


ORIGINAL ARTICLE

Cat allergen exposure in a naturalistic exposure chamber: A prospective observational study in cat-allergic subjects

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Abstract

Background: To determine the proportion and reproducibility of cat-allergic mild asthmatics with early asthmatic response (EAR) during cat allergen exposure in a naturalistic exposure chamber (NEC).

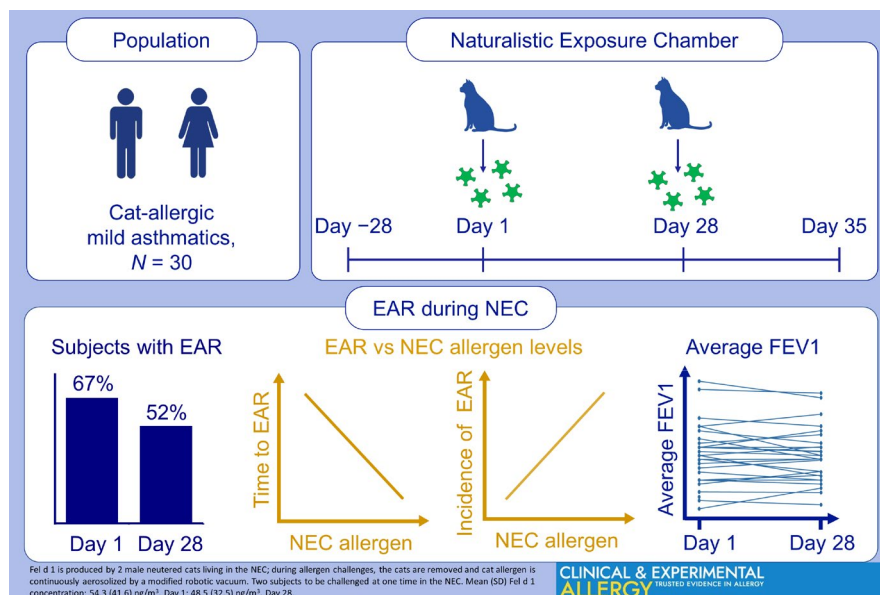
Methods: This was a prospective, observational study in 30 cat-allergic mild asthmatics who received two 180-min cat-allergen (*Felis domesticus* allergen 1 [Fel d 1]) challenges 27 days apart in an NEC.

Results: An EAR ($\geq 20\%$ reduction from baseline in forced expiratory volume in 1 s [FEV1]) was observed in 67% and 52% of subjects at first and second NEC exposure, respectively, with similar median time to EAR; 44% of subjects had an EAR on days 1 and 28. Late asthmatic response ($\geq 15\%$ reduction in FEV1 within 24 h of NEC exit) was observed in 33% of subjects following either exposure. Average FEV1 and total nasal symptom score during NEC exposure were highly correlated within subjects between NEC exposures ($r = 0.91$, $p < 0.0001$; $r = 0.73$, $p < 0.001$), but total ocular symptom score was not. Time to EAR, but not average FEV1, was significantly associated with NEC Fel d 1 concentration, which was variable. There were no serious adverse events; 12/30 subjects experienced 20 adverse events (including asthma, 10%; headache, 10%).

Conclusions: The NEC model demonstrates that average FEV1 change is highly reproducible and has a low correlation with cat allergen levels. However, time to EAR and incidence of EAR are less reproducible and are highly correlated with NEC allergen levels. Average FEV1, rather than incidence of EAR or time to EAR, could be considered as an endpoint for interventional trials testing cat-specific anti-allergy therapies using an NEC.

KEYWORDS

allergy treatment, asthma, challenge tests



GRAPHICAL ABSTRACT

In this prospective, observational study in cat-allergic mild asthmatics who received cat-allergen challenges in an NEC, 67% and 52% of subjects experienced an EAR at first and second exposure. The time to and incidence of EAR were highly correlated with NEC allergen levels, while average FEV1 during NEC challenges was highly correlated within subjects between NEC exposures. Average FEV1 could be a possible endpoint for interventional trials testing cat-specific anti-allergy therapies using an NEC.

1 | INTRODUCTION

Cat allergens are among the most important indoor allergens, and are a common cause of Type 1 (immunoglobulin [Ig] E-mediated) allergic disease world-wide, affecting an estimated 10%–15% of adults presenting with allergic rhinoconjunctivitis and/or asthma.¹ *Felis domesticus* allergen 1 (Fel d 1) in cat hair is produced by the skin, and by the salivary and lacrimal glands of the cat.² Dried saliva and dander from cat hair are spread as small airborne particles into the environment and readily adhere to surfaces such as walls, carpets and furniture. While the highest amount of Fel d 1 allergen is found in households with cats, and the concentration correlates with the number of cats per house,^{3–5} this allergen can be carried on clothes and shoes into homes and schools without cats and may persist in these areas for months to years.⁶ Hence, it is difficult to avoid exposure to cat allergen in the environment.

The association between cat allergy and asthma is significant; ~30% of allergic asthmatics have a concomitant allergy to cats.⁷ Additionally, more than 50% of cat-sensitized subjects have a diagnosis of comorbid asthma, ranging from intermittent mild to potentially life-threatening asthmatic exacerbations requiring treatment with short- and long-acting bronchodilators, inhaled corticosteroids and broad allergen immunotherapy agents.⁸ Patients with high concentrations of cat allergen-specific IgE are at higher risk for ocular and/or asthma symptoms.^{9,10} There is an unmet need for improved prophylaxis of cat allergy to reduce cat allergen-associated asthma exacerbations.

