

ORIGINAL ARTICLE

Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber compared with natural conditions

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Summary

Background Efficacy testing of drugs in seasonal allergic rhinitis (SAR) is often disturbed by seasonal variations of environmental allergens, and assessment of onset and duration of action is hardly possible under natural conditions. Allergen provocation in an environmental challenge chamber (ECC) can be of added value in this respect. However, the specificity, sensitivity, and reproducibility of outcome measures under both settings are unclear.

Objective The aim of this study was to investigate and compare the diagnostic value (specificity, sensitivity, and reproducibility) of clinical end-points and biomarkers both following allergen provocation in an ECC and under natural conditions.

Methods Sixty adult patients with SAR to grass and 60 healthy subjects were exposed twice to grass pollen in an ECC and observed twice during the pollen season. Symptoms, nasal flow, as well as exhaled and nasal nitric oxide (NO) were investigated.

Results The total nasal symptom score (TNSS) in the ECC had the best reproducibility (intraclass correlation coefficient ICC = 0.86) and sensitivity/specificity [area under receiver operating characteristic curve (AUC) = 0.99] of all measures. Symptoms in season also had good sensitivity/specificity but were far less reproducible. Nasal flow in the ECC had good sensitivity/specificity but reproducibility was limited. NO measurements showed good reproducibility but sensitivity/specificity were limited, except for exhaled NO in season (AUC = 0.75).

Conclusion The high reproducibility and sensitivity/specificity in the ECC suggests that TNSS is a valuable outcome measure. While exhaled NO can be considered to monitor airway inflammation, nasal NO appears to be unspecific.

Keywords allergic rhinitis, biomarker, controlled clinical trial, environmental challenge chamber, exhaled nitric oxide, grass pollen, repeatability, reproducibility, sensitivity, specificity

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Introduction

Allergic rhinitis (AR) is a common disease affecting up to 25% of the population world-wide. It leads to a marked reduction of life quality, with considerable health costs [1, 2]. AR is characterized by an inflammatory reaction of the nasal mucosa after sensitization towards common environmental allergens [3]. Acute allergen exposure induces

an early-phase reaction mainly mediated by histamine release from mast cells, with subsequent vasodilatation, oedema, itching, and mucus production [4]. Continuous exposure to allergens leads to a late-phase reaction and a chronic inflammatory response, which is mediated by T cells and effector cells, causing epithelial damage and possibly remodelling of the tissue [5].

Therapy of AR usually includes a pharmacological intervention because allergen avoidance as an integral part in the management of AR is often of limited success,

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especially in seasonal AR (SAR) with a widespread distribution of allergens. Currently, several classes of drug treatments are available like antihistamines, adrenergic agonists, corticosteroids, leukotriene modifiers, cromolyns, and IgE antibodies [6]. These therapies address targets at different stages of the allergic inflammatory reaction, allowing to act synergistically when used in combination. Ideal treatments are effective in most patients, have a rapid onset of action to provide fast relief of symptoms, have a long duration of action to allow for once-daily dosing, prevent or reduce the chronic inflammatory response, are effective against all individual rhinitis symptoms, and display a good safety profile [7]. It is important that physicians have broad information on the different properties of each drug so that they can prescribe the optimal treatment to the individual patient.

For SAR, classical trials that prove the safety and efficacy of investigational new drugs are conducted during the pollen season for up to several weeks [8, 9]. Potentially, differences in pollen levels over the course of the study affect the sensitivity of these clinical studies. Moreover, local variations of pollen levels in particular in multicentre trials add noise to the signal. Importantly, classical trials are limited with regard to detection of the onset and duration of action as well as in detecting differences between doses of a given compound because pollen levels vary and symptom assessments have limitations in frequency and timing [10].

Clinical trials in SAR that use an environmental challenge chamber (ECC) can be of added value because symptoms can be induced under well-controlled and highly standardized conditions. In particular, dose finding, onset of action, and duration of action for investigational new drugs can be determined by means of ECC [11]. Outcome measures include subjective evaluation of symptoms by self-reported scales [12] or objective measurements of e.g. nasal flow by rhinomanometry, assessment of nasal secretions by weighing tissue paper, and monitoring of airway inflammation by exhaled or nasal nitric oxide (NO) [13]. However, the sensitivity, specificity, and reproducibility of these outcome measures assessed in an ECC in comparison with the pollen season have not been determined so far.

Therefore, the aim of this study was to investigate and compare the specificity, sensitivity, and reproducibility of clinical end-points and biomarkers both following allergen provocation in an ECC and under natural conditions. Besides symptom scores, nasal flow, exhaled NO, and nasal NO were analysed.

