

Administering multiple cumulative allergen challenges

An alternative nasal allergen challenge protocol for the Allergic Rhinitis—Clinical Investigator Collaborative



Allergic rhinitis (AR) is an upper respiratory tract inflammatory disease characterized by nasal congestion, rhinorrhea, nasal itch, and sneezing on exposure to relevant aeroallergens. Nasal allergen challenge (NAC) is a human model of AR and involves direct exposure of the nasal mucosa to the allergen of interest while monitoring the participant's subjective AR symptoms and objective airflow measurements, among other parameters.^{1,2} NAC can be used to study the pathophysiology of AR and to assess novel medications at various stages of clinical development, providing proof of efficacy in treating AR symptoms.¹ The Allergic Rhinitis—Clinical Investigator Collaborative (AR-CIC), part of the AllerGen Network Centres of Excellence (AllerGen NCE), has developed and optimized a NAC model for studying AR pathophysiology and evaluation of novel medications.³

The initial protocol used a qualifying allergen challenge (QAC), whereby the final allergen concentration reached at screening was the challenge dose at the NAC visit. Participants qualified when a total nasal symptom score (TNSS) of at least 10 of a possible 12 and/or a peak nasal inspiratory flow (PNIF) reduction of 50% from baseline occurred. A second iteration involved combining all doses received during screening, including the qualifying concentration, as the challenge dose at the NAC visit (cumulative allergen challenge [CAC]).³ In addition, the TNSS requirement was reduced from 10 of 12 to 8 of 12, and a PNIF reduction of 50% from baseline was required to qualify.

Nasal secretions collected at various time points using the QAC and CAC protocols were analyzed, investigating the changes in concentrations of a variety of cytokines after the allergen challenge and comparing allergic vs nonallergic participants.⁴ The protocols were successful in mapping distinct patterns of up-regulation of proinflammatory cytokines, such as interleukin (IL) 5, IL-13, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 β , in the nasal secretions of allergic participants that were largely unchanged in nonallergic patients.⁴

The CAC protocol provided optimal qualifying criteria for screening participants.³ Peak TNSS and PNIF at screening and NAC visits were more reproducible than previous iterations of the protocol, yet there was still a noticeable decrease in the TNSSs between the screening/qualifying challenge visit and the NAC visit. In this study, we aimed to determine whether providing 2 doses of allergen, recapitulating a mini priming effect during the NAC visit, would achieve greater reproducibility of TNSSs between the screening and NAC visits. Participants were challenged with the cumulative concentration of all preceding allergen doses to the QAC, followed by the QAC itself 15 minutes later; this was dubbed the multiple cumulative allergen challenge protocol (MCAC).³

In the present evaluation, 7 ragweed allergic participants were enrolled, with positive skin prick test results to ragweed (≥ 3 mm than negative control) and a minimum 2-year history of ragweed-

induced AR. The study was conducted outside the ragweed season (January to March 2014). Exclusion criteria and washout periods for anti-inflammatory medications were per previous protocols.³ Each nostril was washed with saline 10 times using a specially designed nasal olive. Participants recorded symptoms of sneezing, rhinorrhea, nasal congestion, and nasal itch, each scored from 0 to 3 for a total score of 12 for the TNSS. PNIF was also recorded using a facial mask and meter (InCheck, Clement Clarke International Ltd, Essex, United Kingdom). Diluent control was delivered into each nostril using the Aptar Bidose device (Aptar Group, Congers, New York). Participants were excluded if they recorded a TNSS greater than 2, indicating nonspecific hyperresponsiveness.

The lowest concentration of ragweed allergen (1:128 of a 1:20 wt/vol stock solution; ALK-Abello, Hørsholm, Denmark) was delivered and the TNSS and PNIF recorded 15 minutes later. If a TNSS of 8 of 12 and a PNIF reduction of 50% from baseline was reached, the participant qualified at that concentration; otherwise, they received the next dose, a 4-fold increase, until qualifying criteria were met.³ Twelve nonallergic participants received only the highest allergen concentration (1:2) and otherwise followed the same protocol.

Participants returned 7 to 28 days later for the NAC visit. Baseline TNSS and PNIF were recorded and the nasal cavity washed. Participants received the cumulative allergen dose before the qualifying concentration, followed by the qualifying allergen concentration 15 minutes later, as described above. The TNSS and PNIF measurements were recorded at baseline, 15 minutes, 30 minutes, 1 hour, and hourly up to 12 hours after NAC.