Recommendations for treating allergic rhinitis include allergen avoidance, antihistamines and intra-nasal corticosteroids and

Key messages

- Sixty-seven percent and 52% of subjects experienced an EAR at first and second NEC exposure
- Time to and incidence of EAR are highly correlated with NEC allergen levels
- Average FEV1 during NEC challenges was highly correlated within subjects between NEC exposures

allergen-specific immunotherapy.¹¹ Although antihistamines and intra-nasal corticosteroids are widely used as preventative agents, up to ~50% of patients report poor or only partial symptom control.^{12–15} Specific immunotherapy (SIT), including subcutaneous immunotherapy and sublingual immunotherapy, is indicated when moderate-to-severe symptoms persist despite the use of antihistamines and intra-nasal corticosteroids.^{16–19} However, adverse events (AEs) occur in 40%–50% of patients, ranging from mild reactions (e.g. swelling, injection-site reactions and urticaria) to life-threatening reactions (e.g. asthma exacerbation and anaphylaxis)²⁰; the incidence of severe systemic reactions is estimated to be <1% with conventional immunotherapy and >30% with rush immunotherapy.²¹ Uncontrolled asthma may be exacerbated by SIT and therefore is contraindicated. Given these factors, there is a significant unmet need to develop novel therapies to treat moderate-to-severe allergic rhinoconjunctivitis and asthma due to cat allergy that are efficacious, more rapid and more convenient than currently available therapies, and for novel study designs to determine their efficacy.

Historically, regulatory approval of novel allergy therapeutics targeting aeroallergens has required field studies conducted during the natural allergy season. This type of study is limited by confounding environmental factors (rain, dust, other allergens, etc.) and allergen exposure avoidance by patients.²² Limited paradigms exist to study novel allergy therapeutics targeting perennial allergens such as cat allergen.^{23–25} To overcome the limitations of field studies, environmental exposure units (EEUs) that expose allergic patients to nebulized cat allergen, as well as 'live-cat rooms' that expose allergic patients to cat dander in rooms where cats live, have been developed to standardize the assessment of anti-allergen therapies.^{23,26–28} Novel study designs in the EEU are emerging to determine the efficacy of anti-allergen immunotherapies for the prevention of asthma exacerbations.^{29–33}

It is currently unknown whether the EEU model or the live-cat room model will be developed to obtain regulatory approval of novel allergy therapeutics targeting cat allergy and cat allergen-associated asthma exacerbations. It is also unknown how well allergic and asthma symptoms elicited in an EEU compared to symptoms elicited in a live-cat room. Arvidsson et al. demonstrated an association between bronchoconstriction elicited by a bronchial challenge with nebulized cat allergen extract and by a live-cat room.³⁴ Corren et al. demonstrated that omalizumab significantly reduced bronchoconstriction in patients with cat allergy and moderate asthma who were exposed to cat allergen in a live-cat room.³⁵ The live-cat rooms that were used for these studies were not permanent fixtures, and therefore not maintained over time. The live-cat challenge room, or naturalistic exposure chamber (NEC), that has been established at Red Maple Trials (RMT) has been purposely built and maintained to conduct such studies. The objective of the NEC is to generate a realistic allergen exposure chamber to that experienced in an indoor home environment. This model complements highly controlled allergen exposure chamber studies.

The NEC is a type of natural environmental exposure which offers a more controlled environment than a traditional field study, but more natural allergen exposure than in an EEU, as allergens are produced by living cats. This study was performed to determine whether an NEC, representative of natural cat exposure, may be used to induce an early asthmatic response (EAR) in cat-allergic mild asthmatics in a reproducible manner. Frequent spirometry and subject monitoring for up to 24 h after subjects exited the cat allergen exposure was employed to optimize safety monitoring. These data may be used to inform the design of future interventional clinical trials of cat-specific anti-allergic therapies.

2 | MATERIALS AND METHODS

2.1 | Study design

This prospective, single-centre, observational study evaluated allergic symptoms provoked by exposure to live-cat allergen in an RMT NEC in cat-allergic subjects with mild asthma. The study comprised a 4-week screening period, including skin prick testing for cat hair

and other common allergens, serum for total IgE and specific IgE for Fel d 1 and cat hair, and spirometry testing. The reproducibility of symptom onset following cat allergen exposure was assessed by two cat allergen challenges in the NEC, separated by 27 days, after which subjects were followed up for 1 week. The study design and subject disposition are shown in Figure 1A and B.

The study was conducted in accordance with the 2013 principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The study protocol and all amendments were approved by the institutional review board at the participating study site (Advarra, Pro-0037395). All the participants provided written informed consent prior to enrolment.

2.2 | Study participants

Male and female subjects aged between 18 and 65 years with a self-reported history of at least 2 years of cat allergy and mild asthma (Global Initiative for Asthma stage 1 [GINA 1]),³⁶ with cat sensitization confirmed at screening by: (1) a positive skin prick test (SPT) with cat hair extract (ALK, USA; mean wheal diameter at least 5 mm greater than a negative control) and (2) positive allergen-specific IgE tests for cat hair and Fel d 1 (>0.35 kAU/L at screening). Those with a >10% fall in FEV1 on three consecutive occasions during a 60-min control session with spirometry every 10 min were excluded from the study; only one subject was excluded based on this criterion.

