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Non-invasive sampling methods of inflammatory biomarkers in asthma and allergic rhinitis

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CHAPTER 8

Nasal nitric oxide: longitudinal reproducibility and the effects of a nasal allergen challenge in patients with allergic rhinitis

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Abstract

Background: Exhaled NO (eNO) is a validated non-invasive marker of airway inflammation in asthma. In patients with allergic rhinitis (AR), increased levels of nasal NO (nNO) have also been measured. However, the applicability of nNO as a marker of upper airway inflammation awaits validation.
Aim: To test the longitudinal reproducibility of standardized nNO measurements in patients with AR and the effects of nasal allergen challenge.
Methods: 20 patients with clinically stable, untreated AR participated in a combined study design. First, reproducibility of nNO was tested over 1, 7, and 14-21 days. Subsequently, the effect of nasal allergen challenge on nNO was studied in a placebo-controlled, parallel design. nNO was measured with a chemoluminescence analyzer. Ten subjects randomly underwent a standardized nasal allergen challenge; 10 subjects received placebo. Response to nasal challenge was monitored by composite symptom scores.
Results: There was a good reproducibility of nNO up to 7 days (Coefficient of Variation (CV) over 1 (16.45%) and 7 days (21.5%)), decreasing over time (CV (14-21 days): 38.3%). As compared with placebo, allergen challenge caused a significant increase in symptom scores ($p<0.001$), accompanied by a decrease in nNO at 20 min post-challenge ($p=0.001$). Furthermore, there was a gradual increase in nNO at 7 h, reaching significance at 24 h post-allergen ($p=0.04$).
Conclusions: Similarly to eNO in asthma, nNO is a non-invasive marker, potentially suitable to monitor upper airway inflammation following allergen-induced late response. Present data show a good reproducibility of nNO measurements, decreasing over time, probably due to subclinical seasonal influences.

Introduction

Allergic rhinitis is an IgE-triggered chronic inflammatory disorder of the upper airways with pathophysiological and immunological links to allergic asthma (1). Recent studies providing evidence of systemic cross-talk between upper and lower airway compartments, have resulted in the concepts of "allergic airway disease" or 'combined allergic rhinitis and asthma syndrome' (CARAS) (2). The hallmark of CARAS is chronic airway inflammation, mainly characterized by mast cells, eosinophils and their pro-inflammatory products (3). Historically, airway biopsies have been regarded as the gold standard for the sampling of the allergic airway inflammation. However, the applicability of invasive methods is limited for repeated sampling, such as in clinical monitoring or intervention trials. In addition, biopsies are limited to a very small part of the airways. Therefore, several less or non-invasive methodologies are being developed, some of which have been validated (4). Nitric oxide (NO) is a gaseous molecule synthesized in the respiratory compartment by NO-synthases and can be detected in exhaled air of various species (5,6). Increased levels of eNO – originating from the lower airways - have been measured in asthmatic patients (7), with overall higher levels in allergic asthma (4). In patients with untreated asthma, levels of eNO appeared to correlate with the numbers of eosinophils within the airways (8,9). Likewise, both airway eosinophils and eNO can be reduced by anti-inflammatory therapy (10,11). Alternatively, increased eNO levels following tapering off inhaled corticosteroids (ICS) have been shown to predict asthma exacerbations (12). Hence, eNO measurement is presently a validated tool for non-invasive repeated assessment of the airway inflammation in asthma, both for intervention trials and clinical practice (4,13,14). In patients with AR, nasal eosinophilia and increased nNO levels have been demonstrated (15,16). These increased nNO levels may be the result of enhanced iNOS expression within the nasal epithelium due to persistent mucosal inflammation (17). In the upper airways, paranasal sinuses are major contributors to NO production: direct samplings from paranasal sinuses show substantially higher nNO-levels than nasal samplings (18). The applicability of nNO as a marker of upper airway inflammation, however awaits validation (17). With this study, we wished to test the applicability of nNO as a potential outcome parameter for clinical intervention trials. First, we studied the longitudinal reproducibility of standardized nNO measurements in clinically stable patients with untreated AR (5). Second, the effect of intranasal xylometazoline, a commonly used decongestant, on nNO was assessed. And finally, we performed a nasal allergen challenge in a randomized, placebo-controlled, parallel design, in the same group of patients. Some results have previously been reported in the form of an abstract (19).

