

# Reproducibility of nasal allergen challenge responses in adults with allergic rhinitis

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**Background:** Allergic rhinitis is characterized by nasal inflammation in response to allergen exposure. Nasal allergen challenges are used in clinical trials evaluating drug effects. Reproducibility of nasal secretion cytokine responses and physiological measurements are needed to determine the optimum measurements and power calculations for future studies. We have investigated the reproducibility of nasal cytokine measurements, using ready-to-use polyvinyl acetate sponges to collect nasal secretions, and measurements of nasal physiological responses.

**Methods:** Twelve subjects with allergic rhinitis and no history of respiratory disease, and 12 subjects with asthma and allergic rhinitis underwent a nasal allergen challenge. This was repeated at 7–14 days later.

**Results:** There were increases in IL-5, CCL11, and CXCL8 responses post-challenge (all  $P < 0.05$ ). There was better reproducibility at later time points when higher cytokine levels were detected for IL-5 ( $r_i = 0.64$  at 8 hours) and CXCL8 ( $r_i = 0.91$  at 8 hours). Acoustic rhinometry provided good to excellent reproducibility ( $r_i = 0.66$ – $0.89$ ). Rhinomanometry had lower reproducibility with greater variation ( $r_i = 0.10$ – $0.70$ ), with some subjects unable to perform the measurement. Multiplex immunoassays provided greater sensitivity for CCL11 measurements. There were no differences between allergic rhinitis patients with and without asthma.

**Conclusion:** Polyvinyl acetate sponges are a practical and reproducible way to sample nasal secretions. Acoustic rhinometry is a practical and reproducible method for assessing physiological responses. There were no differences in nasal response due to the presence of concurrent asthma.

**Keywords:** acoustic rhinometry, total nasal symptom score, polyvinyl acetate sponges, IL-5, CXCL8, CCL11

## Introduction

Allergic rhinitis is characterized by nasal inflammation that occurs in response to allergen exposure. T-helper type 2 ( $T_H2$ ) lymphocytes orchestrate this allergic inflammation through the production of cytokines including IL-4, IL-5, and IL-13, which cause a range of pathophysiological effects including  $T_H2$  differentiation, eosinophil recruitment and activation, and tissue remodeling.<sup>1,2</sup> Many patients with allergic rhinitis also suffer with asthma, and allergen exposure in these individuals can trigger both nasal and lung inflammation.<sup>3–5</sup>

Nasal allergen challenges have been used to investigate the pathophysiology of allergic rhinitis and in clinical trials to evaluate the effects of drugs for the treatment of allergic rhinitis.<sup>6–8</sup> Furthermore, nasal allergen challenge can be used as a surrogate model for allergic inflammation in the lungs, for the purpose of evaluating novel drugs for the treatment of asthma.<sup>9</sup> There are no gold standard methods for measuring the

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nasal allergic response. Different methods have been used to sample nasal secretions including microsuction, nasal lavage, filter paper, cotton wool, and absorptive sponges for the purpose of measuring cytokine responses.<sup>10–14</sup>

There are different methods for measuring changes in nasal physiology after allergen challenge, including anterior and posterior rhinomanometry, and acoustic rhinometry.<sup>15,16</sup> Rhinomanometry measures total nasal resistance, while acoustic rhinometry uses sound pulses to quantify the nostril lumen surface area.

It is important to determine the reproducibility of cytokine responses and physiological measurements after nasal allergen challenge, as this allows the optimum measurements to be selected and power calculations to be performed for future studies. For example, Scadding et al demonstrated that absorptive polyurethane sponges provided a superior nasal sampling method compared to synthetic filter paper, and used these polyurethane sponges to assess the reproducibility of cytokine responses to challenges performed on different days.<sup>17</sup>

We investigated nasal cytokine measurements after allergen challenge using polyvinyl acetate sponges to collect secretions.<sup>14</sup> These polyvinyl acetate sponges are purpose built for sampling nasal secretions, as they are pre-sterilized and are designed for ease of manual placement in the nostrils. The main aims of this study were to 1) characterize the time course and reproducibility of cytokine responses measured using polyvinyl acetate sponges and 2) assess the reproducibility of rhinomanometry and acoustic rhinometry. We also compared the nasal allergen responses of two subsets of individuals with allergic rhinitis: individuals with asthma compared to individuals without asthma.