2.3 | Naturalistic exposure chamber challenge

Challenges were performed in an NEC (Red Maple Trials, Inc., Ottawa, Canada), a small room (2.81 m × 2.11 m × 2.44 m) where two male neutered cats (aged 3 years) resided for more than 1 year in an apartment-like setting with furniture, carpets and blankets except during challenges (cats were removed from the NEC prior to challenges; Figure 1C). The NEC was set up for two subjects to be challenged at one time; all challenges were performed in the morning. In addition, the study was conducted during the fall and winter, and all subjects were studied outside of their pollen seasons. The NEC has been approved as a research facility by the Ministry of Agriculture, Food and Rural Affairs of Ontario. The Canadian Council on Animal Care guidelines were followed.³⁷

During the challenges, a modified robotic vacuum (Figure 1C) continuously dispersed particulate containing cat allergen from the floor into the air during the time that the subjects were in the NEC.³⁸ The robot vacuum offered continuous recirculation of cat allergen without interruption of the subject or the exposure room. Airborne cat allergen (Fel d 1) was measured with multiple air sampling pumps (Gillian 5000, Sensidyne, Florida, USA) located throughout the room with cassettes containing filters to capture the antigen; for details, see online Supporting Information. Air samples, chosen randomly and spanning the study, all tested below detection levels for *Dermatophagoides pteronyssinus*

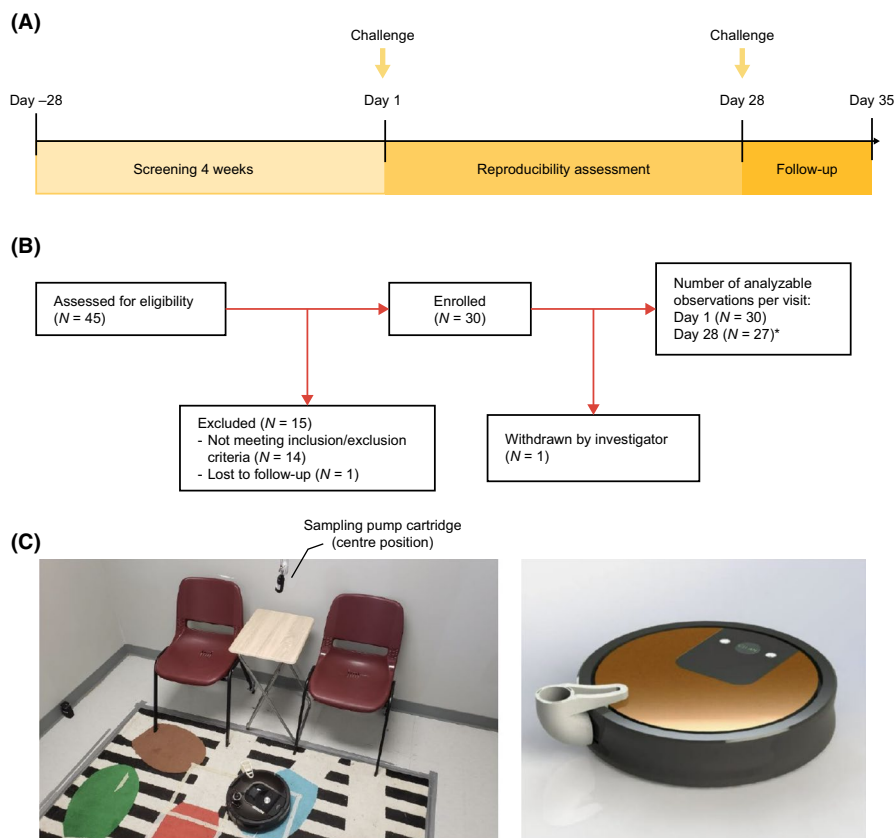


FIGURE 1 Study design, subject disposition and NEC set up. NEC, naturalistic exposure chamber. *Two subjects with unreliable spirometry data were excluded from spirometry-related analyses

allergen 1 (Der p 1 [<3.7 ng/m³]) and *Dermatophagoides farinae* (Der f 1 [<0.8 ng/m³]).

Spirometry was measured just prior to and every 10 min during the challenges using mobile device-linked spirometry (Nuvoair, Sweden). Baseline spirometry was performed outside of the NEC in an area free of cat dander; spirometry measurements during challenges were conducted within the NEC. Rhinoconjunctivitis and respiratory symptoms were captured prior to and every 20 min during the challenges using the same mobile device.

Subjects underwent the challenges for up to 180 min at baseline and 27 days later. Prior to entering the NEC, their eligibility and medication use were reviewed, their Asthma Control Test (ACT) questionnaire³⁹ was completed, and they performed spirometry and peak nasal inspiratory flow tests. They then recorded their nasal (total nasal symptom score [TNSS]),⁴⁰ ocular (total ocular symptom score [TOSS])⁴¹ and chest symptoms (individual questions relating to chest tightness, wheezing, cough, swollen or tight throat, difficulty breathing, itchy throat and itchy skin). If the subject's TNSS score was <2 , meaning they were asymptomatic, ACT score ≥ 20 and forced expiratory volume in 1 s (FEV1) $\geq 70\%$ predicted, signifying stable asthma control prior to cat allergen exposure, they were permitted to undergo the challenge. The subjects left the NEC if they experienced an EAR (FEV1 reduction by 20% of baseline), if their symptoms became intolerable, or if 180 min was reached. Subjects who experienced an EAR and/or had asthma symptoms were treated with salbutamol, a short-acting beta agonist, per protocol, plus

additional treatments if clinically indicated. For details of post NEC departure monitoring, see online Supporting Information.

2.4 | Study endpoints

The primary endpoint was the proportion of cat-allergic asthmatics who attained an EAR within 180 min during the first NEC challenge on day 1. Time to EAR was defined as either the time to a $\geq 20\%$ reduction in FEV1 in the NEC,³² or when the subject voluntarily departed the NEC due to clinically significant allergic or asthma symptoms. Reductions in FEV1 of $\geq 20\%$ were adjudicated by the investigators by blindly evaluating baseline and subsequent spirometry data, including flow volume loops.

Secondary endpoints (see Table S1) included the proportion of cat-allergic asthmatics who attained an EAR within 180 min in the NEC on day 28, the proportion who experienced a late asthmatic response (LAR) within 24 h of being in the NEC, and the incidence rates of AEs and serious AEs through the end of the study.