Methods

SUBJECTS

Twenty multi- sensitized subjects with clinically stable allergic rhinitis participated in the study (Table 1). All subjects had a history of AR (intermittent or persistent) for at least 1 year prior to enrollment (1). Subjects with concomitant stable, intermittent or mild persistent asthma, using inhaled short-acting β2-agonists on demand only, were allowed to participate. During the study, they were asked to withhold the β2-agonists at least 6 hours before each visit. Concomitant maintenance anti-asthma or anti-allergy medication was discontinued at least 6 weeks prior to the study. Atopy was confirmed by a positive skin prick test for at least 2 of 6 common airborne allergens (grass, trees, Dermatophagoides pteronyssinus (HDM), Dermatophagoides farinae, cat-, and dog-dander, ALK Abelló, Nieuwegein, The Netherlands). Potential subjects with any other clinically relevant chronic or acute disorders were excluded. All eligible subjects were sensitized to at least one of the allergens used for nasal provocation, i.e. grasspollen, HDM- or cat-dander extracts. Symptomatic subjects with pollen allergy were tested outside the relevant pollen season (in the Netherlands outside the period May – August) and those with concomitant allergy to pets were only included, provided they had no close contact with pets (e.g. in their homes) during the study. AR symptoms were monitored throughout the study by a composite symptom score validated by Lebel, and subjects with a baseline symptom score of more than 2 on the allergen challenge day (Visit 2) were excluded (20). Furthermore, respiratory tract infections were excluded by patients' history at least 3 weeks prior and during the study. All subjects were non-smokers or ex-smokers (stopped at least 12 months prior to the study with less than 10 pack years). Starting the day before a study visit, all participants had to refrain from strenuous exercise and nitrate rich foods. The study protocol was approved by the Leiden University Medical Centre Ethics Committee and all subjects gave their written informed consent prior to enrolment into the study.

STUDY DESIGN

The study consisted of two parts (Figure 1). First we examined the reproducibility of nNO measurements over 1, 7, and 14-21 days. Second, we studied the effect of intranasal xylometazoline and allergen versus placebo on nNO levels. Before entering the study, the inclusion criteria were examined. Eligible subjects were included into the study and baseline nNO levels were measured (Visit 1). Fourteen to 21 days later (Visit 2), baseline nNO levels were repeated, and all subjects received intranasal xylometazoline (0.1%; Pharmachemie, Haarlem, The Netherlands). Nasal NO levels were recorded 30 min post-xylo-

metazoline. Subsequently, subjects randomly underwent a nasal challenge with either a relevant allergen (n=10) or its diluent (n=10) in a double-blind, parallel fashion. nNO measurements were repeated 20 min and 7 hours post-challenge. Symptom scores were recorded pre-challenge, post-diluent, 10 min after each subsequent challenge dose and hourly, until 7 hours post-challenge (20). Clinically stable subjects were dismissed from the unit 8-9 h post-challenge and returned 24 hours later (Visit 3) for nNO measurements and recording of symptom scores and 7 days later (Visit 4) for a final nNO measurement (Figure 1). The occurrence of adverse events was monitored throughout the study. In order to exclude an allergen-induced late phase bronchoconstriction, the airway response was measured by FEV_1 according to standardized lung function techniques at screening, pre- and 7 h post-allergen (Vmax Spectra, Sensor Medics, Bilthoven, The Netherlands) (21).

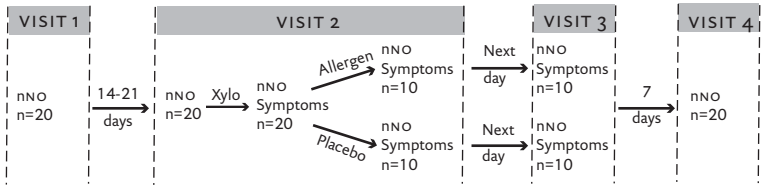

FIGURE 1 Study flowchart. nNO = nasal NO, N = number of subjects, Xylo = xylometazoline administration.