## Materials and methods

### Subjects

Twenty-four subjects participated: 12 subjects with allergic rhinitis and no history of respiratory disease, and 12 subjects with asthma and allergic rhinitis. All subjects were non-smokers with a <1 pack-year history and asthma patients were corticosteroid naïve. Inclusion criteria consisted of age between 18 and 70 years; a positive skin prick test to cat, house dust mite, or grass (ALK, Hørsholm, Denmark) at screening; and no use of medication for asthma in the last 4 weeks other than short-acting beta agonists. Exclusion criteria included uncontrolled asthma, cigarette smoking within the last 12 months, use of nasal medications, respiratory tract infection within 30 days of screening, ongoing symptoms of rhinitis or abnormal nasal examination, skin prick positive to

cat with daily exposure to cats. Written informed consent was obtained and the study was approved by a local research ethics committee (Greater Manchester Central, 10/H1008/86).

### Study design

The study was conducted outside the UK hay fever season. Demographic data were collected including FEV<sub>1</sub> using a spirometer (Vitalograph, Buckinghamshire, UK) and related to reference values.<sup>18</sup> Eligible subjects were administered a nasal allergen challenge using the allergen that had caused the greatest skin prick test reaction at screening. This was either cat, house dust mite, or grass. The nasal allergen challenge involved initial administration of 100 µL of normal saline allergen diluent into each nostril as previously described, and then 100 µL of the selected allergen (50,000 SQ-U/mL concentration) was administered into each nostril by Bidose Liquid nasal spray after 30 minutes (Aptar Pharma, Radolfzell, Germany).<sup>1,19,20</sup> Nasal measurements were determined as follows: total nasal resistance using posterior and anterior rhinomanometry (rhinomanometer; GM Instruments Ltd., Kilwinning, Scotland, UK), and total nasal surface area using acoustic rhinometry (acoustic rhinometer; GM Instruments Ltd.). Rhinomanometry measures air flow and pressure through the nostrils to determine total nasal resistance (kPa/L/s). Acoustic rhinometry uses sound pulses emitted up a hollow, open ended tube sealed onto the nostril, and interprets the reflected sound waves to give a measurement of the nostril lumen surface area (cm<sup>2</sup>) at various distances into the nostril; we measured the surface area at 3 cm from the nostril opening, with the readings of both nostrils added together to give total surface area at 3 cm.<sup>21</sup> For all three methods of nasal measurement, technically acceptable measurements were defined as subjects performing the measurement with reproducible results (three measurements within 20% of each other) at each time point. Nasal secretions were sampled using Ivalon® Post-op Sinus Pack K9 (Ivalon Inc., San Diego, USA) nasal sponges as detailed below. Nasal symptoms were quantified using the total nasal symptom score (TNSS) questionnaire, a 12-point score consisting of four categories rated 0–3: rhinorrhoea, sneezing, nasal itching, and nasal congestion. The nasal symptom score (NSS) questionnaire published by Riechelmann et al, consisting of a 6-point score assessing rhinorrhoea, sneezing, and extra-nasal symptoms, was also used.<sup>22</sup> All measurements and nasal sampling were collected from post-diluent (10 minutes before allergen administration) and up to 8 hours after allergen administration (30, 60, 120, 240, 360, and 480 minutes). This nasal allergen challenge was repeated at 7–14 days later.

## Nasal secretions

Nasal secretions were collected and processed as previously described.<sup>14</sup> A pair of nasal sponges were weighed dry in a 15 mL tube, then inserted, one into each nostril, and left in situ for 5 minutes. On extraction, the sponges were reweighed. They were then irrigated with 3 mL of normal saline to flush out the cytokines and the sealed tube was placed in ice on a roller. After 2 hours, the sponges were inserted into a sterile 5 mL syringe barrel and placed back into the tube. This was then centrifuged at  $1,500\times g$  and  $4^{\circ}\text{C}$  for 15 minutes. The resulting supernatant was aliquoted and stored at  $-80^{\circ}\text{C}$  for later analysis.

## Measurement of nasal secretion supernatant biomarkers

The supernatants were assayed using the following ELISA kits according to manufacturer's instructions: IL-5 (Human IL-5 DuoSet, range of quantification 23.4–2,000 pg/mL; R&D Systems, Abingdon, UK), CXCL8 (Human CXCL8/IL-8 DuoSet, range of quantification 31.2–2,000 pg/mL; R&D Systems), CCL11 (Human CCL11/Eotaxin DuoSet, range of quantification 15.6–1,000 pg/mL; R&D Systems). Supernatants from the second challenge performed on the 12 patients with asthma and allergic rhinitis were also assayed using Mesoscale Sector<sup>®</sup> (from now on referred to as MS) Imager 6000 (Meso Scale Discovery, Rockville, MD, USA) according to manufacturer's instructions for the following cytokines: CCL11, CCL26, CXCL10, CCL2, CCL13, CCL22, CCL4, CCL17, INF- $\gamma$ , IL-1 $\beta$ , IL-2, IL-4, IL-5, CXCL8, IL-10, IL-12p70, IL-13, TNF $\alpha$  (Human TH1/TH2 10-Plex Ultra-Sensitive Kit; Meso Scale Discovery) and CCL11, CCL26, CXCL10, CCL2, CCL13, CCL22, CCL4, CCL17 (Human Chemokine 9-Plex Ultra-Sensitive Kit; Meso Scale Discovery).