During the on-site 6-h observation period, the time to LAR was defined as the time to a $\geq 15\%$ reduction in FEV1 (with spirometry efforts adjudicated by the investigator) from leaving the NEC. During the at-home monitoring period up to 24 h after the subject exited the NEC, time to LAR was defined as time to a $\geq 15\%$ reduction in FEV1³² (confirmed by two spirometry efforts within 5 min) along with either rescue medication use (any medication at any dose)

within 1 h or the presence of chest symptoms within 1 h of the drop in FEV1 (adjudicated by the investigator). A subject could not have more than one LAR within 24 h of leaving the NEC.

2.5 | Statistical analysis

Time to each type of asthma exacerbation was examined using Kaplan–Meier estimates, with times censored at 180 min, 24 h and 27 h, if subjects did not experience EAR, LAR or any asthma response (AAR), respectively. The median times to EAR, LAR and AAR and corresponding 95% confidence intervals (CIs) are provided for each NEC cat allergen challenge.

Average FEV1, TNSS and TOSS for each NEC cat allergen challenge were calculated as the area under the curve of each parameter, based on the trapezoidal rule, divided by the time the subject spent in the NEC at that challenge. Least squares (LS) means and 95% CIs are presented from mixed models with repeated measures, with the Fel d 1 concentration in the NEC and the baseline parameter value prior to the start of the NEC challenge as continuous covariates and the challenge visit as a factor. An unstructured covariance structure was utilized. Pearson's correlation was used to assess the reproducibility of symptoms elicited in the NEC. Lin's concordance correlation coefficient was assessed as a sensitivity analysis; similar results to the Pearson correlation were observed and therefore are not reported here. Spearman's rank correlation was used to evaluate correlations of the allergen concentration and IgE with clinical assessments.

3 | RESULTS

3.1 | Study participants

Among the 45 subjects assessed for eligibility, 30 met the inclusion/exclusion criteria and were enrolled (Figure 1B). Subjects' demographic and baseline characteristics, including response to screening cat challenge and Fel d 1-specific and cat hair-specific IgE levels, are

shown in Table 1. Almost half of the subjects were male, and the mean age of all subjects was 32 years. Mean FEV1 was 3.64 L. In addition to cats, most subjects were also highly sensitized (SPT mean wheal diameter ≥ 5 mm) to timothy grass (15/30, 50%) and dust mite (Der p 1) (20/30, 66.7%). Additional allergens tested and IgE levels to cat-related and other common allergens are shown in Tables S2 and S3.

3.2 | Assessing intra-subject variability to cat allergen challenge in the NEC by examining EAR and symptoms

Overall, 66.7% (20/30) of subjects had an EAR ($\geq 20\%$ reduction in FEV1 within 180 min; 95% CI 47.2%, 82.7%) following exposure to cat dander from live cats during the first NEC challenge (day 1), while 51.9% (14/27; two subjects were excluded from spirometry-related analyses due to unreliable spirometry results; 95% CI 31.9%, 71.3%) had an EAR during the second NEC challenge (day 28, Figure 2A). Only 12 subjects (44.4%) experienced an EAR within 180 min in the NEC during both challenges; eight (29.6%) had an EAR during one of the two visits and seven (25.9%) did not have an EAR at either challenge. The time to EAR was also defined as the time to a subject voluntarily departing the NEC due to clinically significant allergic or asthma symptoms; however, there were no subjects who voluntarily departed the NEC due to intolerability of their symptoms.

Median time to EAR was similar, 132 and 142 min, following the start of NEC cat allergen challenge on days 1 and 28, respectively (Figure 2B). Among subjects who had an EAR at both visits, the time to EAR differed by 37 min on average (mean [standard deviation, SD]; 37.6 [38.6] min); among subjects who had an EAR at one of the two visits, the time in NEC differed by over an hour (65.5 [52.3] min).

An additional supplementary analysis using a 15% reduction in FEV1 as the definition of an EAR showed similar reproducibility. With this definition, 90% (27/30) of subjects had an EAR during the first NEC challenge and 77.8% (21/27) had one during the second challenge. Nineteen subjects (70.4%) had a 15% reduction in FEV1 during both challenges, and one subject (3.7%) did not have a 15%

TABLE 1 Study participants' demographic and baseline characteristics

	Total (N = 30)
Age, years, mean (SD)	32 (11.1)
Male, n (%)	13 (43.3)
Non-Hispanic white, n (%)	27 (90.0)
Screening cat skin test wheal diameter, mm, mean (SD)	8.78 (2.51)
Fel d 1 IgE, Ku/L, median (Q1: Q3)	4.74 (1.26: 10.70)
Cat hair IgE, Ku/L, median (Q1: Q3)	5.64 (1.47: 23.50)
Baseline FEV1, L, mean (SD)	3.64 (0.76)
Baseline FEV1, percent predicted, mean (SD)	97.85 (13.05)
Baseline minute ventilation (L/min), mean (SD)	13.28 (6.80)

Abbreviations: Fel d 1, *Felis domesticus* allergen 1; FEV1, forced expiratory volume in 1 s; Ig, immunoglobulin; SD, standard deviation.

