure feces of both mite species with particle sizes between 25 and 50 µm (Citeq Biologics, Netherlands). For dose determination weight and particle counting of allergen material were done simultaneously. For higher accuracy the procedure of allergen control and adjustment was then changed from counting particles to weighing mite material. Commercially available kits (Indoor Biotechnologies, Charlottesville, VA) were used to determine the concentration of Der p 1 and Der p 2 in the allergen material for VCC3, which was 0.12µg/100mg Der p 1 und

0.1µg/100mg Der p 2. The presence of various HDM allergens in the allergen material was detected by ELISA with sera from rabbits immunized with the respective HDM allergens (for details see methods section in this article's **Online Repository**). All clinically relevant *D. pteronyssinus* allergens were detectable by ELISA (see **table E3**).

TNSS, consisting of nasal itching, sneezing, rhinorrhea and nasal congestion, was evaluated by four point scale⁸ pre- challenge and every 15 minutes during each session. As the symptom nasal blockage was not determined in the trial carried out in VCC2, evaluation of TNSS for purposes of inter chamber variability was done without nasal discharge. Decrease in the sum of nasal airflow measured by active anterior rhinomanometry (RMM) was evaluated pre- challenge and every 30 minutes during challenge session. These measurements were taken for evaluation of retest reproducibility and inter chamber variability.

To define the amount of allergen material required to achieve a plateau TNSS of above 6, in the dose finding trial five challenges (A-E) were conducted with increasing doses of allergen material (**Fig. 1A +B**). The targeted mean TNSS of at least 6 score points⁹ was reached in challenge D (**Fig. 1C**), a further increase of allergen concentration did not lead to further symptom increase, but elevated the percentage of subjects reaching the target TNSS from 70% to 93%. For confirmation of correlation between allergen weight and clinical symptoms six further challenges (F-K) were evaluated. A mean weight of about 0.3g/h mite allergen material turned out to be sufficient to provoke a reproducible TNSS of ≥ 6 . Calculation of Pearson's correlation coefficient showed a strong correlation of mean allergen weight (in g/h) and the average TNSS values of hours 2-6 with $r = 0.961$ (**Fig. 1D**). In one group of subjects a six hour challenge session, with a three hour placebo run in without allergen load, was conducted to evaluate non-specific reactions. Subjects' TNSS was

around 2 for the first three hours and increased to the targeted TNSS of 6 within one hour of mite allergen exposure (**Fig. E1**).

For retest reproducibility, TNSS and decrease in RMM of subjects taking part in trials 1, 2 and 3 in VCC 3, were compared. For inter-chamber variability NSS (=TNSS without nasal discharge) and RMM, documented in 28 placebo-treated volunteers exposed in two different chamber systems (first VCC2, then VCC3), were evaluated. TNSS was scored every 15 minutes during exposure; mean and SD from 2-6 hours were calculated for dose finding and retest reproducibility outcomes, whereas mean NSS from 1-2.5 hours was calculated for inter-chamber variability. Additionally, RMM, measured every 30 minutes during exposure, was evaluated; mean % decrease and SD from baseline from 2-6 hours were calculated for retest reproducibility, and 1- 2.5 hours for inter-chamber variability.

Retest reproducibility was determined with a mean TNSS of 6.7 (**Fig. 2A**) with a SD of 0.5. The mean reduction in nasal flow was 34% with a SD of 3.1 (**Fig. 2B**).

For inter-chamber variability subjects developed a mean NSS of 3.9 in VCC2 and of 4.0 in VCC3 with a SD of 0.2% each (**Fig. 2C**). Reduction in nasal flow sum, evaluated by RMM, showed a mean of 36.3% in VCC2 and a mean of 39.6% in VCC3 with a SD of 1.2 and 2.3, respectively (**Fig. 2D**). 22.4% of all subjects were asthmatics (see **table E2**), 24 (12.9 %) developed a FEV1 decrease of more than 20% as determined by lung function testing with FEV1- measurements during challenge sessions and for further 24 hours at home. Mean decrease was 24.6% (range 21%- 52%) during challenge sessions. After inhalative treatment with Salbutamol no further late phase adverse reactions were observed.

Correlation of mite allergen challenge in the chamber with natural environmental mite exposure of our subjects was not addressed as currently no sufficient method exists to determine environmental airborne mite exposure on an individual level.

A reproducible correlation between house dust mite allergen concentration and TNSS in mite allergic subjects was shown. Retest reproducibility in subjects at different time points was investigated to demonstrate that subjects evolve a comparable clinical picture when exposed repeatedly in different challenge chamber systems¹⁰ and was proven in terms of subjective (TNSS) and objective (RMM) symptom development in different challenge sessions. Inter-chamber variability when exposing the same subjects to dust mite allergens in different chamber systems was clearly demonstrated. The mite allergen challenge chamber model is also safe in asthmatic subjects.