## Statistics

A sample size of 12 subjects per group was chosen for reproducibility experiments, as it allows sample size calculations using the within-subject SD to be performed for future studies with 90% power; these calculations still have 73% power if the true SD is 25% larger than observed.<sup>23</sup> Results were assessed for normality using Kolmogorov–Smirnov test. CCL11 MS, CXCL8 MS, and CXCL10 MS data were normally distributed. Differences in these measurements pre- and post-allergen exposure were compared using one-way ANOVA with Dunnett's multiple comparison post hoc test. All other measurements were not normally distributed, and differences between pre- and post-allergen measurements were compared using Friedman's test with Dunn's multiple

comparison post hoc test. The pre-challenge value was the post-diluent time point. Comparisons between subjects with allergic rhinitis only and those with asthma and allergic rhinitis were performed using Mann–Whitney tests and estimating area under the curve. *P*-values  $<0.05$  were considered significant. The reproducibility of nasal allergen challenges was assessed using intraclass correlation  $r_i$  (ICC) using the Fleiss criteria ( $r_i <0.40$  poor reproducibility, 0.40–0.59 fair, 0.60–0.74 good, and  $>0.74$  excellent reproducibility).<sup>24,25</sup> Results below the lower limit of quantification were assigned a value of half of the lower limit of quantification. For ICC reproducibility analysis, only results above the lower limit of quantification were used. Cytokines measured by MS were assessed for correlation with ELISA data using Pearson's correlation coefficient. Symptom scores were assessed for correlation with physiological measurements at the 30-minute time point using Spearman's correlation coefficient. GraphPad PRISM<sup>®</sup> version 5.02 (GraphPad Software Inc., La Jolla, CA, USA) was used for all analysis except ICC, which was calculated using SPSS version 19 (IBM Corporation, Armonk, NY, USA).

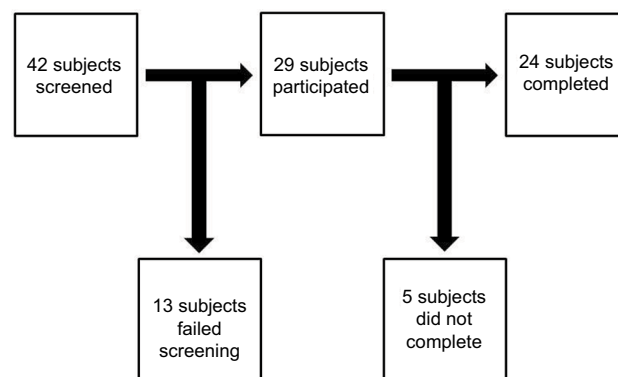
## Results

Figure 1 shows how the study population was recruited. For the five subjects who did not complete the study, two were withdrawn because of a coryzal illness (common cold), while three withdrew for personal reasons.

The demographics of the participants are shown in Table 1. The measurements were performed on all 24 subjects unless stated otherwise.

## Nasal symptom scores

Both NSS ( $n=24$ ) and TNSS ( $n=18$ ) demonstrated significant increases in symptoms at 30 minutes after allergen chal-



**Figure 1** Study population recruitment.

lence that steadily reduced thereafter (Figure 2A, B). The ICC for NSS on different days after nasal challenge varied from 0.52 to 0.69 at different time points (Table S1), with a mean of 0.60 indicating good reproducibility overall. The ICC for TNSS on different days after nasal challenge varied from 0.63 to 0.86 at different time points (Table S2), with a mean of 0.78 indicating excellent reproducibility overall. There was a correlation at 30 minutes post-allergen challenge between nasal symptoms and acoustic rhinometry measurements, eg, for TNSS the Spearman's coefficients ( $r$ ) were  $-0.59$  ( $P=0.014$ ) and  $-0.63$  ( $P=0.005$ ) for the first

and second challenge, respectively. Associations between rhinomanometry and symptoms were mostly not significant (Tables S3 and S4).

