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Nasal allergen challenge (NAC): Practical aspects and applications from an EU/US perspective—a Work Group Report of the AAAAI Rhinitis, Rhinosinusitis and Ocular Allergy Committee

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Nasal allergen challenge (NAC) is applied in a variety of settings (research centers, specialty clinics, and hospitals) as a useful diagnostic and research tool. NAC is indicated for diagnosis of seasonal and perennial allergic rhinitis, local allergic rhinitis, and occupational rhinitis; to design the composition of allergen immunotherapy in patients who are polysensitized; and to investigate the physio-pathological mechanisms of nasal diseases. NAC is currently a safe and reproducible technique, although it is time- and resource-consuming. NAC can be performed by a variety of methods, but the lack of a uniform

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technique for performing and recording the outcomes represents a challenge for those considering NAC as a clinical tool in the office. The availability of standardized allergens for NAC is also different in each country. The objective of this workgroup report is to review the current information about NAC, focusing on the practical aspects and application for diagnosis of difficult rhinitis phenotypes (eg, local allergic rhinitis, occupational rhinitis), taking into account the particular context of practice in the United States and the European Union. (J Allergy Clin Immunol 2023;151:1215-22.)

Key words: *Allergen, challenge, nasal, obstruction, symptoms*

Abbreviations used
AIT: Allergen immunotherapy
AR: Allergic rhinitis
EAACI: European Academy of Allergy and Clinical Immunology
ENT: Ear, nose, and throat
FDA: Food and Drug Administration
HC: Healthy control
HEP: Histamine equivalent prick
HMW: High molecular weight
LAR: Local allergic rhinitis
LMW: Low molecular weight
NAC: Nasal allergen challenge
NAR: Nonallergic rhinitis
NO: Nitric oxide
OA: Occupational asthma
OR: Occupational rhinitis
PNIF: Peak nasal inspiratory flow
RNM: Rhinomanometry
sIgE: Specific IgE
SPT: Skin prick test(ing)
TNSS: Total nasal symptom score
VAS: Visual analog scale

Nasal allergen challenge (NAC) is a very useful diagnostic and research tool. NAC is applied in a variety of settings including research centers, specialty clinics, and hospitals, primarily in Europe.[1,2] NAC can be used for the diagnosis of seasonal and perennial allergic rhinitis (AR), and it is essential for the diagnosis of local allergic rhinitis (LAR).[3] It is also useful to design the composition of allergen immunotherapy (AIT), to monitor the response, and to investigate the physiopathological mechanisms of nasal diseases. It is also used to identify clinically relevant allergens in patients who are polysensitized or those with disagreement between clinical history and skin prick test (SPT)/IgE results, mostly in centers where the use of NAC is routine.[4,5] NAC is a safe and reproducible technique, although it may be time- and resource-consuming.

NAC can be performed by a variety of methods using a variety of allergens. Results are measured by recording symptom scores, using devices to record nasal obstruction, or both. The lack of a uniform technique for performing and recording the outcomes, as well as for choosing allergens for testing, can be challenging when considering NAC as a clinical tool in the office. Moreover, the availability of standardized allergens for NAC is different in each country as are allergens for immunotherapy.[6] In Europe, lyophilized standardized extracts are available and commonly used for NAC, although there are major differences within the European Union due to different regulations. In the United States, glycerinated extracts are mostly used for performing NAC. These extracts are not US Food and Drug Administration (FDA)–approved for use in NAC and are used off-label. Moreover, the regulation and reimbursement differ between the European Union and the United States.

The objective of this workgroup report is to review the current information about NAC, focusing on practical aspects and application for diagnosis of difficult rhinitis phenotypes (eg, LAR, occupational rhinitis [OR]), taking into account the particular context of practice in the United States and the European Union.