Methods

Study design

This study was part of the Allergy Marker Study (ALMA) with the aim to analyse the specificity, sensitivity, and reproducibility of outcome measures for AR under experimental and natural conditions. In this single-centre trial, patients with grass pollen AR and healthy controls were investigated within 1 year (October 2003 to September 2004) and outcome measures were assessed outside (October to April) and during the grass pollen season (May to September) (Fig. 1). During two sessions out of season, separated by two weeks, a grass pollen exposure was performed in an ECC and outcome measures were determined before, during, and after allergen provocation. The same measures were analysed once in the morning at the two visits during the pollen season. At the first in-season visit (May/June), the natural pollen count was 10–50 grass-pollen/m³/24 h, whereas it was lower (4–12 grass pollen/m³/24 h) during the second visit (July/August). Pollen measurements were performed by the official German Pollen Information Service with a Burkhard sampler in the city centre of Hannover.

Subjects

Sixty adult subjects with grass pollen AR and 60 non-sensitized healthy controls of either sex and aged 18–60 years were included in the study. Allergic subjects had to demonstrate a medical history of SAR due to grass pollen for at least 24 months, and a positive skin prick test (SPT) to grass pollen within 12 months of enrolment. Healthy control subjects were included when there was no medical history of allergic diseases and asthma and no sensitization towards allergens determined by SPT (see below).

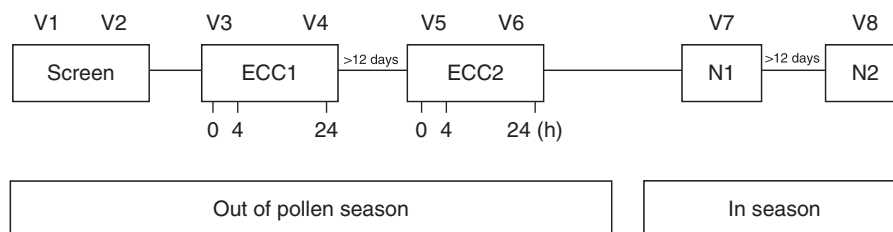


Fig. 1. Study design of the Allergy Marker study. Study subjects were investigated outside of the grass pollen season (visits 1–6) and in the pollen season (visits 7 and 8). Outcome measures were assessed during two sessions (ECC1/ECC2) out of season following allergen provocation in an environmental challenge chamber (ECC) and on two occasions (N1/N2) under natural conditions (N) in the pollen season.

Subjects with persistent asthma according to GINA guidelines (GINA > 1), sensitization towards more than three allergen groups (pollen, animal dander, moulds, mites, food), pregnancy, any chronic and malignant disease, drug abuse, and psychiatric disease or immunotherapy within the last 5 years were excluded. Patients were not allowed to use corticosteroids, cromolyn, leukotriene-modifiers, and long-acting β -agonists. Antihistamines, short-acting β -agonists, anticholinergics, and theophyllin had to be stopped 3 days before a visit.

The study protocol was approved by the Ethics Committee of the Hannover Medical School, Germany. The study was conducted in accordance with the Declaration of Helsinki (Somerset West Amendment, 1996) and the ICH Guideline on Good Clinical Practice (Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), 17 January 1997) and performed at the Clinical Research Unit of the Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany. Written informed consent was obtained from each subject before any study-related procedures started.

Allergen provocation in the environmental challenge chamber

Subjects were exposed twice outside the pollen season to a grass pollen atmosphere of 4000 pollen grains (*Dactylis glomerata*; Biopol Laboratory Inc., Spokane, WA, USA) per cubic metre for 4 h in the validated Fraunhofer ECC [14]. The ECC accommodates up to 18 subjects who are under constant supervision by medical staff. At each exposure session, an equal number of patients with AR and healthy volunteers were sent to the ECC. Pollen concentration, temperature, and humidity are continuously monitored according to SOPs, allowing for a constant humidity ($40 \pm 10\%$), temperature ($21 \pm 2^\circ\text{C}$), and pollen concentration (4000 ± 300 grains/m³) to be maintained throughout the exposure period. Symptoms, nasal flow, exhaled NO, and nasal NO were assessed as indicated below.

Symptom scores (total nasal symptom score and visual analogue scale)

Symptoms were scored at baseline before entering the ECC (0 h) and every 20 min until the end of the 4-h exposure with the score at 4 h taken for analysis (4 h) and after 24 h (24 h). At the in-season visits, symptoms were assessed once in the morning. The total nasal symptom score (TNSS) was assessed by the subject by filling in a questionnaire and was the sum of the four major nasal symptoms of running nose, blocked nose, sneezing, and itching. Each item was assessed on a 0–3 scale (0 = none; 1 = mild; 2 = moderate; 3 = severe) as recommended by the Food and Drug Administration [8]. Care was taken that

subjects had no access to their scores from any previous time-point to avoid bias. In addition, a visual analogue scale (VAS) was used to assess the total patient discomfort ('How would you rate your discomfort as a whole?').