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Figure legends:

Fig. 1:

A) allergen count (in particles/slide), B) allergen weight (in g/h), C) TNSS (average value of hours 2-6; 17 scores) during dose finding and dose confirmation challenges A-K and D) Scatterplot of the Correlation between mean allergen weight (g/h) and mean TNSS (average values of hours 2-6) $r = .96$

Fig. 2:

A +B: Retest reproducibility assessed for 360 minutes in 3 trials:

(A) TNSS and (B) RMM (flow decrease in percentage)

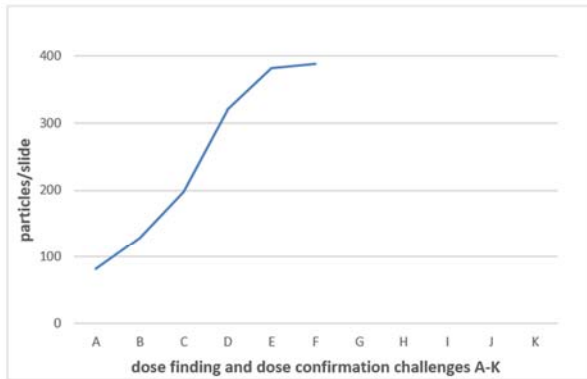
blue line – Trial 1; green line – Trial 2; purple line – Trial 3; red dotted line – mean curve of trials 1-3

C +D: Inter chamber variability assessed for 150 minutes in trial 4:

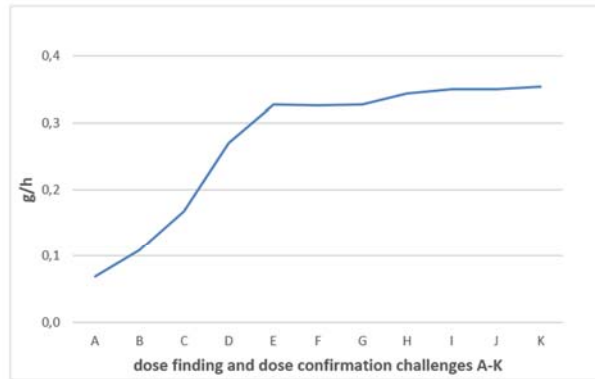
(C) NSS and (D) RMM (flow decrease in percentage)