METHODS FOR PERFORMING NAC

NAC consists of applying allergens to the nasal mucosa and monitoring the responses. NAC should be conducted under standardized, reproducible, and controlled conditions.[7]

Indications and contraindications of NAC

In both Europe and the United States, NAC has been used primarily to elucidate pathophysiologic mechanisms[8-11] and to investigate efficacy and mechanisms of action of different antiallergic medications[12-14] and of AIT[15] In addition to acquiring mechanistic answers about the nasal allergic response, NAC in Europe is also used in the clinic in selected patients to confirm allergen reactivity in patients who are polysensitized prior to initiating AIT[4,16] mostly in centers where NAC is routinely used. More recently, NAC has been used to confirm the diagnosis of LAR[17] and plays an important role in the diagnosis of OR.[18]

Absolute, relative, and temporary contraindications to NAC are listed in Table I.[1,19-25] Some nasal pathologies affect nasal patency, leading to difficulties in the objective measurement of nasal obstruction and therefore affecting the result of the allergen challenge, so it is crucial to explore the nasal cavity before provocation. These pathologies include septal perforation, nasal polyps, or severe septal deviation among others.[1]

The use of NAC has advantages and disadvantages that have been summarized in Fig E1 in this article's Online Repository (available at www.jacionline.org).

Extracts and allergens

In Europe, the use of glycerinated extracts is not recommended because it may produce nonspecific reactions in the nose, and nonglycerinated extracts are available.[26] The units of measure of allergen concentration of the extracts used for NAC are variable and include standard quality unit , standard biological unit, allergen unit, and histamine equivalent prick—all expressed per milliliter; protein nitrogen unit; bioequivalent allergy unit, or weight/volume (wt/vol%).[1] Because the content of protein allergen is different in these preparations, it is difficult to compare the results of NACs using the various units. Ideally, concentration of allergen in μg/mL should be specified for each challenge, allowing comparisons among techniques. In Europe, there are some companies that commercialize standardized extracts for nasal challenge. EU regulations require the control of allergen product potency using a validated assay.[27] In the United States, the allergen extracts used for NAC are usually purchased from companies that manufacture them for the purposes of skin testing or immunotherapy. Allergen extracts are not US FDA-approved for use in the nose. Therefore,

TABLE I. Indications and contraindications for NAC

Indications	
	Diagnosis of seasonal and perennial AR
	Diagnose LAR
	Confirm allergen reactivity in polysensitized patients prior to initiating AIT
	Diagnose OR
	Elucidate pathophysiologic mechanisms
	Investigate efficacy and mechanisms of action of different anti-allergic medications
Absolute contraindications	
	Acute inflammation in the nose or sinuses (sinusitis)
	Poorly controlled asthma or chronic obstructive pulmonary disease (possible bronchospasm)
	Severe comorbidities (cardiopulmonary diseases, impairment of lung capacity) that can be worsened by the test
	Pregnancy
Relative and temporary contraindications	
	Children younger than 5 years (mostly for lack of collaboration)
	Nasal or sinus surgery within the previous 6-8 weeks (false-positive response due to baseline inflammation)
	Recent infection or vaccination (false-positive response due to baseline inflammation)
	Use of alcohol or tobacco 24-48 hours prior to NAC (false-positive response for airway irritation)

in a clinical research setting, an investigational new drug application would need approval by the US FDA before using the extracts for NAC. However, these commercial allergen extracts can be used for NAC in clinical practice as an off-label application. Mostly 1:1, 1:10, and 1:100 dilutions of skin test concentration are used for NAC in clinical practice. It is considered a safe approach the use of 1:10 and 1: 100 for most patients,[4] but in individual cases where a high sensitivity is suspected, the 1:1000 can be used to avoid a strong reaction.