NASAL NO MEASUREMENTS

Nasal NO measurements were performed according to current ATS recommendations (5), using a chemoluminescence analyzer (Ecomedics CLD88sp, Duernten, Switzerland) with a nasal sampling tube. Briefly, subjects seated in an upright position were instructed to inhale "NO free" air (<1 ppb) through their mouth to total lung capacity and subsequently exhale through their mouth with a constant expiratory flow of 50 mL/s using online visual monitoring. An expiratory resistance was applied to ensure soft palate closure to exclude NO originating from the lower airways (eNO). During exhalation a Teflon tube connected to the NO analyzer aspirated nasal air with a constant flow rate of 0.3 L/min. During the study, nNO was measured in the same nostril, which was completely occluded with a foam plug (containing the Teflon tube) to prevent leakage of air. The contralateral nostril remained unobstructed. To minimize diurnal variation, all measurements were performed at the same time of the day (±2 hours). During each measurement, plateau nNO levels were determined and expressed as parts per billion (ppb). nNO levels were measured until three acceptable measures were obtained (within 10%) and the mean ppb-value was implicated into the analysis.

TABLE 1 SUBJECTS' CHARACTERISTICS

SUBJECT NR	AGE (YRS)	GENDER (M/F)	ATOPY	ASTHMA (+/-)
1	21	M	Cat, HDM, grass, trees, dog, D. Far	+
2	21	M	HDM, D. Far, dog	+
3	24	F	Grass, trees, Dog	-
4	24	F	Cat, HDM, trees, grass, D. Far	+
5	21	M	HDM, D. Far, dog	-
6	22	M	Grass, cat, dog, D. Far., trees, HDM	+
7	19	F	HDM, D. Far, trees, cat, dog	-
8	27	F	Cat, HDM, dog, D. Far	+
9	27	M	Cat, HDM, D. Far, grass, dog	+
10	50	F	Cat, dog, grass, trees	+
11	35	F	HDM, D. Far	-
12	32	M	HDM, D. Far	-
13	20	M	D. Far, HDM, grass, trees, cat, dog	+
14	22	F	Cat, HDM, D. Far, grass, dog	+
15	24	M	Grass, HDM, D. Far, cat, dog	+
16	21	M	HDM, D. Far, grass, trees, cat, dog	-
17	21	F	Grass, dog	-
18	19	F	Cat, HDM, D. Far, dog	-
19	43	F	D. Far, HDM, cat, dog	-
20	23	M	Grass, trees, dog	-

HDM = Dermatophagoides pteronyssinus, D. Far = Dermatophagoides farinae.

NASAL ALLERGEN CHALLENGE

Nasal allergen challenges were performed according to the previously validated protocol by de Graaf-in't Veld *et al* (22). Briefly, the procedure can be described as follows: selection of the relevant allergen was based on whichever allergen caused the largest wheal in the SPT in combination with clinical symptoms and depending on the season. Pre-challenge, subjects acclimatized for 30 minutes in the 'challenge' room and each nostril was inspected for accessibility using a nasal speculum. Subsequently, subjects received 1

puff of xylometazoline 0.1% in each nostril. Approximately 20 min later, the allergen's diluent (phosphate-buffered saline containing HSA 0.03% in benzoalkonium chloride 0.05%, ALK Abelló, Nieuwegein, The Netherlands) was sprayed in both nostrils (1 puff/nostril), followed by the allergen in 3 increasing concentrations of 100, 1,000 and 10,000 BU/mL (1 puff/nostril) each at 10 minutes intervals. Subjects were challenged with HDM, grasspollen or cat dander (ALK Abelló, Nieuwegein, The Netherlands). During placebo challenge, subjects received 4 times the allergen's diluent intranasally. The puffs were delivered as distally as possible into the nasal cavity using a nasal pump, delivering a fixed dose of 0.125 mL solution per puff (ALK Abelló, Nieuwegein, The Netherlands). Subjects were instructed not to inhale too deeply to prevent the solution to enter the lower airways. The nasal response at the respective time points was quantified by composite symptom scores validated by Lebel *et al* (20). Symptoms were recorded using the following scoring system: sneezes ≤2 = 0, sneezes 3-4 = 1 point, sneezes ≥5 = 3 points, anterior rhinorrhoea = 1 point, posterior rhinorrhoea = 1 point, difficult breathing = 1 point, one blocked nostril = 2 point, two blocked nostrils = 3 points, pruritus in the nose = 1 point, pruritus of palate or ear = 1 point, conjunctivitis = 1 point (total score 0-11). Symptom scores were recorded 10 min after each allergen dose and further dosing was discontinued at a total score of 6 or more, or after the highest dose was given (10,000 BU/mL) (22).