Nasal secretions were collected at baseline, 1 hour, and 6 hours using preweighed synthetic absorbent matrices (SAMs; Accuwik, Pall Corporation, New York, New York), with 2 strips placed back to back onto the inferior turbinate of 1 nostril for 2 minutes. SAMs were then placed in 0.5% bovine serum albumin in Dulbecco's phosphate-buffered saline and centrifuged, aliquoted, and stored at -80°C . Cytokine levels were measured from the SAM samples using the Bio-Plex Pro Human Cytokine 17-plex Assay (Bio-Rad Laboratory, Hercules, California) on a Bio-Plex 200 system (Luminex xMAP Technology). Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software Inc, La Jolla, California).

Participants experienced an initial peak in TNSS at 15 minutes after allergen challenge, followed by a gradual decrease in symptoms to near baseline (Fig 1), demonstrating a similar pattern to that seen using the QAC and CAC protocols.³ The 15-minute time point was significantly higher compared with baseline ($P < .001$, 1-way analysis of variance and Tukey corrections), 1 hour ($P < .01$), and from the second to the 12th hour ($P < .001$). The TNSS for ragweed allergic participants was significantly higher than for nonallergic participants at 15 minutes to 2 hours ($P < .001$) and at 3 hours ($P < .05$).

PNIF was initially reduced at 15 minutes followed by a gradual return to baseline flow measurements, which was statistically significant at 15 and 30 minute ($P < .01$) (Fig 1). Two-way repeated measures analysis of variance and Bonferroni corrections compared TNSS and PNIF for allergic vs nonallergic participants. Comparing peak TNSS at screening to that achieved at the NAC visit, MCAC participants experienced similar mean scores at both visits, a 1.56% mean reduction in TNSS (with no statistical significance between scores; $P = .84$, 2-tailed paired t test), and an improvement on previous studies (QAC $P < .01$, 14.6% mean reduction in TNSS; CAC $P = .07$, 21.2% mean reduction in TNSS).

Disclosures: In the past 12 months, Dr Ellis has participated in advisory boards for Circassia Ltd, GSK, Merck, and Novartis; she has been a speaker for Novartis, Merck, Pfizer, Takeda, and AstraZeneca; and her institution received research grants from GSK, Circassia Ltd, SunPharma, Merck, Pfizer, and Novartis. Dr Ellis is also a consultant to Ora Inc and ALK-Abello, Canada. Dr Neighbour has received support from GSK and Adiga Lifesciences and has served on the speaker's bureau and received honoraria from Merck, Astra-Zeneca, GSK, and Novartis. No other disclosures were reported.

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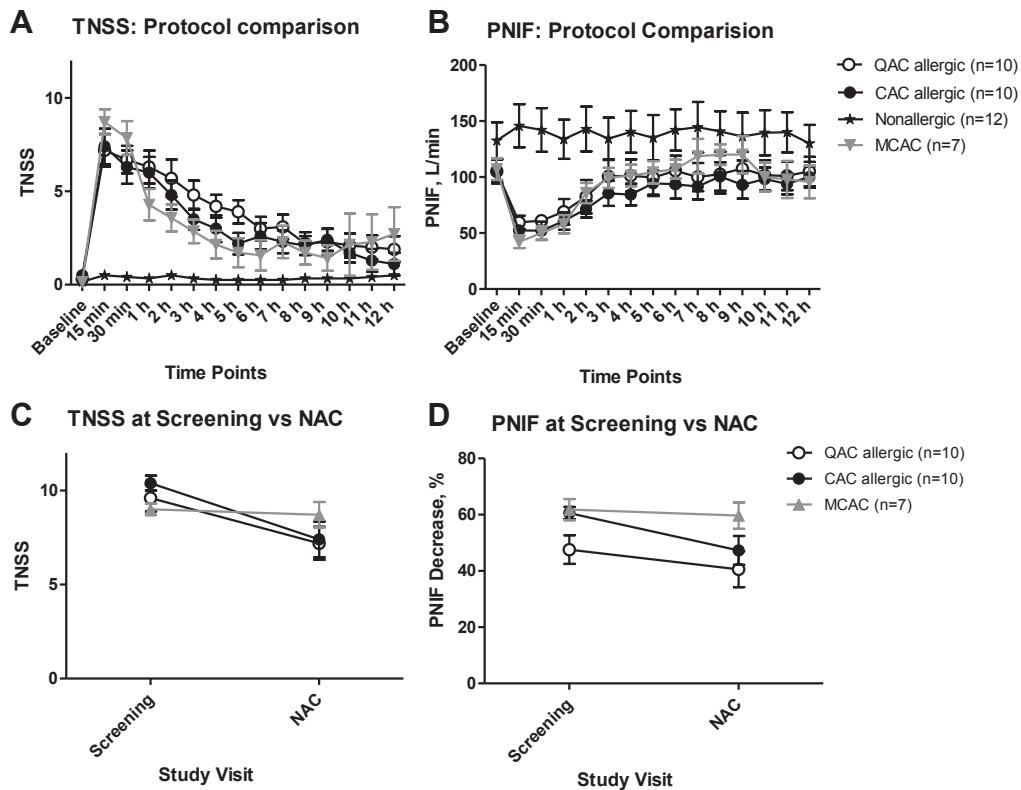