Purple line- VCC2, blue line – VCC3

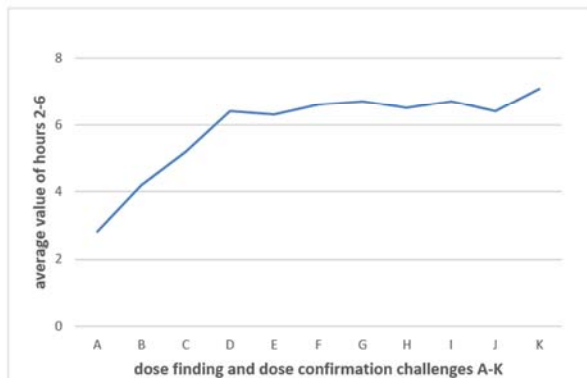
A: Allergen count



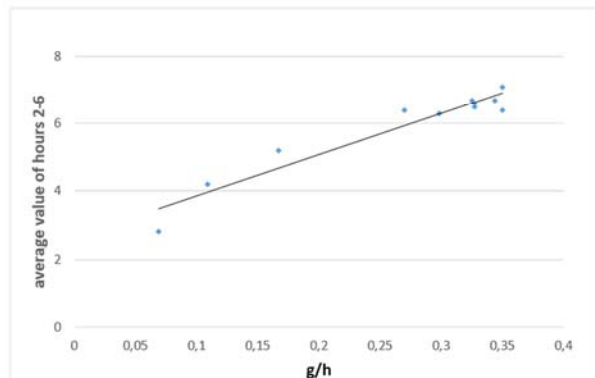
B: Allergen weight



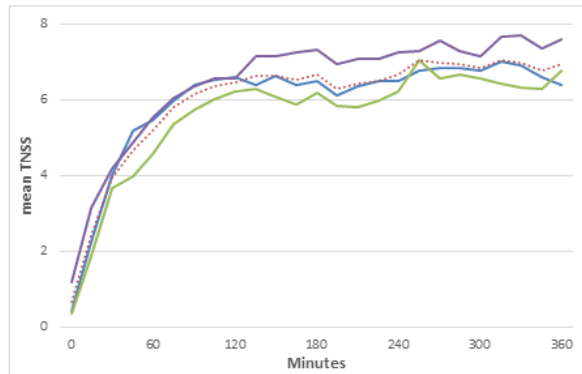
C: TNSS



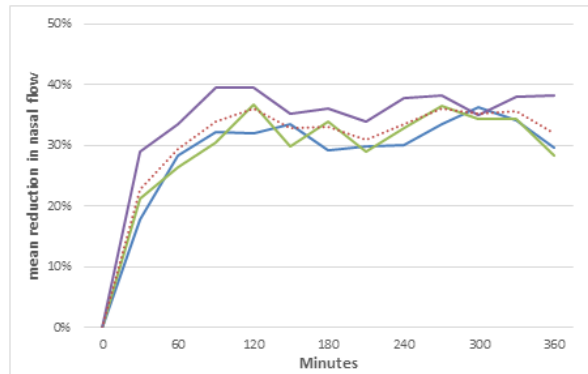
D: Correlation of allergen weight and TNSS



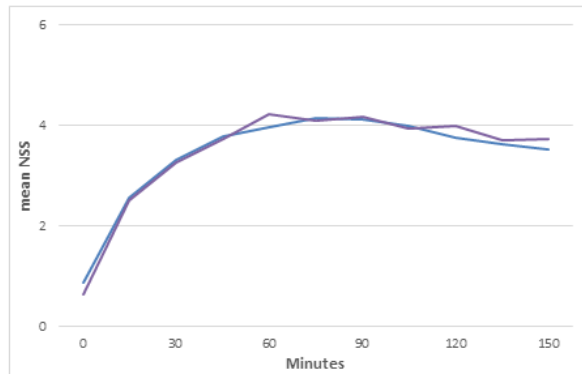
A: Total Nasal Symptom Score (TNSS)



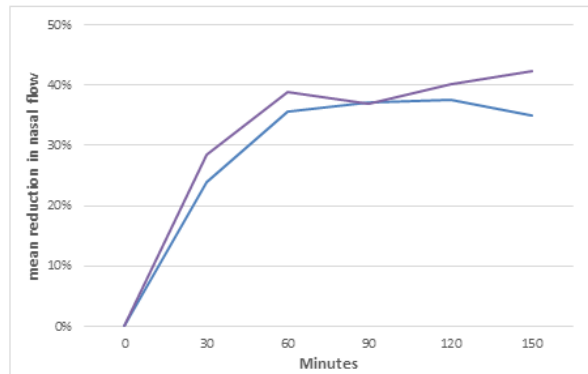
B: Active anterior rhinomanometry (RMM)



C: Nasal Symptom Score (NSS; TNSS without nasal discharge)



D: Active anterior rhinomanometry (RMM)



Methods:

Technical description of VCC

The Vienna Challenge Chamber (VCC) is an enclosed system with constant climatic conditions. Patients can be challenged simultaneously under controlled and reproducible conditions for several hours (usually 2 to 8 hours, with respect of the target of the trial). Windows and an intercom system between the chamber and the surrounding working area allow the constant observation of the challenged subjects. Constant allergen load leads to a stable allergen concentration over hours.

Climatic overview of VCC 3

An automatic air supply system guarantees permanent air conditioning. Filtered fresh air is brought into the chamber by the air conditioning machinery. The climatic conditions of the chamber can be adjusted, but normally, conditions of 24°C with 40% humidity are used for clinical trials. For recording the climatic conditions six temperature/humidity sensors are fixed to the ceiling. Used allergen loaded air is constantly sucked off through 8 openings close to the floor (see also ³).

Allergen material and dispersion

The allergen input proceeds by an additional air system. For each different allergen a separate supply is installed to avoid contamination of different allergens within the delivery tubes. Genuine house-dust mite allergens are continuously fed into the system. Two special dispensers spread out the allergens from the ceiling into the chamber. These outlets and the slightly turbulent air flow in the chamber guarantee a homogeneous dispersion of the allergen all over the chamber. Consumption of

allergen material is determined every five minutes by weighing the allergen container before and after the supply of allergen material to the chamber.

Detection of HDM allergens in HDM extracts

Commercially available kits (Indoor Biotechnologies, Charlottesville, VA) were used to determine the concentration of Der p 1 and Der p 2 in the allergen material for VCC 3. The measurements were performed according to the manuals of the manufacturer. The presence of various HDM allergens in the allergen material was detected with sera from rabbits immunized with the respective HDM allergens in ELISA assays. For this purpose ELISA plates were coated with 50µg/ml of the allergen material and incubated overnight at 4°C. After washing twice with PBST and blocking with blocking buffer (PBST, 1%[w/v] BSA) for 3 h at room temperature, plates were incubated overnight at 4°C with rabbit anti-Der p 1, 2, 4, 5, 7, 10, 11, 14, 15, 18, 21 and anti-Der p 23 antisera diluted 1:10.000 with PBST. Bound allergen-specific antibodies were detected with 1:2000 diluted HRP-conjugated donkey anti-rabbit IgG antibodies (GE Healthcare, Buckinghamshire, United Kingdom) as described ^{E1}. ODs were measured in an ELISA reader (Dynatech, Denkendorf, Germany) at 405 nm.