Rhinomanometry

Nasal flow was measured using anterior rhinomanometry via a face mask (Master Scope Rhino, Viasys Healthcare, Hoechberg, Germany) before (0 h), every 60 min during allergen exposure in the ECC with the measurement at 4 h taken for analysis (4 h), and after 24 h (24 h). At the visits during the pollen season, rhinomanometry was measured once. Flow rates at 150 Pa were obtained separately for the right and left nostrils (mL/s). The sum of the flow rates of both nostrils was calculated from the two measurements.

Nitric oxide measurements

Fractionated exhaled NO (FeNO) and nasal NO (NNO) were measured using the NIOX[®] system (Aerocrine, Solna, Sweden) according to the manufacturer's instructions and ATS/ERS guidelines [15]. Measurements were performed within 1 h before (0 h) and immediately after the 4-h exposure (4 h) as well as 24 h after the allergen exposure (24 h) in the ECC and once at the visits during the pollen season. Nasal NO was recorded three times per nostril and every measurement was assessed for acceptance in a blinded manner. Measurements were accepted when there was a logistic growth of the curve with a stable plateau. The mean plateau of acceptable curves was reported.

Spirometry

Lung function [forced expiratory volume in 1 s (FEV₁)] was measured using a portable electronic asthma monitor AM-1 (Viasys Healthcare) at the visits during the pollen season and before and every 60 min during allergen exposure in the ECC. To ensure the safety of the participants, they continued to measure their lung function at home every 2 h until retiring to bed. They received salbutamol aerosol as rescue medication in case of a decline of FEV₁ > 20% from baseline.

Skin prick test and immunoglobulin E

SPTs were performed according to study site SOPs. Test solutions were provided by HAL-Allergy (Haarlem, the Netherlands) and ALK-Scherax (Hamburg, Germany). A battery of 15 major aeroallergens [*Dactylis glomerata*, grass mix, rye, tree mix (early and late), mugwort, ribwort, ragweed, *Dermatophagoides pteronyssinus et farinae*, dog hair, cat fur, horse epithelium, fungi mix, *Alternaria alternata*, *Cladosporium herbarum*] as well as positive and negative controls were tested on the volar surface of

the forearms. After 15 min, the largest diameter of the weal reaction was measured. A test was considered positive when the weal diameter was 3 mm greater than the corresponding negative control.

Serum samples were analysed for the total IgE levels. For values < 150 IU/mL, a sandwich radio-immuno assay (RIA) was used [16]; values > 150 IU/mL were determined with a competitive RIA [17].

Statistics

All calculations were performed using SAS 9.1.3. Basic tests of significance were performed using Mann-Whitney's or Wilcoxon's test for unpaired/paired quantitative data and Fisher's test for categorical data. The level of significance was set at 5% in all cases. Intra-class correlation coefficients (ICC) were calculated using ANOVA sum of squares. Area under the curve (AUC) for the receiver operating characteristic (ROC)-analyses were performed using logistic regression. Correlations were carried out using Spearman's procedure. Bland-Altman Plots were used to visualize repeatability in a specific example [18].

Results

Study population

Sixty subjects with SAR and 60 healthy controls were included in the study. There was one drop-out due to non-medical reasons in the SAR group. Demographic data and baseline conditions of the per-protocol population are shown in Table 1. There were no significant differences in age, sex, body mass index (BMI), and FEV₁. As expected, the level of IgE and the numbers of eosinophils in peripheral blood were significantly increased in the allergic subjects.

Table 1. Demographic data and baseline characteristics of the study population

	Grass pollen allergic rhinitis (n = 59)	Healthy (n = 60)
Sex, n (%)		
Male	22 (37)	20 (33)
Female	37 (63)	40 (67)
Age (years)	31.9 ± 8.5	33.3 ± 10.2
BMI (kg/m ²)	24.0 ± 3.6	24.2 ± 3.3
FEV ₁ (% predicted)	99.4 ± 10.7	100.8 ± 11.5
Total IgE (IU/mL)	248.4 ± 307.8*	32.4 ± 49.4
Eosinophils (%)	2.8 ± 1.9*	2.1 ± 1.6

Mean ± SD.

**P* < 0.05.

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; SD,

standard deviation.