Many allergens are available for routine use in Europe, being different depending on the relevance of the allergen in each country. The most commonly used are house dust mites (mixed or individual, eg, *Dermatophagoides pteronyssinus* in concentrations up to 4 μg/mL depending on the brand), grass pollen (either mix or individual such as *Phleum pratense* up to 0.1 μg/mL), *Olea europaea* (olive tree pollen up to 0.6 μg/mL) and *Parietaria judaica* in the Mediterranean area, molds (mainly *Alternaria alternata* up to 0.25 μg/mL), and animal dander (cat and dog) at 30 histamine equivalent prick per milliliter. In the diagnostic algorithm for LAR, a single full dose of those allergens without serial dilution (1:1) can be safely used.[28]

Allergen application methodology: pros and cons

Most challenge techniques involve using a metered dose spray device to deliver a specific amount of allergen to the nasal mucosa (Fig 1, *A*). Allergen is administered with the patient holding their breath to minimize chances of accidental bronchial aspiration. This method is easy to use, it is not expensive, it is easy to obtain everywhere, and there is no need for special training. However, the allergen can be accidentally delivered to the lower airways. A micropipette can be used for nasal challenge. This method enables the precise deposition of a specific volume of allergen solution to the nasal mucosa, with less leakage into the bronchi (Fig 1, *A*).[25] Local application of allergen via filter paper has also been used. This is primarily to evaluate reflex responses by applying allergen to 1 nostril (placing the filter paper on inferior turbinate) and monitoring bilateral responses.[9] Both micropipette and filter paper methods have recently been demonstrated to be very safe, with a low rate of local adverse events.[4] However, these methods require some training such as proper use of nasal speculum.

TABLE II. Contraindicated medications for the performance of NAC

Medication	Period to hold before NAC (washout period)*
Topical antihistamines	4-5 d
Topical corticosteroids	2-4 d
Topical mast cell stabilizers	7-21 d
Systemic antihistamines	7 d
Systemic corticosteroids	14-21 d
Systemic NSAIDs	7 d
Leukotriene modifiers	No recommendation
Topical and systemic decongestants	2 d
Tricyclic antidepressants	14-21 d
Clonidine and other central-acting	21 d

NSAIDs, Nonsteroidal anti-inflammatory drugs.
*Washout periods recommended by convention.

Performance of NAC

It is recommended to perform NACs in patients who are asymptomatic or mildly symptomatic. For seasonal allergens, NAC should be performed outside of the allergy season (minimum of 4 weeks after pollen season) to eliminate the confounding influence of ongoing seasonal allergic inflammation.[1] Subjects should avoid coffee, spicy foods, tobacco, alcohol, and exercise the day of the challenge because they can either cause nasal symptoms such as nasal congestion or runny nose or increase allergen response (alcohol or exercise), therefore causing nonspecific hyperresponsiveness or false positive results. Drugs that affect the nasal response should be discontinued prior to NAC. Medications that influence NAC results are listed in Table II.[7]

Patient should acclimatize for 20-30 minutes in the room where the NAC will be performed before initiating the challenge.[7] Baseline nasal symptoms and signs are recorded followed by challenge with a control solution (vehicle in which the allergen extract is diluted), which is used to assess nonspecific reactivity of the nasal mucosa. There are several published criteria that define nonspecific reactivity and positive/negative response will depend on the protocol used.[1,25] Briefly, NAC should start with the application of an inert substance (the same diluent used to prepare the allergen dilution, avoiding irritant substances including glycerol). Fifteen minutes later, the nasal response is assessed (eg, symptom score, rhinoscopy, rhinometry), and if there is a significant decrease in patency and or symptoms, the test should be interrupted. If there is no positive response to the diluent, this is followed by challenge with an allergen. Some protocols use increasing doses of allergen to create a dose response curve and others use a screening challenge that employs multiple increasing doses to determine a threshold that will produce allergic signs and symptoms and later use that dose for subsequent challenges.[29]

When performing serial dose challenges, the doses are administered 10 minutes apart and responses are usually measured between doses and before application of the next dose.[8-16] Some protocols interested in late phase responses will monitor responses after the initial challenge at various intervals (normally every 60 minutes) extending to up to 6-8 hours after challenge.[16,30] The

FIG 1. Methods of allergen delivery and measurement of nasal obstruction. **A,** Metered dose spray device and micropipette. **B,** PNIF meter with facemask. **C,** Acoustic RNM. **D** and **E,** Active anterior RNM.

response is usually monitored by subjective (symptom score) and objective responses using various techniques.