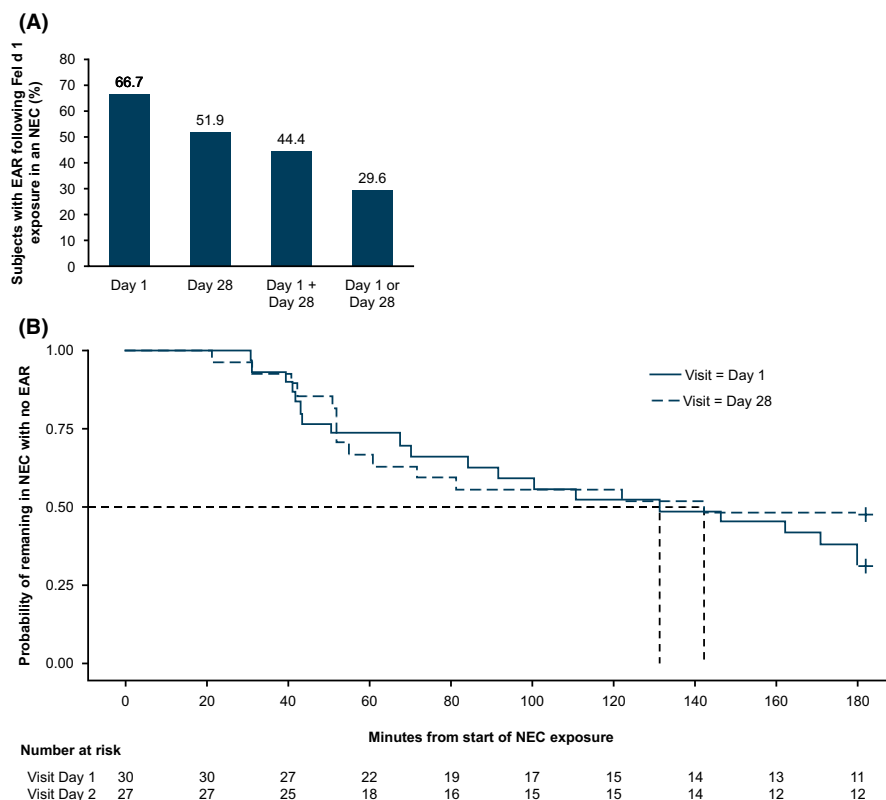


FIGURE 2 (A) Proportion of subjects experiencing an EAR in the NEC and (B) median time to EAR at days 1 and 28 following NEC challenge. EAR, early asthmatic response; Fel d 1, *Felis domesticus* allergen 1; NEC, naturalistic exposure chamber. "Day 1 or day 28" represents the proportion of subjects who experienced an EAR on either day 1 or day 28, but not both. Dotted line corresponds to the median time to EAR on days 1 and 28

reduction during either challenge. The median time to a 15% reduction in FEV1 was 65 and 50 min on days 1 and 28, respectively. Among subjects who had a 15% reduction at both visits, the time differed by 30 min on average (mean [SD]; 29.5 [35.4] min); among subjects who had an EAR at one of the two visits, the time in NEC differed by 49 min on average (48.6 [32.4] min).

Average FEV1 was highly correlated between day 1 and day 28 NEC exposure ($r = 0.92$, $p < 0.0001$; Figure 3) and was similar at the aggregate level (LS mean [95% CI] 3.10 L [3.01, 3.20] vs. 3.11 L [3.02, 3.20]). The maximum reduction in FEV1 (median [minimum, maximum]) during NEC exposures on day 1 (20.7% [11.5, 38.7%]) and day 28 (20.2% [7.0%, 41.8%]) ranged between 7% and 42%. Subjects were removed from the NEC once FEV1 dropped by 20% or more.

The Fel d 1 concentration in the NEC was variable in the two NEC exposures. Fel d 1 concentration measured at the subject shoulder (referred to as subject pump) ranged from 15.4 to 167.5 ng/m³ over the first, mean (SD) 54.3 (41.6) ng/m³, and second exposure, 48.5 (32.5) ng/m³ (Figure 4A). Notably, the time to EAR was significantly associated with the concentration of Fel d 1 in the NEC, as calculated from the room and subject pumps (Figure 4B and C), but average FEV1 did not correlate with Fel d 1 concentration (room pump: $r = 0.02$, $p = 0.88$; subject pump: $r = -0.08$, $p = 0.55$). On average, subjects tolerated a similar quantity of cat allergen in nanograms (ng) during both NEC exposures, as assessed by NEC Fel d 1 concentration over time (ng/m³) \times min ventilation (L/min) \times time in NEC (min) (where 1 L/min = 1/1000 m³/min). Cat allergen quantity (ng) tolerated on day 1 vs. day 28 (LS mean [95% CI]) was 53.5 ng (36.4, 70.5) vs. 48.8 ng (33.4, 64.1).