ANALYSIS

Prior to analysis, nNO data were tested for normal distribution using several tests, including the Kolmogorov-Smirnov test. These tests showed no significant deviation of normal distribution; hence, data were not log transformed. To assess the reproducibility of nNO measurements the within subject variation between visits was calculated and expressed as a CV (= the standard deviation expressed as percentage of the mean). The necessary change in nNO for the detection of an intervention effect was calculated with a power calculation program nQuery (nQuery advisor 3.0) using the within subject CV to estimate the standard deviation of the difference and based on a cross-over study with power = 80% and α=0.05, two-sided. The data were analyzed with a mixed model analysis of covariance (ANCOVA) with intervention, time, and intervention by time as fixed factors, the subject as a random factor and the measurement before challenge/placebo but after xylometazoline as covariate. Contrasts between placebo and allergen challenge were calculated at various timepoints. A treatment effect or contrast with a p-value less than 0.05 was considered statistically significant. All calculations were performed using SAS for windows V9.1.2 (SAS Institute Inc., Cary, NC, USA).

Results

REPRODUCIBILITY OF nNO

All subjects were able to perform the nNO maneuvers. On average (mean ± SD) 6.1 ± 2.4 maneuvers during maximally 7 minutes were needed to obtain three acceptable measurements (good plateau response and within 10%). Although comparable mean nNO levels were measured on visits 1 and 2 (mean ± SD 1017 ± 477 ppb and 1104 ± 496 ppb, respectively), there was a substantial within subject variability (CV = 38.3%; Figure 2, Table 2). Better reproducibility was found over shorter time intervals: 1 day and up to 7 days later, i.e. between Visit 2 and 3; and between Visit 2 and 4 (Table 2). To exclude potential influence of nasal allergen challenge, the reproducibility of nNO over 1 and 7 days was analyzed in the placebo group only.

The necessary change in nNO for the detection of a significant intervention effect (e.g. following drug treatment) was calculated using the within subject CV for several sample sizes in a cross-over study design (Table 3).

TABLE 2 REPRODUCIBILITY OF NASAL NO OVER VARIOUS TIME POINTS (MEAN ± SD).

	NUMBER OF SUBJECTS	FIRST MEASUREMENT	SECOND MEASUREMENT	CV (%) WITHIN SUBJECTS
Δ 14-21 days (Visit 1 - 2)	20	1017 ± 477 ppb	1104 ± 496 ppb	38.3
Δ 14-21 days (Visit 1 - 2)	10*	874 ± 310 ppb	977 ± 531 ppb	33.5
Δ 7 days (Visit 2 - 4)	10*	977 ± 531 ppb	1038 ± 285 ppb	21.5
Δ 1 day (Visit 2 –3)	10*	977 ± 531 ppb	968 ± 373 ppb	16.5

*n=10 subjects in the placebo challenge group only. cv = coefficient of variation.

TABLE 3 CHANGE IN NASAL NO FOR DETECTION OF A SIGNIFICANT INTERVENTION EFFECT

NO. OF SUBJECTS	Δ 7 DAYS	Δ 14-21 DAYS
12 subjects	261 ppb	510 ppb
16 subjects	220 ppb	430 ppb
20 subjects	194 ppb	379 ppb

Calculations based on a cross-over study with power = 80% and α=0.05, two-sided.

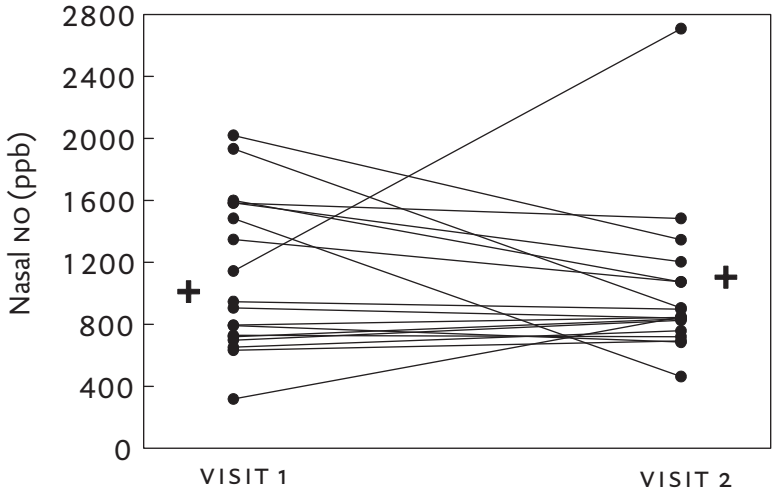

FIGURE 2 Individual baseline values of nasal no on visit 1 and 2 (Δ 14-21 days) for all subjects (N=20). + = mean nasal no level.