Figure 1. A comparison of the total nasal symptom score (TNSS) and peak nasal inspiratory flow (PNIF) profiles among the 3 nasal allergen challenge (NAC) protocols. Mean TNSSs and PNIF scores (A and B) with standard error of the mean (SEM), including nonallergic participants. Allergic participants experienced the highest scores at 15 minutes (multiple cumulative allergen challenge [MCAC]) TNSS (15 minutes vs 1 hour $P < .01$; 15 minutes vs 2 hours to 12 hours $P < .001$) compared with nonallergic participants who did not experience any significant change from baseline. PNIF scores followed a similar pattern (MCAC PNIF: 15 minutes vs 2 hours $P < .05$; 15 minutes vs 3 hours to 12 hours $P < .001$). Although there were significant differences between the time points for allergic participants (2-way analysis of variance $P < .001$ for both TNSS and PNIF), no such differences were experienced by nonallergic participants. Participants in the qualifying allergen challenge (QAC) and cumulative allergen challenge (CAC) studies (C and D) recorded lower peak TNSSs during the NAC visit compared with the screening visit, whereas MCAC participants recorded almost the same scores.

Most cytokine levels tested were close to or below the detection limit and did not change after allergen challenge. Nasal secretions from the QAC and CAC protocols, on the other hand, had measurable cytokine responses, such as IL-5, IL-13, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 β , increasing significantly after allergen administration and significantly higher than nonallergic samples.⁴

The current study met its main aim: to determine whether the peak TNSS achieved at screening was reproducible during the NAC visit. Allergic participants had a significantly different progression of symptoms and PNIF than nonallergic participants, following a similar pattern to that observed with previous iterations of the protocol. TNSSs reached a peak 15 minutes after the allergen challenge followed by a gradual decrease in symptoms, whereas nonallergic participants did not report such a change, resulting in a statistical difference between the 2 groups. The protocol was able to demonstrate this with a small number of participants.

The mean peak TNSS recorded during the NAC visit using the MCAC protocol was not statistically greater compared with other protocols.³ The MCAC was successful in achieving similar peak TNSS at both visits ($P = .84$, paired, 2-tailed t test), an improvement on previous protocols in which the peak TNSS was reduced at NAC compared with the screening values. Thus, the MCAC protocol was successful from the clinical standpoint.

The cytokine outcomes from MCAC were below the levels of the QAC and CAC protocols, however.⁴ One possible hypothesis is the delivery of multiple challenges released proteases in the nasal mucosa after the first allergen challenge, which could break down

cytokines, following their action on cell migration and inflammation.^{5,6} Different cytokines can be preserved using several enzyme inhibitors, although this may not be feasible when evaluating a wide variety of targets.⁷

Administering multiple allergen doses during the NAC visit has improved TNSS and PNIF outcomes, achieving scores similar to those recorded at screening, an improvement on previous protocols. Increasing repeatability of TNSS between visits might also be achieved by modifying the interval between visits as suggested by Ciprandi et al.⁸ Such a method should be investigated as a possible modification on the previous successful AR-CIC protocols, aiming to achieve clinical repeatability while preserving nasal sample quality for analysis. Future studies will focus on evaluation of nasal symptom and cytokine repeatability based on a fixed 3- to 4-week interval using the CAC protocol.³