One limitation of NAC with a single allergen per session is that it is time-consuming. However, the use of a NAC protocol with multiple allergens sequentially administrated at their maximum concentration has significantly reduced the number of visits and shortened the diagnostic work-up, facilitating the use of NAC in clinical practice to diagnose LAR in patients with no evidence of systemic allergy (negative skin test or serum allergen-specific IgE [sIgE]). Moreover, NAC with multiple allergens is highly reproducible and specific compared with NAC with single allergens.[28]

TABLE III. TNSS and VAS

	TNSS	
Symptom	**Scale**	**Interpretation**
Sneezing	0-3	0 = None
Rhinorrhea	0-3	1 = Mild
Congestion	0-3	2 = Moderate
Nasal itching	0-3	3 = Severe
Sum total	0-12	
	VAS	
Measurement	**Interpretation**	
0-40 mm	Mild	
41-70 mm	Moderate	
71-100 mm	Severe	

TNSS is the sum total of sneezing, rhinorrhea, congestion, and itching.

Safety and reproducibility of NAC

Most of studies on NAC safety and reproducibility include a small number of adult patients, do not include children, and do not properly represent the different rhinitis phenotypes. However, a recent study showed the results of a prospective analysis of repeated NACs performed at 1-2-month intervals in patients with AR, LAR, or nonallergic rhinitis (NAR) and in healthy controls.[4] The reproducibility and positive/negative predictive values of 3 consecutive NACs performed in 710 subjects were 97.32%, 100%, and 92.91%, respectively. These data demonstrate the absence of false positive results in patients

TABLE IV. Advantage and disadvantage of objective measurement of nasal obstruction

	Advantage	Disadvantage
PNIF	— Simple and well tolerated — Facilitating repetitive measurements — Obtain a simple indication of obstruction — Affordable	— Largely effort-dependent — Possible effect of nasal valve collapse — Comparisons between individuals are not informative
Acoustic rhinometry	— More reliable than peak nasal inspiratory flow — Less dependent on individual effort — Relatively quick and easy to perform — Correlates well with the size of the inferior turbinate — Percentage (%) of volume 2-6 cm alone can confirm and rule out nasal allergen-specific reactivity	— Relatively expensive
Rhinomanometry	— More reliable than PNIF — Less dependent on individual effort — 4-phase-RNM is considered the most reliable method to quantify nasal patency	— Relatively expensive — Time-consuming compared to other methods

with NAR and in healthy controls. Moreover, the retrospective evaluation of 11,499 NACs conducted in 518 children and 5,830 adults showed that only 4 local adverse events including uvular edema occurred, and 99.97% of NACs were well tolerated. These results show that NAC is a reasonably reproducible and safe technique.

OBJECTIVE AND SUBJECTIVE METHODS OF EVALUATION OF NAC RESPONSE

Evaluation of nasal response

A combination of subjective symptom assessments and objective measures of nasal patency should be used to evaluate NAC outcomes.[1,2] Objective measures do not always correlate with clinical symptoms.[31]

Subjective evaluation: symptom scores. Standardized symptom scores and visual analog scales (VASs) quantify clinical symptoms. The total nasal symptom score (TNSS) is commonly used to assess nasal symptoms. It is the sum of sneezing, rhinorrhea, congestion, and nasal itching, each scored on a scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe; total 0-12) (Table III).[29,32] Ocular symptoms are not measured by the TNSS but are included in other scoring methods such as the Linder and Lebel scores,[2] which are fully described in this article's Online Repository (available at www.jacionline.org).