EFFECTS OF INTRANASAL XYLOMETAZOLINE AND NASAL CHALLENGES

All intranasal interventions (xylometazoline, allergen and the allergen's diluent) were well-tolerated by all subjects and no clinically significant adverse events occurred. Pre-challenge, none of the subjects had lower airway complaints and all FEV_1 values were similar to screening. At 7 h post-challenge, there was no significant fall in baseline FEV_1 following either intervention (mean ± SD: -0.03 ± 0.13 L (Allergen (A)); p=0.43; -0.03 ± 0.09 L (Placebo (P)); p=0.39). Neither were the changes in FEV_1 significantly different between the two challenges (p=0.88).

As compared with baseline measurements, 30 min post-administration, xylometazoline significantly decreased nNO levels in all patients by on average 24% (265 ppb; p=0.001).

For evaluation of the effect of nasal allergen challenge, post-xylometazoline symptom scores and nNO levels were used as baseline measurements in the analysis. Ten patients were randomized to intranasal allergen (2 subjects received cat-allergen; 2 grass-pollen; 6 HDM) and ten to placebo. As compared with placebo, intranasal allergen induced a nasal early allergic response (nasal EAR) in all subjects with a mean symptom score ± SD: 0.70 ± 0.82 (P) and 7.25 ± 1.58 (A) at 10 min post-challenge (Figure 3). In addition, all subjects had a nasal late allergic response (nasal LAR), defined as a symptom score above baseline on two consecutive timepoints between 3-7 h post-allergen

(mean ± SD: 0.24 ± 0.66 (P) and 1.3 ± 1.0 (A) (Figure 3) (22). The changes in symptom scores between allergen and placebo were significantly different ($p<0.001$).

As compared with placebo, nasal allergen challenge induced a significant decrease in nNO at 20 min post-challenge (estimated difference –514 ppb, 95% confidence interval (CI) -780 to -247 ppb; p=0.001). Seven hours post-allergen, both nNO levels and symptom scores were slightly increased as compared to placebo. At 24 hours post-challenge, there was a further increase in nNO levels following allergen as compared with placebo (estimated difference 344 ppb, 95% CI 24 to 664 ppb; p=0.04) (Table 4). Mean changes (± 95% CI) in symptom scores and nNO levels following allergen and placebo are shown in Figures 3 and 4.

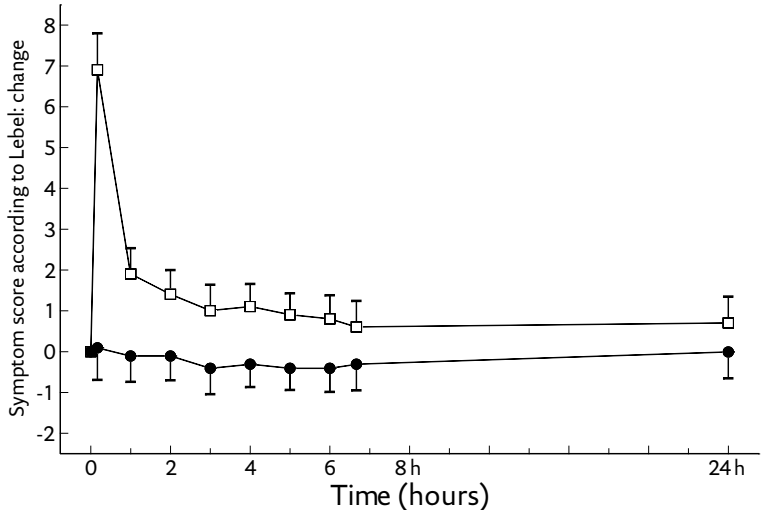

FIGURE 3 Mean change (± 95% confidence interval) in composite symptom scores following nasal allergen versus placebo challenge. Open squares: relevant allergen, closed dots: placebo.