The AR-CIC's NAC protocols continue to provide robust methods for the study of AR pathophysiology through collecting and investigating various biological samples and analyzing them in relation to clinical parameters. As an AllerGen NCE collaborative, the AR-CIC provides a useful tool for the study of novel medications in multicenter trials at several sites across Canada.^{9,10}

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Variations in pollen counts between Indianapolis, IN, and Dayton, OH, in spring 2013 and 2014



Approximately 20% to 40% of the US population has allergic rhinitis. Because pollens contribute significantly to allergic rhinitis, monitoring local pollen counts can help patients and clinicians better manage this condition.¹ Identification of local pollens can help clinicians more accurately select allergens for testing and immunotherapy and improve advice to sensitized patients on environmental control measures.

Certified pollen counting stations can be found on the National Allergy Bureau (NAB) website. Currently, there are 82 NAB stations in the United States but none in Indiana. The closest station to Indianapolis listed on the NAB website is 120 miles east, in Dayton, OH.² Dayton and Indianapolis are considered to be in the Appalachian floristic province within the North Atlantic region. Pollen counts in cities in the same region are assumed to be similar.³ Prior work comparing pollen counts between cities 11 km apart found similarities in pollen counts.⁴ However, pollen counts can be affected by precipitation, temperature, wind, and other environmental factors.⁵

To determine whether pollen reports from 120 miles away correctly reflect local pollen activity, we compared differences in pollen species and pollen counts between Indianapolis and Dayton from April through June in 2013 and 2014. We chose the spring season because this timeframe is associated with peak public interest in pollen allergies.⁶

Pollen data for Indianapolis were collected from the semirural backyard of a pediatric allergist with a Rotorod sampler (model 40; Rotorod, IMS, Plymouth Meeting, Pennsylvania). The sampler was located on an unobstructed flat surface, 1 story above ground, as per the NAB and American Academy of Allergy, Asthma, and Immunology (AAAAI) guidelines.⁷ One allergist, who has studied pollen counting at the AAAAI and is eligible for the AAAAI aerobiology certification examination, counted and recorded all pollen data. Pollen counts were posted daily to his website.⁸ The Regional Air

Pollution Control Agency collected and reported the data for Dayton to the NAB, using a Burkard sampler at the public library in downtown Dayton.² The laboratory technician performing the 2013 and 2014 pollen counts for Dayton was not NAB certified (A.R., personal e-mail communication, February 29, 2016). Both sites reported daily overall pollen count (grains per cubic meters of air) and counts for each tree, grass, and weed species collected. Meteorologic data were obtained from the website of the Midwestern Regional Climate Center from the University of Illinois.⁹ Analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina), with Wilcoxon nonparametric tests being used because of data skewness.

Pollen data for Dayton were available for 62 days for the periods in 2013 and 2014. Pollen data for Indianapolis were available for 84 days in 2013 and 89 days in 2014. Pollen counts were available for 20 to 22 days each month for Dayton. Tree, grass, and weed pollens were detected in Indianapolis and Dayton during this period in both 2013 and 2014 (Fig 1). In 2013, the daily median pollen count was significantly higher in Dayton (232.5 grains/m³ vs 154.5 grains/m³; $P = .02$). Dayton's daily median pollen count was higher in 2013 compared with 2014 (232.5 grains/m³ vs 123 grains/m³; $P = .02$).

Indianapolis had a higher total number of grass pollen days in 2013 (57/84 days [68%] vs 31/62 days [50%]; $P < .001$), with grass pollens present almost daily in May. In addition, Indianapolis had more days in June where weed pollens were identified (11/28 days vs 0/20 days; $P < .001$). In contrast, tree pollens were detected on significantly more days in Dayton in June (20/20 days vs 8/28 days; $P < .002$).

In 2014, weed pollens were identified more frequently in Indianapolis compared with Dayton (29/89 days [33%] vs 10/62 days [16%]; $P < .001$). In addition, during June in Indianapolis, grass and weed pollens were identified on most of the days sampled (29/30 days vs 14/21 days for grasses and 22/30 days vs 6/21 days for weeds; $P < .002$ for each pollen). In contrast, tree pollens were noted on significantly more in Dayton during June (21/21 days vs