## Nasal secretion weight

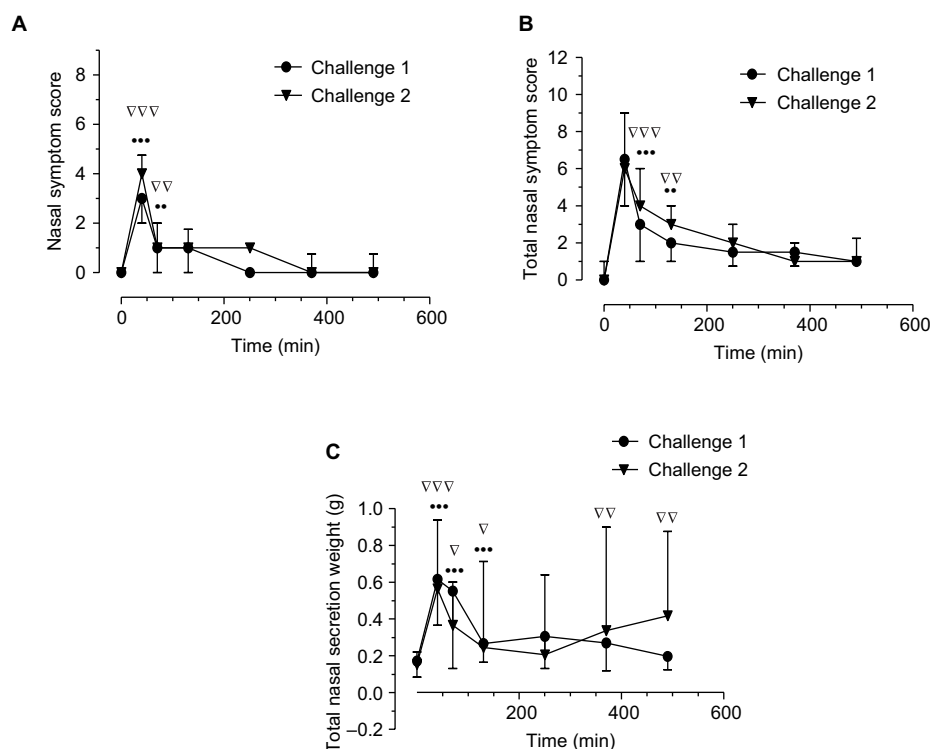
There were significant increases in nasal secretion weights after allergen challenge (Figure 2C). Nasal secretion weights peaked 30 minutes after nasal challenge. The ICC for nasal challenge on different days varied from 0.63 to 0.87 at different time points (Table S5), with a mean of 0.74 indicating excellent reproducibility overall.

**Table 1** Subjects' clinical features

Characteristics	Asthma with allergic rhinitis subjects (n=12)	Allergic rhinitis only subjects (n=12)
Age (years)	40 (21–53)*	34 (20–55)*
Sex	7 M, 5 F	7 M, 5 F
FEV <sub>1</sub> (L)	3.22±0.69	3.71±1.23
FEV <sub>1</sub> % pred	92.5±9.5	99.3±15.3
BMI	28.4±4.6	27.1±6.8

**Notes:** All values are expressed as mean ± SD apart from those indicated by \*, which are expressed as median with minimum and maximum.

**Abbreviations:** BMI, body mass index; F, female; M, male.



**Figure 2** Post-nasal allergen challenge symptom and nasal secretion changes in allergic rhinitis patients.

**Notes:** Physiological changes were measured before (0 minutes) and at various time points post-challenge. Measurements were nasal symptom score (A), total nasal symptom score (B), and nasal secretion weight (C). Each subject underwent two allergen challenges with results being presented separately. Data are presented as median values with interquartile range. Nasal allergen was administered at 10 minutes. Measurements post-allergen challenge were compared to their respective pre-challenge concentrations (time 0) using Friedman's test with Dunn's multiple comparison post hoc test: ▽  $P<0.05$ ; ▽▽▽  $P<0.001$ ; ●●● or ▽▽▽  $P<0.001$ ; ● represents challenge 1 and ▽ represents challenge 2.

## Cytokines

No subjects produced detectable IL-5 concentrations before allergen challenge. IL-5 concentrations increased significantly after both nasal allergen challenges (Figure 3A), with 12 and 16 subjects producing detectable IL-5 concentrations after the first and second nasal allergen challenges, respectively, at 480 minutes. The ICC for IL-5 measurements after nasal challenge on different days varied from 0.17 to 0.64 at different time points (Table S6), with good reproducibility (ICC = 0.64) at 480 minutes when the highest IL-5 levels were observed.

CXCL8 was detected in all subjects before and after allergen challenge. CXCL8 concentrations increased significantly after both nasal allergen challenges (Figure 3B). The ICC ranged from 0.51 to 0.91 at different time points (Table S7), with excellent reproducibility at the later time points (360 and 480 minutes) when the highest CXCL8 levels were observed.