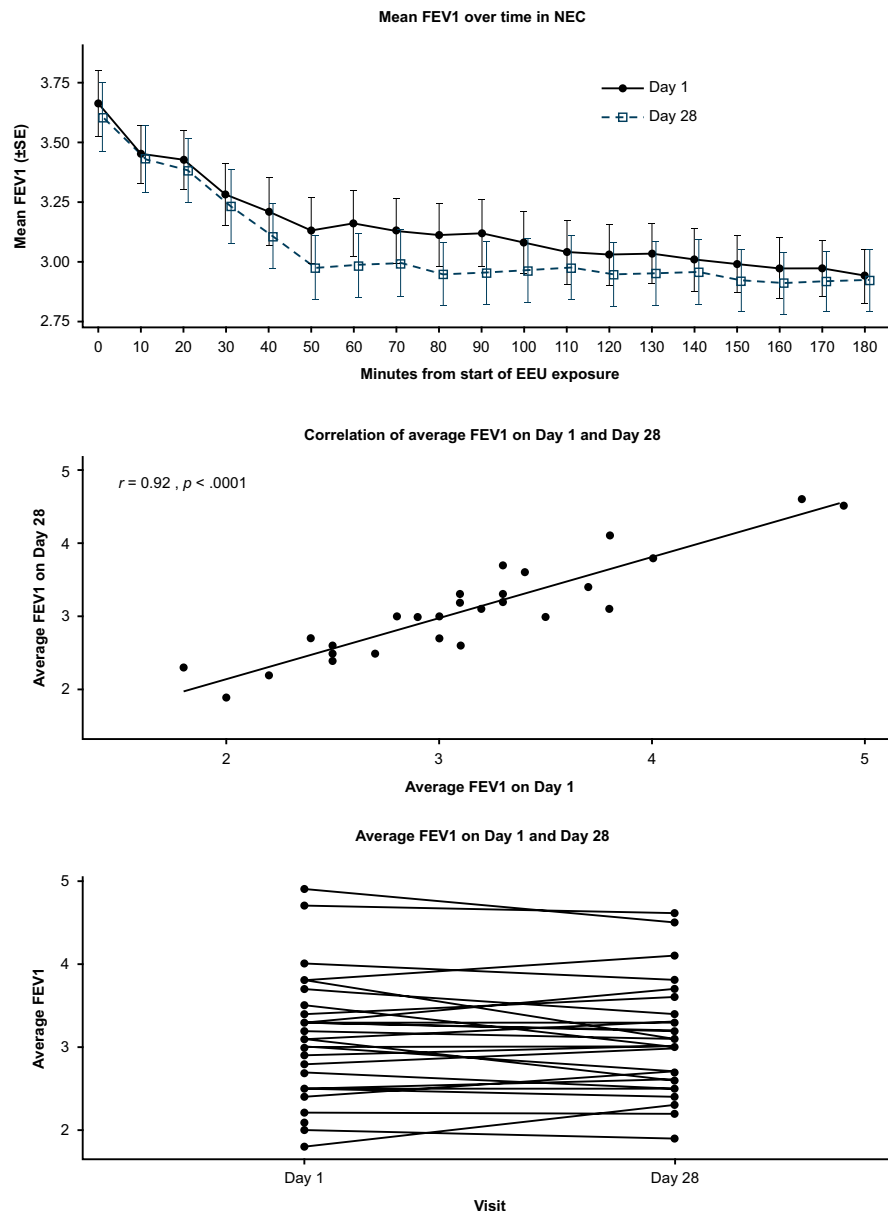
Average TNSSs induced by cat allergen challenge in the NEC were low but similar during exposures on day 1 and day 28 (LS mean [95% CI]: 3.17 [2.51, 3.84] vs. 2.65 [1.91, 3.39]), with a strong intra-subject correlation between the average TNSS on day 1 and day 28 ($r = 0.73$; $p < 0.001$). By contrast, although the average TOSS induced by a cat allergen challenge in the NEC was not significantly different on days 1 and 28 (LS mean [95% CI]: 0.77 [0.30, 1.23] vs. 1.04 [0.38, 1.69]), no significant intra-subject correlation was observed ($r = 0.28$; $p = 0.32$).

Average chest symptoms observed were similar during exposures on days 1 and 28, with the highest symptom score observed for symptoms related to chest tightness and cough (Table S4). A strong intra-subject correlation was observed for symptoms related to cough, itchy skin, and wheezing, and moderate correlation was observed for the other symptoms assessed (Figure S1).

3.3 | IgE and SPT, and relationship with time to EAR and symptom scores

Baseline characteristics of SPT and IgE are included in Table 1. Correlation between SPT and IgE with time to EAR and symptom scores was evaluated. No significant correlation was observed between the SPT mean wheal diameter or cat dander IgE with the time to EAR (Table S5). A weak-to-moderate correlation with Fel d 1 specific-IgE and total IgE with time to EAR was observed, with statistical significance achieved on day 1 for Fel d 1-specific IgE and on day 28 for total IgE (Table S5). No significant correlations were observed between cat dander IgE, Fel d 1-specific IgE, total IgE or SPT mean wheal diameter with average FEV1, TNSS or TOSS.

FIGURE 3 FEV1 during day 1 and day 28 NEC exposure. FEV1, forced expiratory volume in 1 s; NEC, naturalistic exposure chamber; SE, standard error. Top plot shows the mean FEV1 (\pm SE) across subjects every 10 min for each NEC exposure. Middle plot shows the correlation of average FEV1 on day 1 vs. day 28, with Pearson correlation coefficient, *p*-value and the line of best fit (linear least squares). Lower plot shows the average FEV1 per subject on day 1 vs. day 28; the lines connect the values for the same subject



3.4 | Reproducibility of subjects experiencing LAR within 24 h of NEC challenge

Almost a quarter of the subjects (23.3%) had an LAR (within 24 h) following day 1 NEC cat allergen exposure, and fewer (14.8%) had one following day 28 NEC cat allergen exposure. The frequency of LAR was higher among the subjects who did not have an EAR than among those who had had an EAR on either day 1 ([3/10] 30%) vs. [4/20] 20%) or day 28 ([3/13] 23.1% vs. [1/14] 7.1%); no statistically significant difference was shown. The majority of subjects (66.7%) did not have an LAR following either day 1 or day 28 NEC exposure, 29.6% had an LAR at one of the two exposures, and only one subject (3.7%) had an LAR after both exposures. Since most subjects did not experience an LAR, the median time to LAR could not be estimated for either day 1 or day 28. For subjects who experienced an LAR, the maximum reduction in FEV1 (*n*, median [minimum, maximum]) on day 1 (*n* = 7, 16.8% [15.8%, 22.2%]) and day 28 (*n* = 4, 21.4% [18.3%,

23.1%]) ranged between 15.8% and 23.1%. Of the LARs experienced by subjects, the mean (SD) time to LAR after exiting the NEC was 6.2 (5.4) h, with the majority (72%) occurring between 1 and 9 h after NEC exit.