Outcome measures following allergen provocation in the environmental challenge chamber and under natural conditions

Symptoms. The total nasal symptom scores were low at baseline before allergen exposure in the ECC (Fig. 2). At the end of the 4-h exposure in the ECC, there was a pronounced increase of TNSS in the allergic patients while in healthy subjects TNSS was significantly lower compared with the pre-challenge values (Fig. 2). Twenty-four hours after allergen exposure, TNSS in allergic patients remained slightly elevated compared with baseline. However, this elevation was only significant at the first allergen exposure (ECC1). While the difference between allergic patients and healthy subjects was not significant at baseline, there was a significant group difference at 4 h after allergen exposure. At 24 h, the group difference was only significant at the first ECC session. In general, the response upon allergen exposure was highly reproducible (ICC = 0.86, Table 2). The TNSS response showed perfect separation between groups with high sensitivity and specificity, resulting in an area under the ROC curve (AUC) of 0.99 (Table 2).

Under natural conditions in the grass pollen season, there was also a clear separation between groups with an AUC of 0.89. However, the ICC was considerably lower (0.47), suggesting limited reproducibility of TNSS in season. Bland-Altman plots (Fig. 3) confirm a lower variability of TNSS during allergen exposure in the ECC

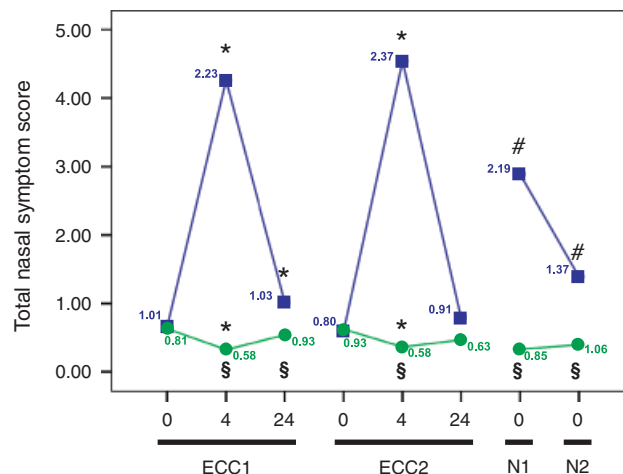


Fig. 2. Total nasal symptom score in patients with grass pollen allergic rhinitis (blue squares) and healthy volunteers (green circles) during two sessions (ECC1/ECC2) out of season following allergen provocation in an environmental challenge chamber (ECC) before challenge (0), at the end of the 4-h-challenge (4), and after 24 h (24), and on two occasions under natural conditions (N1/N2) in the pollen season. Symbols represent means and standard deviation is given as exact value. * *P* < 0.05 compared with the respective baseline (0), # *P* < 0.05 for comparisons between baseline at ECC1 and values at each 0-h time-point. \$ *P* < 0.05 between groups.

Table 2. Sensitivity/Specificity and repeatability of outcome measures following allergen provocation in an environmental challenge chamber (ECC) and under natural conditions (N)

Outcome measure	ECC		N	
	AUC	ICC	AUC	ICC
TNSS	0.99	0.86	0.89	0.47
Running	0.91	0.82	0.77	0.44
Blockage	0.87	0.77	0.74	0.49
Sneezing	0.84	0.66	0.73	0.26
Itching	0.87	0.73	0.78	0.39
VAS (total complaints)	0.97	0.80	0.84	0.40
Nasal Flow	0.83	0.51	0.67	0.59
FeNO	0.57	0.77	0.75	0.72
NNO	0.57	0.86	0.56	0.80

The area under the curve (AUC) of the receiver operating characteristic of the first measurement in or out of season and the intraclass correlation coefficient (ICC) between ECC sessions and between natural conditions are shown.

TNSS, total nasal symptom score; VAS, visual analogue scale; FeNO, fractionated exhaled nitric oxide; NNO, nasal nitric oxide.

Table 3. Correlation of outcome measures in patients with grass pollen allergic rhinitis following allergen provocation in an environmental challenge chamber (ECC) and under natural conditions (N)

ECC1	N1				
	TNSS	VAS	Rhino	FeNO	NNO
TNSS-(4)	0.25	0.16	-0.17	-0.07	-0.03
VAS-(4)	0.06	0.12	-0.04	-0.10	-0.17
Rhino-(4)	-0.13	0.04	0.41*	-0.04	-0.01
FeNO-(24)	-0.05	-0.04	-0.02	0.77*	0.47*
NNO-(24)	-0.03	-0.08	-0.03	0.54*	0.46*

*Significant correlation between ECC1 and N1 ($p < 0.05$).

Measurements of the first session (1) in or out of season were used for comparison.