In the VAS, subjects place a mark on a 100 mm line to indicate the severity of individual or overall symptoms on a scale from 0 (none) to 100 (severe).[30,31]

The 2018 European Academy of Allergy and Clinical Immunology (EAACI) position paper on the standardization of NACs recommends both Linder and Lebel scores because they include nasal and ocular symptoms. According to the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines, the use of VAS to evaluate congestion, sneezing, itching, and rhinorrhea is a clear and easy-to-use method for measuring severity of AR.[1]

Objective evaluation: measurement of nasal obstruction. Peak nasal inspiratory flow (PNIF), rhinomanometry (RNM), and acoustic rhinometry measure nasal patency, and each has advantages and disadvantages (Fig 1, *B-E*, Table IV). PNIF is simple and well tolerated, facilitating repetitive measurements.[32,33] Measurements can be generated from a single inspiratory maneuverer using both nostrils simultaneously and obtain a simple indication of obstruction. Although this method is largely effort-dependent, there is the possible effect of nasal valve collapse, and comparisons between individuals are not informative,[2,33] PNIF is widely used as an objective measurement of nasal obstruction in most institutions. Acoustic rhinometry and RNM are more reliable methods, mostly used for research purposes although they are also used as routine diagnostic methods in some specialized clinical settings. Acoustic rhinometry uses transmitted sound waves to measure the cross-sectional area of the nasal airway, usually 2-6 cm distal to the anterior opening of the nasal airway.[1,25] It is less dependent on individual effort, relatively quick and easy to perform, and correlates well with the size of the inferior turbinate.[32,33] Acoustic rhinometry was standardized by the European Rhinology Society in 2005.[1,34] RNM measures transnasal pressure, with anterior, posterior, or combined anterior and posterior pressure measurements depending on the location of the nasal sensor. It is also classified as active or passive, depending on whether the test subject uses a specified breathing pattern or is breathing spontaneously.[2,32,33] Ascending and descending curves in inspiration and expiration can be measured with 4-phase rhinomanometry.[35] Active anterior RNM and 4-phase rhinomanometry may be the most established RNM approaches, and 4-phase rhinomanometry is considered to be the most reliable method to quantify nasal patency according to published guidelines.[35] However, a recent publication based on NAC performed in 1165 patients with AR, 369 who have NAR, and 361 healthy controls (HCs) demonstrates that the percentage (%) of volume 2-6 cm measurement obtained by acoustic

TABLE V. Selected positivity criteria for NAC

Method	Positive response (moderately to strongly positive)
Subjective	
TNSS	Increase ≥3-5 points
VAS	Increase ≥25-55 mm
Objective	
PNIF	Decrease by ≥20%-40%
Acoustic rhinometry	Decease in cross-sectional area at the inferior turbinate/nasal cavity volume from 2 to 6 cm from the nostril ≥25%-40%
Active anterior RNM	Decrease in flow ≥20%-40% at 150 pascals
4-phase-RNM	Increase of logarithmic effective resistance ≥20%-40%

The lower ranges indicate moderately positive responses while the higher ranges indicate strongly positive responses. Adapted from Augé et al.[1]

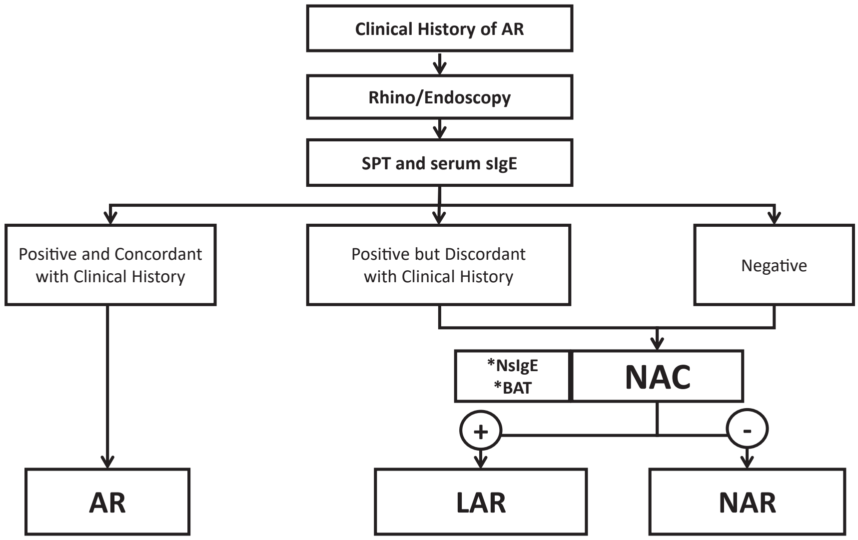

FIG 2. Diagnostic algorithm of LAR. *BAT*, Basophil activation test; *NsIgE*, nasal sIgE.