Median time to AAR was similar, being 139 (95% CI 84, 395) and 142 min (95% CI 61.2, not reached), during NEC cat allergen challenge on days 1 and 28, respectively.

3.5 | Safety outcomes

Naturalistic exposure chamber cat allergen exposure was generally well tolerated. All subjects who experienced an EAR received salbutamol after the end of the exposure. Ten subjects on day 1 and six subjects on day 28 required more than one treatment with salbutamol in the 24 h after leaving the NEC. Twelve subjects on day 1 and two subjects on day 28 required salbutamol to treat an LAR during

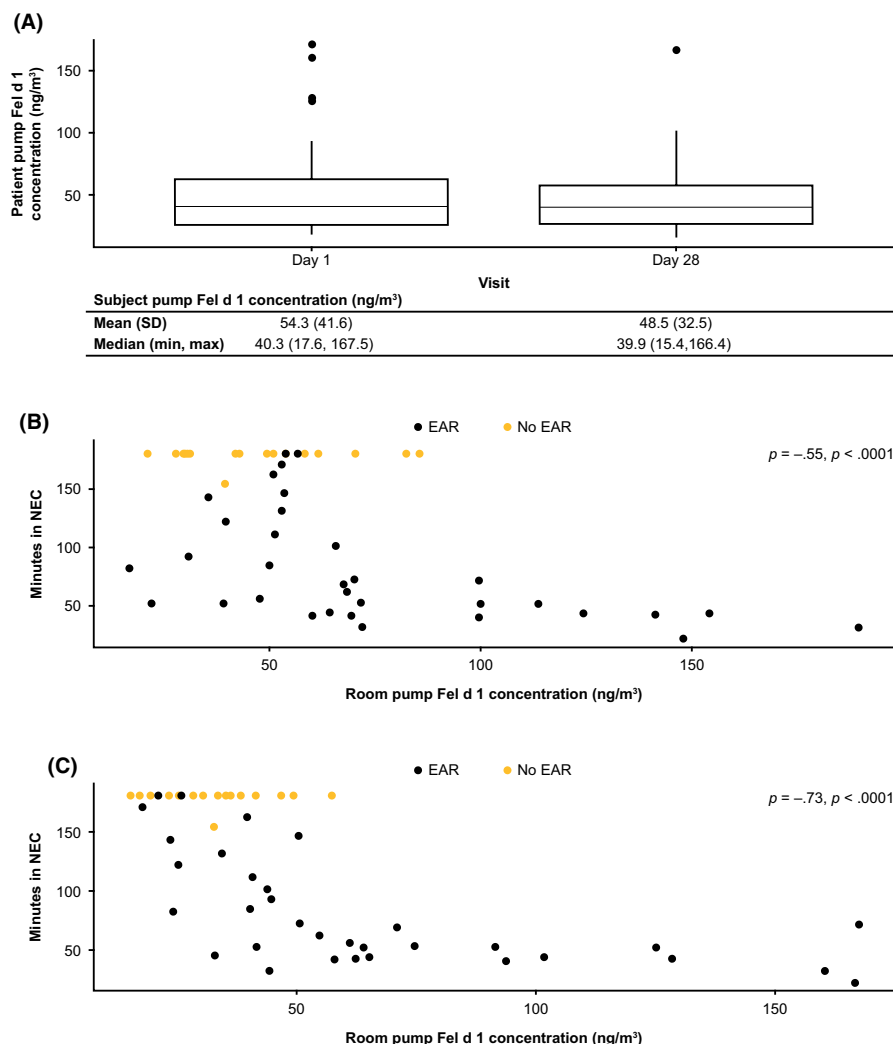


FIGURE 4 (A) Fel d 1 concentration during NEC exposure and, (B) and (C), association between time to EAR and Fel d 1 concentration in the NEC. C, concentration; EAR, early asthmatic response; Fel d 1, *Felis domesticus* allergen 1; m, collected mass, NEC, naturalistic exposure chamber; Q, pump flow rate (typically 0.004 m³/min); SD, standard deviation; t, challenge duration. The calculated subject pump Fel d 1 air concentration in ng/m³ is averaged over the challenge duration: $C = m/(Q \cdot t)$. The calculated room pump Fel d 1 air concentration in ng/m³ averaged over the challenge duration, collected from the pump cartridge(s) suspended in the room nearest the subject, was $C = m/(Q \cdot t)$

the 6-h observation period post NEC, and one subject required budesonide/formoterol to treat an LAR during the observation period on day 1. Less than 30% of subjects required rescue medication at home within 24 h after each NEC exposure (Table 2). No subjects required asthma care outside of the clinical trial unit. Overall, 20 AEs were reported by 12 (40%) of 30 subjects during the study (Table 2). The most frequent AEs were asthma (2/3 mild, 1/3 moderate), headache (3/3 mild) and nasopharyngitis, which occurred with incidences of 10%, 10% and 6.7%, respectively.