ECC1, environmental challenge chamber (first session); N1, natural conditions (first occasion); TNSS, total nasal symptom score; VAS, visual analogue scale; Rhino, nasal flow; FeNO, fractionated exhaled nitric oxide; NNO, nasal nitric oxide; 4, measured at 4 h, i.e. the end of allergen provocation; 24, measured at 24 h.

(Fig. 3a) compared with natural exposure (Fig. 3b). While the mean TNSS difference ECC1-ECC2 is very close to zero, suggesting good reproducibility, the mean TNSS difference N1-N2 is around +2, suggesting higher symptoms at N1. In patients with SAR, there was no correlation of TNSS in the ECC at 4 h with the TNSS in-season ($r = 0.25$, Table 3). However, TNSS at 24 h (1.02 ± 1.03) significantly correlated with TNSS in-season ($r = 0.41$), although TNSS was almost back to the pre-challenge values (0.66 ± 1.01).

Overall discomfort assessed by VAS provided a very comparable picture compared with TNSS. VAS was low at baseline (healthy: 0.34 ± 0.55 ; rhinitis: 0.40 ± 0.54) and 24 h after allergen exposure in the ECC (healthy: 0.26 ± 0.53 ; rhinitis: 0.49 ± 0.71) and not different between groups. At the end of the 4-h exposure, there was a pronounced increase of VAS in the allergic patients (ECC1: 2.36 ± 1.59 ; ECC2: 2.47 ± 1.86), which had a good reproducibility (ICC = 0.80, Table 2). Compared with TNSS, the sensitivity and specificity was slightly inferior, both after ECC (AUC = 0.97) and in-season (0.84) and similar to TNSS. As with TNSS, reproducibility in-season was poor, with an ICC of 0.40. There was no correlation of VAS between seasons ($r = 0.12$, Table 3).

Nasal flow. Nasal flow was around 300 mL/s in healthy subjects and 270 mL/s in patients with SAR, with no difference between groups at baseline (Fig. 4). At the end of the 4-h exposure, there was a pronounced reduction of nasal flow in the allergic patients and the magnitude of flow reduction appeared similar between ECC sessions at 4 h (ECC1: 159 ± 110 mL/s; ECC2: 174 ± 111 mL/s). However, reproducibility was only limited (ICC = 0.51, Table 2). Nasal flow at the end of allergen provocation showed acceptable sensitivity and specificity, resulting in an AUC of the ROC of 0.83 (Table 2). In season, there was only a

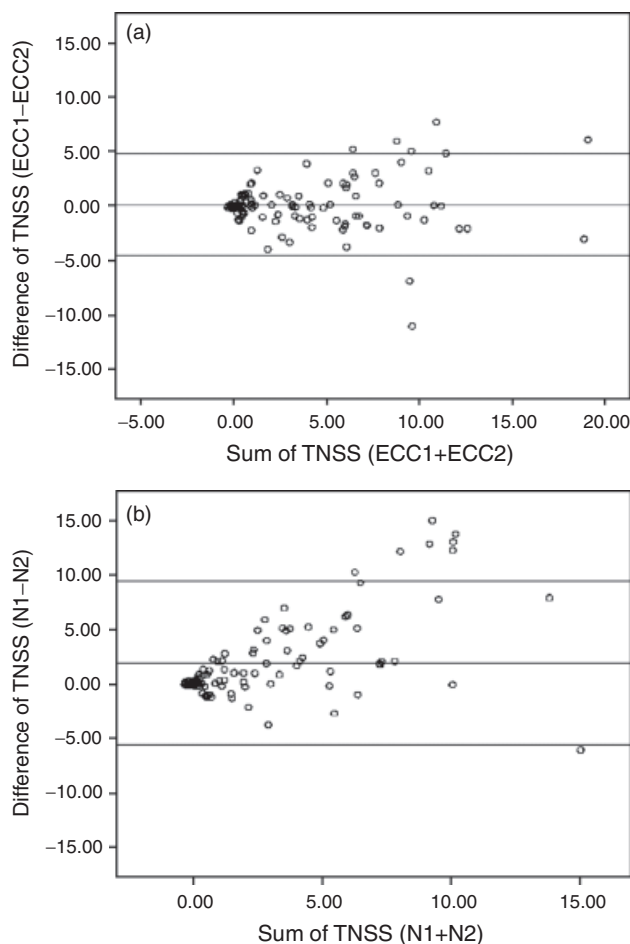


Fig. 3. Bland-Altman plots of total nasal symptom scores (TNSS) during (a) two sessions in the environmental challenge chamber (ECC) measured at the end of the 4-h allergen exposure and (b) two occasions under natural conditions (N).