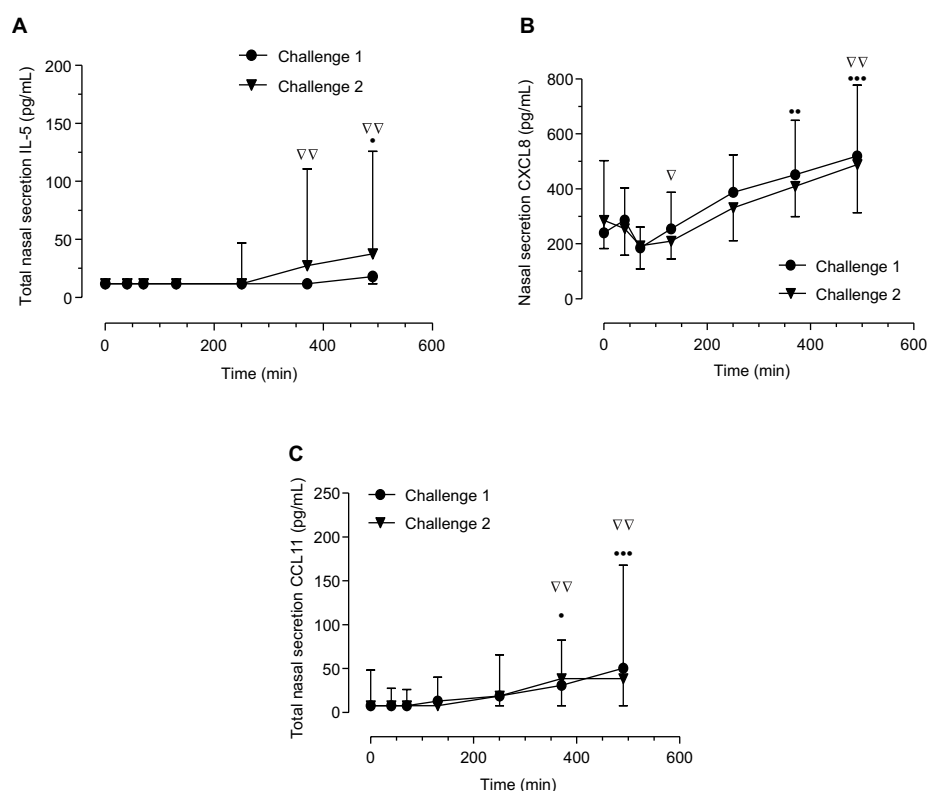
CCL11 concentrations increased significantly after both nasal allergen challenges (Figure 3C). CCL11 was detectable in eight subjects before the first challenge, and six of these subjects before the second challenge. Eighteen and 19 sub-

jects produced detectable concentrations of CCL11 after the first and second nasal allergen challenges, respectively. The ICC at different time points varied from 0.45 to 0.94 (Table S8), with better reproducibility at the earlier time points (30–240 minutes) when low CCL11 levels were observed.

Analysis of MS data for samples collected during the second allergen challenge in 12 asthma patients with allergic rhinitis showed statistically significant increases in CCL2, CCL4, CCL11, CCL17, CCL26, IL-1 $\beta$ , IL-5, IL-13, CCL13, TNF $\alpha$ , and CXCL8 (Figure 4 and Figure S1). Other cytokines showed no statistically significant increases. The Pearson's coefficients ( $r$ ) of the MS and ELISA cytokine data for IL-5 ( $r=0.97$ ,  $P<0.0001$ ) and CXCL8 ( $r=0.58$ ,  $P<0.0001$ ) demonstrated good correlation between methods, while for CCL11 ( $r=0.46$ ,  $P=0.021$ ) the correlation was lower (Figure S1). The MS CCL11 results were higher compared to the ELISA data at every time point.

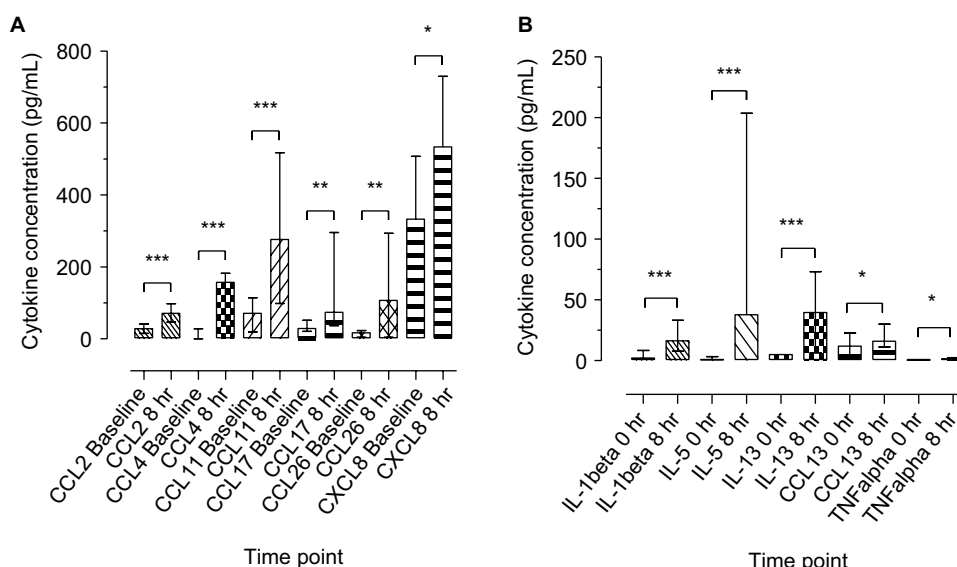
## Posterior and anterior rhinomanometry