rhinometry is able to distinguish AR from NAR and HC ($P < .001$), establishing a decrease ≥24.48% as optimal cutoff point.[32] Correlations with subjective nasal symptoms are variable for PNIF, acoustic rhinometry, and rhinomanometry.[33]

Objective measurement of allergic/inflammatory responses

Nasal secretion analysis, nasal scraping/brushing, nasal nitric oxide (NO) measurement, and nasal biopsies permit assessments of allergic and inflammatory responses during NAC. These measurements are performed to investigate the mechanism of the disease and certain drug responses to NAC in a research setting and are not commonly used in clinical setting. Secretion analysis has been used most extensively and is discussed with NO and biopsy in the Online Repository.

Interpretation of results

Different guidelines recommended the combination of a positive objective plus a positive subjective response to NAC as positivity criteria. Recently the ear, nose, and throat (ENT) section of EAACI has proposed another positivity criterion consistent in a strongly positive objective response, a strongly positive subjective response, or a combination of moderately positive subjective and objective responses.[1] Selected positivity criteria are listed in Table V.[2,20,25,32,36]

False-positive/-negative outcomes

False-positive results can occur due to a respiratory infection within 3-6 weeks, nasal hyperreactivity, recent allergen exposure, or fluctuations in the nasal cycle. Also, allergen extract solutions may contribute to false positives through irritants/preservatives, irritating pH levels, hypo- or hyperosmolality of the extracts, temperature variations, or inaccurate delivery of solution.[1,20]

False-negative results can occur due to low allergen concentration, faulty delivery of the test allergen, recent nasal or sinus surgery, lack of experience/training for evaluation nasal response, current limitations of assessment tools, obstruction from nasal polyposis, mucosal abnormalities associated with atrophic rhinitis, or use of medications that influence the test result (Table II).[1,25,37,38]

APPLICATION OF NAC FOR DIAGNOSIS OF OTHER RHINITIS PHENOTYPES: LAR AND OR

Local allergic rhinitis

LAR is a confined nasal allergic response in the absence of systemic atopy (negative sIgE or SPT to allergens) that is characterized by a positive NAC, with release of inflammatory mediators in nasal secretions including tryptase and eosinophil cationic protein.[3,39] LAR is a chronic respiratory disease with a natural evolution toward severe phenotype with asthmatic symptoms and a good response to AIT.[40] In a proportion of patients with LAR, nasal sIgE can be detected in nasal secretions using different methods of collection and measurements, in general with a rather low sensitivity.[41] Moreover, a positive basophil activation test is found in 50% of patients with LAR who are house dust mite–sensitized and in 66% of those who are olive tree pollen–sensitized with specificity > 90% for both allergens.[42]

In the diagnostic approach of patients with LAR, NAC plays a principal role in the diagnosis of the disease and is considered the gold standard. The classical diagnostic approach with SPT and serum sIgE is insufficient and leads to misdiagnosis in these patients. NAC is essential for differentiation between LAR and NAR. The diagnostic approach is shown in detail in Fig 2.[3]

An important proportion of subjects with LAR develop their first symptoms during childhood. In the last 5 years a significant number of studies regarding LAR in pediatric populations have been published, involving close to 400 children who were recruited.[23,43-47] In line with what has been observed in adults, prevalence of LAR among children with rhinitis symptoms, negative SPT/sIgE, and positive NAC is higher in Western countries (range 36.7%-66.6%)[43,45,46,48] compared to Asian countries (range 3.7%-25%).[23,47,48] House dust mite is the most common allergen in Asian countries.[47,49] In summary, LAR is also important in the differential diagnosis within the pediatric population and must be ruled out in children with typical AR symptoms and negative SPT/sIgE.