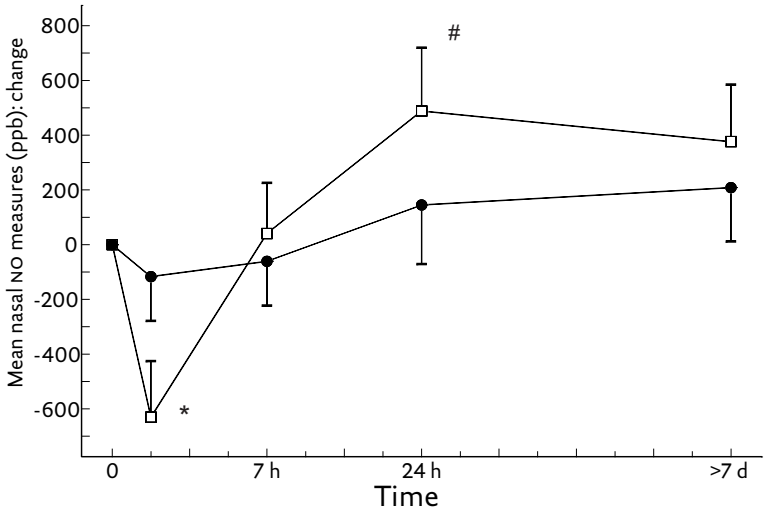

FIGURE 4 Mean change (± 95% confidence interval) in nasal NO concentrations (PPB) following nasal allergen versus placebo challenge. Baseline was set after xylometazoline administration in all subjects. Open squares: relevant allergen, closed dots: placebo. *Indicates a significant difference between allergen and placebo at 20 min post-challenge (P=0.001), #indicates a significant difference between allergen and placebo at 24 hours post-challenge (P=0.04).

TABLE 4 EFFECT OF NASAL ALLERGEN/PLACEBO CHALLENGE ON NASAL NO LEVELS (MEAN ± SD).

	PRE-CHALLENGE	20 MIN POST-CHALLENGE	7 H POST-CHALLENGE	24 H POST-CHALLENGE	7 DAYS POST-CHALLENGE
Allergen					
Nasal NO	980 ± 345 ppb	336 ± 287 ppb	946 ± 267 ppb	1393 ± 307 ppb	1272 ± 294 ppb
Placebo					
Nasal NO	717 ± 358 ppb	682 ± 258 ppb	752 ± 220 ppb	968 ± 373 ppb	1038 ± 285 ppb

Discussion

We have demonstrated that nNO is a reproducible parameter over short time intervals in clinically stable patients with untreated AR. A potential explanation for the increased variability over time in our study could be due to (subclinical) seasonal effects. Moreover, we showed that unlike placebo challenge, intranasal decongestant and allergen challenge significantly affected nNO levels. Overall, our data suggest that nNO may be a potential outcome parameter in clinical intervention trials in patients with AR.

Our findings confirm and extend data from previous studies in both healthy subjects and patients with AR applying different nNO sampling methods (23,24). While in these studies recordings were made during breath holding, we measured nNO according to ATS recommendations (5,23,24). This method holds sampling of nNO during oral exhalation against a fixed resistance for optimal closure of the velum to prevent mixing from other (airway) compartments. Applying this technique, baseline nNO levels on both visits 1 and 2 were higher to those reported in two other studies in patients with untreated AR (25,26). This discrepancy can be ascribed to the higher sampling flow rate applied in our study, as comparable nNO levels have been measured in another study in patients with AR applying a similar flow rate (0.25 L/min compared to our 0.3 L/min) (27).

We found good reproducibility of nNO levels in patients with untreated AR up to 7 days. Comparable data have been reported in healthy subjects over a similar time period (23). The decay in reproducibility over time (14 to 21 days) may be caused by subclinical seasonal or weather influences and hence, should be taken into account when calculating the necessary sample size. Based on our findings, it can be anticipated that in clinical intervention trials outside pollen season, a change in nNO levels beyond baseline variation will be needed to detect a potential drug effect. So far, there are few data on nNO as an outcome parameter in clinical intervention trials. In a comparative study mean nNO levels were approximately 55% lower (mean difference of 846 ppb) in patients with AR treated with nasal corticosteroids as compared to nNO measured in patients with untreated AR (16). Although this was not a placebo-controlled study, it provides further evidence on potential applicability of nNO as a biomarker of upper airway allergic inflammation, even over longer (treatment)-periods. Finally, in accordance with other studies, we found marked inter-individual differences in nNO (Figure 2) (27). In order to minimize the effect of between-subject variability, a cross-over design is recommended.