Patients` description

Eligible subjects were males and females of 18-60 years of age who had suffered, for at least 2 years, from mite allergic rhinitis as documented in the subject's history, and with such severity that it required pharmacological intervention. All subjects had to have sIgE >0.7 kU/l and/or positive skin-prick tests (SPT ≥ 4mm diameter) performed within the last year for *D. pteronyssinus* and/or *D. farinae* allergens (Table E2). Inclusion and exclusion criteria were applied as given in the respective trial protocol.

All investigations were conducted in accordance with Good Clinical Practice Guidelines (International Conference on Harmonization) and the Declaration of Helsinki. They were approved by the competent authority and responsible Ethics Committee. Written Informed consent was obtained from each subject before screening or entry into the respective clinical trial.

Data were obtained exclusively from placebo- or non-treated volunteers of the studies described below.

Trial 1 (carried out in VCC3) was the allergen dose finding study. Data were taken from 85 subjects exposed to mite allergen material for 6 hours.

Trial 2 (carried out in VCC3) was a double blind placebo- controlled cross over study for validation purpose. In this trial 39 subjects were challenged for 6 hours. Desloratadine 5mg was given as active treatment (manuscript in preparation). Data out of this trial were evaluated for proof of dose response and retest reliability.

Trial 3 (carried out in VCC3) was a double blind placebo controlled dose finding study to investigate a house dust mite SLIT tablet^{E2}. 34 placebo treated subjects were challenged for 6 hours. Data out of this trial were evaluated for proof of retest reliability (ClinicalTrials.gov identifier: NCT01644617).

Trial 4 (carried out in VCC2 & 3) was a double blind placebo- controlled evaluation of a subcutaneous mite allergen immunotherapy (data unpublished). 28 placebo treated subjects were challenged for 2.5 hours. Data out of this trial were evaluated for inter chamber variability.

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Table and Figure legends:

Table E1: Technical information about Vienna Challenge Chambers VCC2 & VCC3

Table E2: Demographics of placebo subjects of the 4 presented trials

Table E3: Semi quantitative analysis of mite allergen content by ELISA assay of mite material used in VCC3. Values indicate optical density (OD)

Figure E1: TNSS (y- axis) of 6 hour challenge (x- axis 0-360 minutes) with a 3 hour Placebo run in (n=9)

TABLE E1

	VCC2	VCC3
size	~37m ³	~58m ³
Capacity (number of subjects)	Up to 12	Up to 20
ventilation	Air enters through openings in the ceiling; used air is sucked out at floor level	Filtered fresh air enters through fan coils in the ceiling; used air is sucked out at floor level
Dispersion system	Second air circuit for allergen loaded air, delivered from dispensers next to ventilation outlets	Second air circuit for allergen loaded air, delivered from dispensers next to fan coils
Mite allergen load	~220 particles/5min Der p (feces)	~350 particles/5min mixture feces/whole culture Der p & Der f
Allergen load monitoring	Burkhard trap – every 5mins	Burkhard trap – every 5mins; load by weighing

TABLE E2

	Trial 1	Trial 2	Trial 3	Trial 4
No of subjects	85	39	34	28
Female, n (%)	36 (52.9)	23 (59)	15 (44.1)	14 (50)
Mean age, y (range)	26 (18-44)	27 (18-52)	26 (18-43)	23 (20-30)
Mean height, cm (range)	172.2 (152-190)	171 (157-190)	172.4 (154-189)	172.5 (158-192)
Mean weight, kg (range)	69,7 (49-117)	67,9 (51-100)	68,6 (49-100)	67,6 (49-95)
Subjects with asthma, n (%)	18 (26.5)	11 (28.2)	7 (20.6)	4 (14.3)
Mean duration AR/C, y (range)	12.8 (1-35)	15.3 (1-44)	15.7 (3-34)	8.9 (1-18)
Sensitization type, n (%)				
HDM only	4 (5.9)	6 (15.4)	4 (11.8)	5 (17.9)
HDM & others	64 (94.1)	33 (84.6)	30 (88.2)	23 (82.1)

TABLE E3

Allergen	OD
Der p 1	3,581
Der p 2	0,969
Der p 4	0,377
Der p 5	1,878
Der p 7	2,368
Der p 10	3,449
Der p 11	3,600
Der p 14	0,460
Der p 15	2,512
Der p 18	1,514
Der p 21	0,630
Der p 23	0,843

Total Nasal Symptom Score (TNSS)