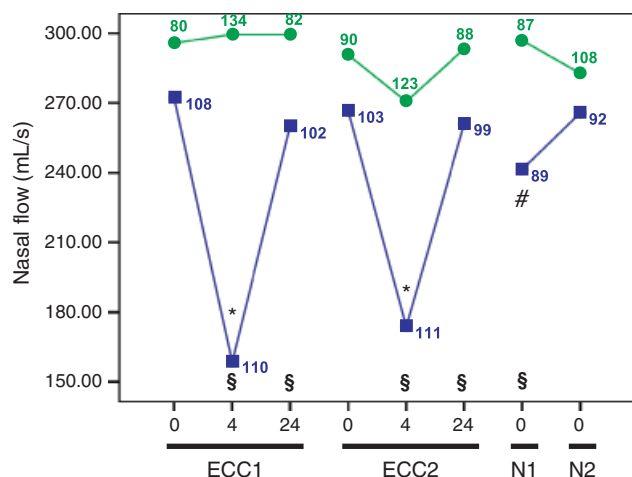


Fig. 4. Nasal flow measured by anterior rhinomanometry in patients with grass pollen allergic rhinitis (blue squares) and healthy volunteers (green circles) during two sessions (ECC1/ECC2) out of season following allergen provocation in an environmental challenge chamber (ECC) before challenge (0), at the end of the 4-h-challenge (4), and after 24 h (24), and on two occasions under natural conditions (N1/N2) in the pollen season. Symbols represent means and standard deviation is given as an exact value. * $P < 0.05$ compared with respective baseline (0), # $P < 0.05$ for comparisons between baseline at ECC1 and values at each 0-h time-point. § $P < 0.05$ between groups.

limited separation between groups: at the first in-season visit, nasal flow was 241 ± 89 mL/s in allergic patients and 297 ± 87 mL/s in healthy subjects (N1, Fig. 4), with an AUC of 0.67 (Table 2). At the second in-season visit, this difference became even smaller and the AUC was only 0.56. The ICC for nasal flow in-season was 0.59 (Table 2). Nasal flow in the ECC significantly correlated with nasal flow in-season ($r = 0.47$, Table 3).

Fractionated exhaled nitric oxide. Fractionated exhaled nitric oxide (FeNO) was 15 ± 7 ppb in healthy subjects and 19 ± 12 ppb in patients with SAR, with a significant difference between groups (Fig. 5a). At the end of every 4-h ECC exposure, exhaled NO was lower compared with baseline but this difference was only significant in allergic subjects at the first ECC session. Twenty-four hours after allergen exposure, FeNO significantly increased both in allergic patients and in healthy subjects. The difference between groups at 24 h was significant. The FeNO level in allergic patients during the second ECC session was generally higher compared with the first ECC. Importantly, baseline FeNO at ECC1 was significantly higher compared with baseline at ECC1. Despite a higher level at the second ECC, reproducibility showed an ICC of 0.76 (Table 2). FeNO measured 24 h after the end of allergen provocation had an AUC in the ROC analysis of only 0.57 (Table 2). In season, FeNO was significantly higher in allergic patients compared with healthy subjects and compared with baseline levels out of season. The AUC was higher in-season (AUC = 0.76) and reproducibility