Out of 23 subjects, 19 produced technically acceptable posterior rhinomanometry measurements. There was an increase



**Figure 3** Changes in cytokine concentrations post-nasal allergen challenge in nasal secretions of allergic rhinitis patients.

**Notes:** Nasal secretion samples were collected before (0 minute) and at various time points post-challenge. Concentrations of IL-5 (A), CXCL8 (B), and CCL11 (C) were measured by ELISA. Each subject underwent two allergen challenges with results being presented separately. Nasal allergen was administered at 10 minutes. Data are presented as median values with interquartile range. Cytokine concentrations post-allergen challenge were compared to their respective pre-challenge concentrations (time 0) using Friedman's test with Dunn's multiple comparison post hoc test: • $P<0.05$ ; ∇ $P<0.01$ ; •• $P<0.001$ ; • represents challenge 1 and ∇ represents challenge 2.



**Figure 4** Changes in cytokine concentrations post-nasal allergen challenge in nasal secretions measured by Mesoscale Sector Imager.

**Notes:** Data from the second nasal allergen challenge (of 12 asthma patients with allergic rhinitis) of nasal secretion samples collected before (0 minutes) and at 8 hours post-challenge of CCL2, CCL4, CCL11, CCL17, CCL26, and CXCL8 (**A**); IL-1 $\beta$ , IL-5, IL-13, CCL13, and TNF $\alpha$  (**B**). Data are presented as median values with interquartile range. Cytokine concentrations post-allergen challenge were compared to their respective pre-challenge concentrations (time 0) using Friedman's test with Dunn's multiple comparison post hoc test: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

in total nasal resistance 30 minutes after nasal allergen administration (Figure 5A and Table S9). The range of ICC for posterior rhinomanometry at different time points varied from 0.32 to 0.56 (Table S9) with a mean of 0.43 indicating fair reproducibility overall.

Out of 22 subjects, 12 produced technically acceptable anterior rhinomanometry data. There was a significant increase in total nasal resistance 30 minutes after nasal allergen administration (Figure 5B and Table S10). The range of ICC for anterior rhinomanometry at different time points varied from 0.10 to 0.70 (Table S10) with a mean of 0.36 indicating poor reproducibility overall.

## Acoustic rhinometry

All subjects who performed acoustic rhinometry produced technically acceptable data ( $n=21$ ). There were significant decreases in total nasal area 30 minutes after allergen challenge (Figure 5C and Table S11). The range of ICC at different time points after nasal challenge varied from 0.66 to 0.89 (Table S11) with a mean of 0.81 indicating excellent reproducibility overall.

## Allergic rhinitis versus asthma and allergic rhinitis subjects

There was no statistical difference found between the 12 allergic rhinitis subjects with asthma and the 12 allergic rhinitis