Occupational rhinitis

Like occupational asthma (OA), OR cases should be evaluated and confirmed to be work-related.[18] The literature is replete with case reports and case series of confirmed OR secondary to high molecular weight (HMW) and low molecular weight (LMW) agents similar to methods proposed for OA.[50-52] Current guidelines address the different approaches to preparing and applying the suspected OR inducer and the various methodologies used to confirm objective nasal responses including anterior rhinomanometry, peak inspiratory nasal flow rates, and acoustic rhinometry, as well as methods for assessing changes in nasal inflammation, blood flow, temperature, and pH.[18,25,50] Furthermore, there are several validated patient-reported outcome scales that reliably measure symptom scores pre- and postprovocation.[25] In cases where a HMW agent is suspected, either skin testing or serologic testing, if available, should be performed to determine whether an IgE-mediated mechanism of action is responsible.[53] For LMW, skin or serologic testing is more problematic, as is nasal provocation, because inciting agents are mostly chemical irritants or noxious odorants. However, there are some LMW agents such as trimellitic anhydride and platinum salts that elicit sIgE-mediated responses where provocation is possible in a controlled setting performed by experienced personnel. In subjects with OR and concomitant asthma, NAC should be performed carefully to avoid inducing an asthma exacerbation. It is important to make an accurate diagnosis of OR because this condition is a well-known precursor for the development of OA.

APPLICATION OF NAC IN DIFFERENT SETTINGS AND THE REAL-WORLD CHALLENGES FOR PERFORMING NAC

Clinical practice: Challenges for performing NAC

Use of NAC in the clinical practice environment serves as an additional tool to assess patients with a clinical history strongly suggestive of aeroallergen sensitivity, despite a lack of identifiable systemic IgE. As published, 1 year of immunotherapy inhibits allergen-induced immediate and late nasal symptoms in patients with LAR who are allergic to house dust mites,[40] birch pollen,[54] or grass pollen.[55] Moreover, in some European countries, NACs are also performed before starting AIT to confirm the clinical relevance of the allergen sensitization.[4] In addition to clinical indications, subjects should be examined for nasal patency and demonstrate comprehension of procedure instructions and the ability to complete objective and subjective assessments. It is most advantageous for the procedure to occur directly after negative skin testing, because interfering medications may have been already held. While the optimal dose of intranasal allergen has not been verified by studies, the EAACI position paper recommends using commercially available, standardized solutions.[1]

Economic considerations in performing NAC include the cost of new materials and potentially objective assessment equipment, training staff, and use of clinic space while testing occurs. However, the test is easy to administer and the learning curve for staff and patients is short. In contrast with Europe, the available test materials in the United States are mostly glycerine-based, but these extracts might be too viscous for some spray devices if not properly diluted or may cause nonspecific reactions. However, if properly diluted, glycerinated extracts can be delivered by the spray method or paper disk method can be used. NAC can be done in a typical practicing allergist's office. The time to perform the test is generally 60-120 minutes for each allergen (20-30 minutes to acclimatize patients to the local, controlled environment plus negative control, then intervals of 10-15 minutes for single allergen with single or multiple concentrations). Testing will take longer if multiple allergens are used. The Current Procedural Terminology code 95065 is applicable to direct nasal mucous membrane tests; however some insurance carriers term the procedure "experimental" and thus non-reimbursable. Counselling that occurs during and after the procedure, spirometry for subjects with asthma or in the event of acute lower respiratory symptoms, and AIT are potential sources of revenue related to NACs. In Europe, most health insurance companies do reimburse the NAC expenses, although NACs are not widely performed in private settings mostly due to staff shortage or the expense of the objective measurements (RNM, acoustic rhinometry, PIF). In some European countries, NACs are performed in public university hospitals mostly by allergists in selected sites.