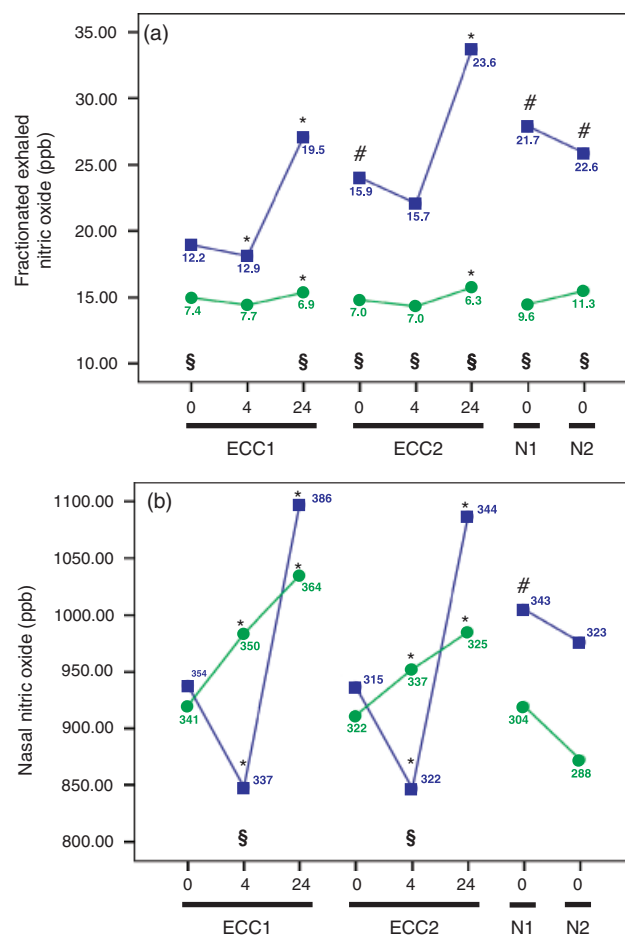


Fig. 5. Exhaled nitric oxide (a) and nasal nitric oxide (b) in patients with grass pollen allergic rhinitis (blue squares) and healthy volunteers (green circles) during two sessions (ECC1/ECC2) out of season following allergen provocation in an environmental challenge chamber (ECC) before challenge (0), at the end of the 4-h-challenge (4), and after 24 h (24), and on two occasions under natural conditions (N1/N2) in the pollen season. Symbols represent means and standard deviation is given as an exact value. * $P < 0.05$ compared with respective baseline (0), # $P < 0.05$ for comparisons between baseline at ECC1 and values at each 0-h time-point. § $P < 0.05$ between groups.

in-season (ICC = 0.76) was comparable to out of season (ICC = 0.72, Table 2). FeNO in the ECC significantly correlated to the FeNO in-season ($r = 0.77$, Table 3).

Nasal nitric oxide. NNO was 920 ± 341 ppb in healthy subjects and 938 ± 354 ppb in patients with SAR at the first baseline visit out of season, with no difference between groups (Fig. 5b). At the end of every 4-h ECC exposure, NNO was significantly lower in allergic patients while NNO significantly increased in healthy subjects, leading to a significant group difference at 4 h. Twenty-four hours after allergen exposure, NNO was significantly increased compared with baseline both in allergic patients and in healthy subjects, with no significant difference between groups. Reproducibility showed an ICC of 0.86 (Table 2). NNO measured 24 h after the end of allergen

provocation had an AUC in the ROC analysis of 0.57, while NNO after 4 h had a higher AUC (0.61). In season, NNO was higher in allergic patients compared with healthy subjects but the difference between groups was not significant. The sensitivity/specificity in-season (AUC = 0.56) and reproducibility in-season (ICC = 0.80) was comparable to out-of-season (Table 2). NNO in the ECC significantly correlated to the NNO in-season ($r = 0.46$, Table 3).

Safety

No adverse or serious adverse events were observed. Pulmonary function did not differ between groups and remained stable throughout each allergen provocation in the ECC. There was a non-clinically relevant significant difference of FEV₁ before ECC2 provocation in both groups compared with baseline at ECC1. In healthy subjects, FEV₁ declined from $100.9 \pm 12.4\%$ predicted at baseline ECC1 to $98.9 \pm 12.0\%$ predicted at baseline ECC2 ($P < 0.0001$) and in allergic patients, FEV₁ declined from $97.6 \pm 10.3\%$ predicted at baseline ECC1 to $97.3 \pm 10.9\%$ predicted at baseline ECC2 ($P = 0.02$).

Discussion

The aim of this study was to investigate and compare the specificity, sensitivity, and reproducibility of outcome measures both following allergen provocation in an ECC and under natural conditions. To the best of our knowledge, this is the first study that simultaneously evaluates several clinical end-points and biomarkers for SAR both off-season during a controlled allergen exposure in an ECC and during the natural exposure in a diseased population in comparison with healthy controls.

The outcome measure with the highest sensitivity/specificity and retest reliability was the TNSS measured during ECC provocation. Under this experimental setting, a pollen count of 4000 pollen per cubic metre in the ECC induced a moderate TNSS of around 4.5 (of a maximum of 12). The chosen pollen concentration mimics the natural condition because pollen counts of several thousands per cubic metre have been reported when measurements are performed over a meadow [19] (and own unpublished data). It is important to note that the study population was not pre-selected according to their symptom scores during a screening exposure visit, which explains why the average TNSS is lower compared with other ECC studies where pre-selection of responders is part of the screening procedure in order to reduce the variability of the outcome measure [20, 21].

Currently, TNSS determined during the pollen season is the recommended end-point by the authorities to be used in double-blind, placebo-controlled trials. In our study, TNSS during the season had a good sensitivity/specificity.