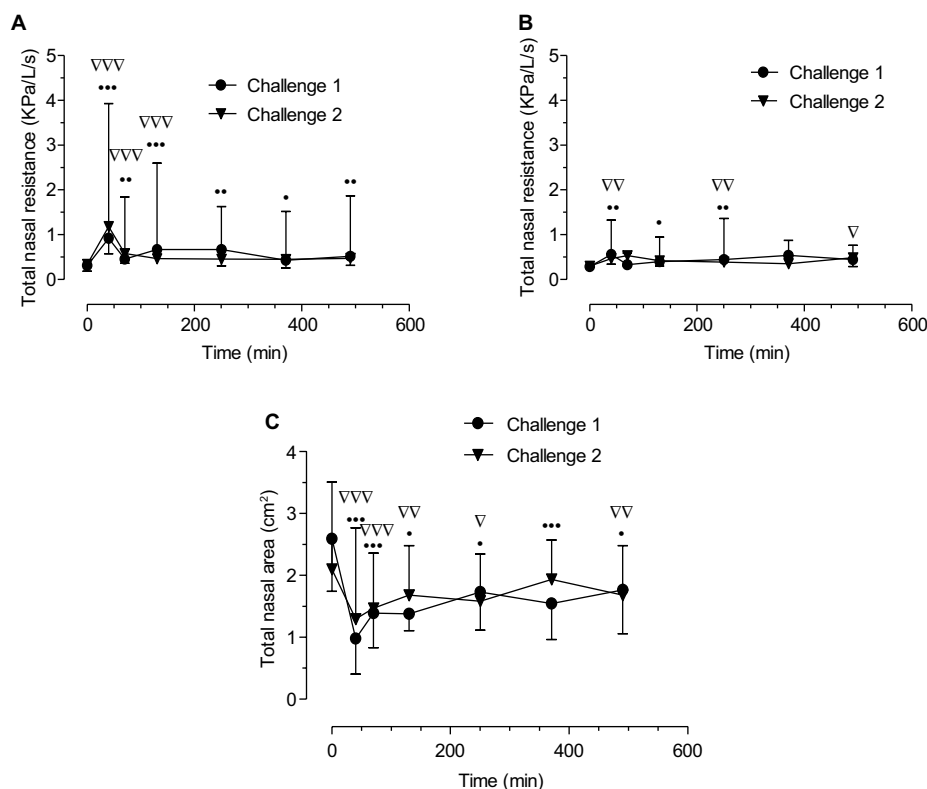
subjects without asthma on any measurement (Figures S2–S5, which also show area under the curve analysis).

## Discussion

The main novel aspects of this study were to document the reproducibility of nasal cytokine measurements after allergen challenge using polyvinyl acetate sponges, and to compare the practicality and reproducibility of nasal physiological measurements during challenges. We observed good reproducibility for IL-5, CXCL8, and CCL11 levels after allergen challenge, but this reproducibility varied according to the time point. For IL-5 and CXCL8, there was better reproducibility at later time points during the late allergic response when the cytokine response was higher. This highlights the importance of selecting the optimum time points to perform these measurements. Furthermore, we demonstrated that acoustic rhinometry is the method that all subjects were able to perform and provided good to excellent reproducibility. In contrast, rhinomanometry was less practical and had lower reproducibility. Acoustic rhinometry was also more closely associated with nasal symptoms compared to rhinomanometry.

The physiological response to allergen challenge has been measured using a variety of methods, but there is no accepted gold standard.<sup>26–30</sup> We investigated three methods of monitoring nasal physiological responses after allergen





**Figure 5** Physiological changes post-nasal allergen challenge in allergic rhinitis patients.

**Notes:** Physiological changes were measured before (0 minutes) and at various time points post-challenge. Measurements were taken using posterior rhinomanometry (A), anterior rhinomanometry (B), and acoustic rhinometry (C). Each subject underwent two allergen challenges with results being presented separately. Nasal allergen was administered at 10 minutes. Data are presented as median values with interquartile range. Measurements post-allergen challenge were compared to their respective pre-challenge concentrations (time 0) using Friedman's test with Dunn's multiple comparison post hoc test: • or ∇  $P < 0.05$ ; •• or ∇∇  $P < 0.01$ ; ••• or ∇∇∇  $P < 0.001$ ; • represents challenge 1 and ∇ represents challenge 2.

challenge: posterior rhinomanometry, anterior rhinomanometry, and acoustic rhinometry. In terms of reproducibility and practicality, we found that acoustic rhinometry was the superior technique. Posterior rhinomanometry required subject participation in holding their throat and tongue still, which some participants were unable to do. Anterior rhinomanometry does not require subject participation, but suffered from missing data at time points when rhinorrhea occurred which prevented sticking of the tape required to occlude the nostril. Additionally, in some cases, complete nasal congestion occurred, so the anterior rhinomanometer could not register a nasal pressure gradient. In contrast, acoustic rhinometry was an easy procedure for participants to learn and had excellent reproducibility. It also was the only physiological method with significant correlation with TNSS at 30 minutes in both challenges. An association between physiological changes and nasal symptoms has previously been reported.<sup>15</sup>

We chose to measure IL-5, CXCL8, and CCL11 based on consistent previous literature showing an elevation in the levels of these mediators after nasal allergen challenge.<sup>6,7,17,31,32</sup> We used standard immunoassay techniques for measuring

cytokines published in the literature.<sup>6,7,14,32</sup> Based on previous studies, we used a washout period of 7–21 days, which is also a common washout period in crossover clinical trials investigating drug effects.<sup>6,7,32</sup>

We did not set out to characterize the early-phase response in detail, but focused on sampling during the late-phase response. For both IL-5 and CXCL8, there were clear late-phase responses after both challenges, as shown in previous studies.<sup>6,13,17,31,32</sup> IL-5 was not measurable before allergen challenge in any individual, making it easier to observe the late-phase response. At early time points, only small concentrations were being secreted or were not quantifiable as in the case of IL-5.<sup>6,32</sup> This produces a relatively wide range of concentrations compared to the mean at early time points, reducing the ICC. At later time points, the concentrations secreted increase, thereby reducing the between-patient variability and improving ICC.