Since xylometazoline is often incorporated in a nasal allergen challenge protocol when measurements of pro-inflammatory biomarkers (e.g. in nasal

lavage) are performed, we wished to evaluate its effect on pre-allergen nNO levels as part of the analysis (28,29). We found a 24% mean fall in nNO from baseline levels, at 30 min following intranasal xylometazoline. In correspondence with our findings, previous studies in healthy volunteers and patients with AR showed a mean decrease in nNO levels by 12-14% and 20-25%, respectively, 10 minutes following administration of intranasal decongestants with comparable pharmacological properties as xylometazoline (25). Moreover, a comparable fall in nNO has been encountered in subjects with an upper respiratory tract infection following intranasal oxymetazoline (30). The reduction in nNO is possibly caused by the xylometazoline-induced vasoconstriction, decreasing NO-diffusion (25). In addition, evidence from in vitro animal studies suggests additional anti-inflammatory effects through inhibition of the NO-producing enzymes (31). However, prolonged use of nasal decongestants in humans is associated with unwanted side effects, surpassing these potentially beneficial properties (32).

In contrast to sham challenge, nasal allergen challenge (irrespective of the relevant allergen applied) caused similar symptoms and signs of acute rhinoconjunctivitis in all subjects, consistent with a nasal EAR (22). In agreement with previous studies in allergic rhinitis, we found a significant fall in nNO at 20 minutes post-allergen (16). At least one possible explanation for this phenomenon may be the allergen-induced swelling of the nasal mucosa during the nasal EAR – not occurring after placebo – resulting in hampered NO diffusion (33). Our data are consistent with findings by Colantonio *et al* in patients with allergic rhinitis and polyposis nasi, in whom - despite iNOS-upregulation – decreased nNO levels have been found (34). In this study, nNO levels appeared to be inversely correlated to the polyp size and raised following treatment of the polyps; suggestive of a mechanic blockade of NO diffusion from the sinuses. In addition, similar observations have been reported for the lower airways, measuring eNO following bronchial allergen challenge in asthma (35,36). In these studies, there was an (albeit statistically non-significant) decrease in eNO accompanied by allergen-induced bronchial obstruction during the early asthmatic response

In agreement with previous data on eNO following allergen-induced bronchial LAR, we found a (gradual) increase in nNO at 7 h (i.e. during the nasal LAR), reaching statistical significance at 24 h post-allergen (35,37). However, it should be noted that pre-challenge administration of intranasal xylometazoline in the present study protocol, may account for potentially underestimated values of both the symptom scores and nNO up to 7 h post-allergen (38). Indeed, at 24 h post-challenge when the xylometazoline effect had worn off, the increase in nNO became statistically significant. Similarly, Hanazawa *et al* found increased nNO levels in patients with AR at 24 h following intrana-

sal administration of eotaxin (39). In addition, an increase in nasal eosinophils was measured at 8 h post-eotaxin; accompanied by maximal levels of nNO (no pretreatment with nasal decongestant) (39). Likewise, during allergen-induced LAR in asthma, increases in eNO appeared to be accompanied by sputum eosinophilia (37). Based on these data, nNO may be a useful outcome parameter for allergen-induced airway inflammation during the LAR in patients with AR, preferably not pre-treated with nasal decongestant.
Based on the link between the upper and lower airways, it could be anticipated that nasal allergen challenge may affect lung function especially in patients with allergic rhinitis and asthma (40). Although in the present study there were no significant changes in FEV_1, this does not entirely rule out co-existent pro-inflammatory events within the lower airways induced by nasal allergen. However, assessments of the lower airway inflammation were kept outside the scope of this study. Moreover, including a limited number of AR patients in combination with different phenotypes (with and without concomitant asthma) precludes a heterogeneous lower airway response to nasal allergen.
In conclusion, nNO measurement is a quick and non-invasive tool in patients with allergic rhinitis. Based on our data and previous other findings in AR patients, nNO may potentially serve as a biomarker of (allergen-induced) nasal inflammatory response during "the stable phase", such as the nasal LAR (27,39). However, confounding factors such as acute allergen-induced nasal response resulting in massive rhinorrhea and occlusion of the paranasal sinuses or pretreatment with nasal decongestants or corticosteroids may limit the applicability of nNO during the nasal EAR.

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