4 | DISCUSSION

The results of this study demonstrate that the NEC model may be used to provoke asthma exacerbations in cat-allergic mild asthmatics. All provoked asthma exacerbations were resolved with salbutamol, with the exception of one subject who received budesonide/formoterol for asthma treatment, during the on-site observation period, with resolution. Average FEV₁, rather than time to EAR, was the most reproducible measurement of the provoked asthma exacerbation, exhibiting a high correlation between days 1 and 28 NEC exposure, and the least associated with cat allergen levels in the

NEC. Incidence of EAR and time to EAR were less reproducible and highly associated with cat allergen levels in the NEC. An EAR was defined as a $\geq 20\%$ reduction in FEV₁ during allergen exposure in the NEC, or when the subject voluntarily departed the NEC due to clinically significant allergic or asthma symptoms. In a pre-specified analysis, when the threshold for defining EAR was $\geq 15\%$ reduction, rather than $\geq 20\%$ reduction, reproducibility of incidence of EAR and time to EAR may be slightly improved. Because average FEV₁ was the most reproducible measure and the least susceptible to effects by variable cat allergen levels, this measurement may be the most reliable outcome measure for use in this type of cat-allergen intervention. Furthermore, the definition of EAR as $\geq 20\%$ reduction in FEV₁ is taken from the definition for bronchoalveolar challenge,³⁴ and the 3-h period in the NEC was applied as this duration of exposure was previously shown to stimulate allergic rhinitis and conjunctivitis symptoms in an EEU⁴²; however, these parameters may not be optimal for the provocation of allergic and asthma symptoms in the NEC as the kinetics of allergic rhinitis/conjunctivitis and EAR may differ in these different experimental conditions. A limitation of the study is that EAR occurred in most subjects by 2–3 h in the NEC, which is the time when allergic rhinitis and conjunctivitis symptoms rise and plateau.^{42,43} Therefore, the opportunity to measure allergic rhinitis and

TABLE 2 Safety analysis: use of rescue medication and incidence of AEs

Timing	Rescue medication, N (%)	Day 1 (N = 30)	Day 28 (N = 27)
At NEC exit	Any rescue medication	20 (66.7)	14 (51.9)
	Salbutamol	20 (66.7)	14 (51.9)
Observation period	Any rescue medication	15 (50.0)	6 (22.2)
	Budesonide formoterol	1 (3.3)	0 (0.0)
	Cetirizine hydrochloride	1 (3.3)	1 (3.7)
	Loratadine	3 (10.0)	3 (11.1)
	Olopatadine	1 (3.3)	1 (3.7)
	Salbutamol	12 (40.0)	2 (7.4)
At-home monitoring	Any rescue medication	7 (23.3)	8 (29.6)
	Loratadine	0 (0.0)	3 (11.1)
	Salbutamol	7 (23.3)	7 (25.9)
AE, n (%)		Total (N = 30)	
Asthma		3 (10.0)	
Dyspnoea		1 (3.3)	
Nasal congestion		1 (3.3)	
Nasal oedema		1 (3.3)	
Rhinitis allergic		1 (3.3)	
Headache		3 (10.0)	
Persistent postural-perceptual dizziness		1 (3.3)	
Syncope		1 (3.3)	
Nasopharyngitis		2 (6.7)	
Influenza		1 (3.3)	
Pneumonia		1 (3.3)	
Pyrexia		1 (3.3)	

Abbreviations: AE, adverse event; NEC, naturalistic exposure chamber.

conjunctivitis symptoms was confounded by the earlier kinetics of the EAR. Another limitation is that the time in the EAR was capped at 3 h. Longer or higher allergen level exposures in the NEC might have revealed more cat allergen-induced asthma exacerbations. In addition, the subjects were selected based on their asthma symptoms rather than on the severity of their rhinoconjunctivitis symptoms, as is done in studies specifically evaluating rhinoconjunctivitis during challenge.^{7,44}

Environmental exposure units and live-cat rooms have been used to study anti-allergic treatments for allergic rhinitis/conjunctivitis and asthma.^{24,26,28,35,45} With respect to asthma provocation by cat allergen, in one study it was demonstrated that, of the 62 cat-allergic asthmatics studied, 100% achieved an EAR with a bronchial challenge with cat allergen (13.75 standardized quality [SQ] up to a maximum cumulative dose of 7026 SQ) while only 60% of the same patients achieved an EAR in a live-cat room exposure for 3 h (Fel d 1 13.9 [1.5–40] ng/m³) and approximately half of patients

in both exposures developed an LAR (FEV1 reduction $\geq 15\%$ within 3–24 h).³⁴ Similarly, it was reported that approximately half of subjects exposed to aerosolized allergen extract had an EAR alone, while the other half experienced both an EAR and LAR.⁴⁶ These data suggest that the aerodynamic characteristics of the particles, and possibly the antigen concentration, affect the likelihood of an EAR. Particles in aerosols of allergen extract are spherical, with constrained diameters, while natural cat dander is platelike and of a wider range of sizes, likely resulting in a different pattern of deposition in the airways and a different bronchoconstriction response. In another report, a controlled cat room was used to demonstrate that omalizumab significantly reduced the change in FEV1 and increased the time the patient with cat allergy and moderate asthma was able to remain in the EEU during cat allergen exposure for up to 1 h (median Fel d 1 425 and 429 ng/m³ at baseline and Week 16, respectively).³⁵ Our study findings complement these results and suggest that average FEV1, rather than incidence of EAR or time to EAR, may be the most reproducible measure in mild cat-allergic asthmatics exposed to cat allergen in a NEC and, therefore, would be the most relevant for studying a cat allergy-specific intervention.

5 | CONCLUSIONS

This study establishes the safety and feasibility of the NEC to provoke a reproducible EAR and a highly reproducible average FEV1 in cat-allergic mild asthmatics, and demonstrates the effectiveness of aerosolizing Fel d 1 using a modified vacuum cleaner. Our study findings may inform the design of interventional clinical trials of novel cat-specific anti-allergic therapies for the prevention of allergic rhinitis and asthma in the cat-allergic population.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

The authors were involved in the study design and collection, analysis and interpretation of data. All authors had full access to all the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis. All authors critically reviewed and edited the manuscript. All authors approved the final version.

DATA AVAILABILITY STATEMENT

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing once the product and indication has been approved by major health authorities (e.g. FDA, EMA and PMDA), if there is legal authority to share the data and there is not a reasonable likelihood of participant re-identification. Submit requests to <https://vivli.org/>.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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