For CCL11, the ELISA measurements were able to demonstrate the late-phase response, but the reproducibility was lower at later time points after allergen challenge. Some previous studies have shown no significant increase

after allergen challenge for this eosinophil chemoattractant, although some studies have shown a positive response.<sup>6,13,33–35</sup> The wide variation in the late-phase CCL11 concentrations appears to be due to some individuals producing CCL11 and those that are “non-producers”. The low CCL11 concentrations at early time points combined with the use of half the lower limit of quantification for data points of subjects not producing CCL11 means that the ICC values at early time points are driven by low/absent values. ICC decreased as the mean concentration increased at later time points. These differences in the literature and in reproducibility may be related to the sensitivity of the immunoassays to detect CCL11; we found that MS measurements provided greater sensitivity for CCL11 measurements and were able to demonstrate a significant increase during the late phase. This highlights the importance of using sensitive immunoassays to measure cytokine responses after nasal allergen challenge.

The MS measurements demonstrated significant increases in T<sub>H</sub>2 cytokines (IL-5, IL-13, CCL11, and CCL26) during the late-phase response. This is in agreement with previous publications.<sup>6,12,13</sup> IL-1 $\beta$ , CCL2, CCL4, CCL13, CCL17, CXCL8, and TNF $\alpha$  were also found to significantly increase post-allergen challenge, although for CCL13 and TNF $\alpha$ , these increases were numerically small. Due to budget constraints, further MS measurements were not performed.

There is limited evidence in the literature concerning reproducibility of nasal allergen challenges. Proud et al investigated the reproducibility of nasal allergen challenges following intranasal corticosteroid treatment.<sup>20</sup> There was excellent reproducibility for sneezing and lysozyme secretion, good reproducibility for symptom scores and albumin release, but only poor reproducibility for kinin release. Scadding et al investigated the nasal responses of 20 grass pollen allergic subjects, comparing three mediums for collecting nasal samples: Accuwick Ultra (fibrous hydroxylated polyester), 111 (100% cellulose fibers), and synthetic polyurethane sponge. The different mediums were tested in pairs in each subject and inserted into different nostrils using forceps. The polyurethane sponge was reported to be the best medium for collection of nasal secretions.<sup>17</sup> All of the methods used required insertion with forceps, which risks trauma to the nasal mucosa. We used nasal sponges made of stiff, sterilized polyvinyl acetate, and were able to efficiently absorb nasal secretions without the need for forceps. We found these sponges easy to use, and they were able to collect sufficient material for immunoassay measurements. A potential limitation is the cost; these sponges are purchased already pre-packaged in sterilized foil packets in pairs.

We have presented the within-subject SD for the measurements performed (see Supplement). These can be used to perform power calculations for future studies, including clinical trials of drugs designed to modulate the nasal allergen challenge response.

There was no clear difference in the nasal allergen challenge responses of asthma patients with allergic rhinitis compared to subjects with allergic rhinitis only. This indicates that subjects with and without asthma could be recruited into studies using the nasal allergen challenge model, and little differences are expected due to the presence of asthma. Area under the curve analysis (see Supplement) highlighted that there are some individuals with very high cytokine responses relative to the rest of the group, and the limited sample size of this sub-analysis means that some differences in allergen response due to the presence of asthma could not be completely ruled out.

Possible future improvements to the nasal supernatant analysis after allergen challenge include normalization of protein levels to secreted substances that are less affected by nasal allergen such as urea. Also, we were not able to investigate other relevant mediators due to limitations of sample volume for analysis, such as histamine, immunoglobulin E, and leukotriene C4. These could be investigated in future work along with more detailed study of the early-phase response to nasal allergen.

## Conclusion

In summary, we show that polyvinyl acetate sponges can be used to collect nasal samples after allergen challenge, with reproducible cytokine responses. A panel of T<sub>H</sub>2 cytokines can be detected using this method. We also show acoustic rhinometry to be a practical and reproducible measurement after nasal allergen challenge.

## Ethics approval and consent to participate

This study was approved by a local research ethics committee (Greater Manchester Central, 10/H1008/86) and all subjects provided written informed consent. This research was performed in accordance with the Declaration of Helsinki.

## Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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## Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

## Disclosure

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