However, it was not highly reproducible because it is dependent on the variable natural pollen concentration, which was low at the second seasonal visit in our study. This observation emphasizes that a high allergen concentration is mandatory for a good reliability of the TNSS in season. This could be one reason why the TNSS measured during ECC provocation did not correlate with TNSS during natural exposure in the SAR patients. Another possibility for the lack of a positive correlation might be that induction of symptoms during an ECC challenge is primarily driven by an early-phase reaction, while symptoms during natural pollen exposure rather reflect an ongoing late-phase reaction. Interestingly, TNSS 24 h after ECC exposure correlated with TNSS during natural exposure. Therefore, symptoms at this later time-point seem to be more reflective of a late-phase response or ongoing allergic inflammation that resembles the natural situation. This view is also supported by data on exhaled NO, which were found to be elevated 24 h after allergen provocation in the ECC. Potentially, outcome measures in the ECC during instead of out of the pollen season might better reflect ongoing inflammation with a better correlation to natural exposure. Studies that compare ECC outcome measures in season with out of the grass pollen season are ongoing.

In healthy subjects, TNSS in the ECC was significantly lower at the end of the allergen exposure compared with the baseline values. Most of all, nasal blockade improved by 0.25 points, suggesting that clean air with well-controlled temperature and humidity is beneficial to nasal patency [22]. However, improvement of this subjective measure was not mirrored by changes in nasal flow.

We found nasal flow to be a poor outcome measure with low reproducibility. Upon allergen provocation in the ECC, sensitivity and specificity were acceptable, suggesting that this objective variable is at least capable of reflecting immediate effects during the early phase. However, no correlations with symptoms both off season and in season were found. This confirms the findings of other authors who found rhinomanometry to be inferior to other objective measures [23].

FeNO levels 24 h after the ECC provocation were significantly elevated, and expectedly, the magnitude of response was significantly greater in allergic subjects compared with healthy controls. The group difference was smaller at the first allergen exposure compared with the second allergen exposure, suggesting that detection of group differences with a single allergen exposure to environmental levels of pollen is of limited diagnostic value. This is confirmed by the low AUC of 0.57 (Table 2). While FeNO was of limited diagnostic value out of the pollen season, the sensitivity and specificity of FeNO was acceptable in season to clearly separate allergic patients from healthy controls. Although reproducibility was acceptable both in and out of the pollen season, it is

important to note that the FeNO response during the second allergen exposure in the ECC was on a higher level, with a significantly elevated baseline value before challenge. It has been demonstrated that repetitive low-dose allergen challenge gives rise to FeNO [24] and that this outcome measure indicates ongoing airway inflammation [15]. We conclude from our repeat ECC exposure two weeks apart that FeNO is reflective of an ongoing lower airway inflammation that leads to a carry-over effect with elevated FeNO levels.

Interestingly, FeNO values at the end of allergen exposure were always lower compared with the baseline. This is in line with previous reports that found that FeNO levels decreased during the acute phase of airway challenges where bronchoconstriction or mucus production occurred [25, 26]. However, in our setting, this change of FeNO was not consistently significant and pulmonary function was stable.

Our data confirm that nasal NO is a poor outcome measure for the differentiation between allergic patients and healthy subjects both in and out of the pollen season. It has been reported before that there is no difference in NNO between subjects with SAR and healthy controls and no seasonal variation was noted [27]. Allergen exposure in the ECC did not add discriminative power because nasal NO increased both in allergic patients and in healthy subjects 24 h after allergen exposure in the ECC. Despite good reproducibility, this unspecific behaviour of NNO after allergen exposure questions whether NNO can provide useful information in the setting of an ECC. To our knowledge, this is the first report on NNO in healthy subjects who have been subjected to an environmental allergen challenge. Of interest is the opposite response of NNO after a 4-h ECC exposure with a decrease of NNO in allergic patients while NNO increased in healthy subjects. The decrease of NNO following allergen challenge has been described before [28] and is probably due to the swelling of the nasal mucosa with reduced release of NO from the paranasal sinuses [29]. Because swelling does not occur in healthy subjects, NNO levels at 4 h are readily elevated in response to unspecific irritation of the nasal mucosa by pollen particles.

In conclusion, several outcome measures were prospectively evaluated in patients with SAR in the present study. TNSS, as determined during a standardized provocation in the ECC, was the measure with the best diagnostic value and repeatability. However, it did correlate only poorly to symptoms in-season, suggesting that the ECC paradigm addresses predominantly the acute-phase reaction upon allergen provocation while lacking information on the late-phase inflammatory response. In this respect, FeNO might be of added value to detect ongoing lower airway inflammation while NNO is of limited use because of its unspecificity. Taken together, this study provides useful information on the specificity, sensitivity, and

reproducibility of outcome measures for SAR that should be taken into account when planning clinical trials in the future.

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