When NAC is properly performed in a practice setting, patients seem to appreciate the extra effort in identifying the source of their symptoms. These patients feel "validated" that the cause of their clinical condition has been identified, and therefore they are highly motivated to pursue AIT.

NAC in research and clinical trials and environmental challenges

NAC in research and clinical trials and environmental challenges are discussed in the Online Repository.

CONCLUSIONS

NAC is a safe and reproducible technique, used in both clinical and research settings. There are numerous indications including identification of difficult rhinitis phenotypes, the evaluation of the clinical significance of allergens, and the diagnosis of OR. The interpretation of NAC results should rely on a combination of subjective and objective measurements using validated methods. There are some differences between the European Union and United States, particularly regarding allergen availability, regulations, and reimbursement.

We conclude that NAC is a valuable diagnostic and research tool for the evaluation of nasal allergic diseases, and NAC can be widely used today.

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SUBJECTIVE EVALUATION: SYMPTOM SCORES

The Linder score ranges from 0 to 13 points, and includes sneezing (0-2 = 0, 3-4 = 1, ≥5 = 3), pruritus (nose = +1, palate = +1, ear = +1), rhinorrhea (0-3), obstruction (0-3), and ocular symptoms (present = +1, absent = 0). The Lebel score is similar, ranging from 0 to 11 points: sneezing (0-2 = 0, 3-4 = 1, ≥5 = 3), pruritus (nose = +1, palate and/or ear = +1), rhinorrhea (anterior = +1, posterior = +1), obstruction (difficult nasal breathing = +1, 1 nostril blocked = +2, both nostrils blocked = +3), and ocular symptoms (present = +1).[E1,E2]

OBJECTIVE MEASUREMENT OF ALLERGIC/ INFLAMMATORY RESPONSES

Nasal secretion analysis, nasal scraping/brushing, nasal NO measurement and nasal biopsies permit assessments of allergic and inflammatory responses during NAC. These measurements are performed to investigate the mechanism of the disease and certain drug responses to NAC in a research setting and are not commonly used in clinical setting.

Nasal secretion analysis

Nasal secretions contain inflammatory mediators, markers, and cells that can be analyzed and quantified before, during, and after NAC. Depending on the timing of collection, secretions may contain early or late phase inflammatory mediators (Table E1).[E3,E4] Cells can also be obtained by different methods such as nasal lavage, blown secretions, or brushings. Nasal lavage is simple and minimally invasive but results in a diluted sample. The magnitude of this dilution varies among subjects and is influenced by nasal anatomy and technique. Normalization of lavage solutions to total protein or albumin content attempts to correct for dilutional effects.[E1,E3] However, the nasal lavage dilutional effect is of no concern when cellular work is conducted because the cells will be spun down and the cell yield will end up being higher with higher nasal lavage volume. Nasal lavage allows both total and differential cell counting compared to blown secretions and brushings that only allow the calculation of differential cell percentages. Scrapings and brushings collect cells and mediators on the surface of the nasal mucosa, usually by using a plastic device applied to the medial or inferior surface of the inferior turbinate. Collection of secretions using a matrix, such as foam or filter paper, corrects for the dilution of lavage.[E5,E6] However, the material used, nasal placement, and mucosal contact time vary among studies, making comparisons and standardization difficult. Blown secretions, including nasal cytology, are minimally invasive, but the sample size is highly effort-dependent and may be inadequate in many individuals.[E6]

Nasal NO

Nasal NO is increased in AR and eosinophilic nasal polyposis. It increases 24 hours after NAC.[E7] However, reproducibility is inconsistent and may be affected by variations in nasal patency.[E8,E9] Nasal obstruction due to various factors can decrease measured nasal NO levels as it is primarily produced by the mucosal epithelium of the paranasal sinuses.[E8] Nasal NO is also decreased in subjects with primary ciliary dyskinesia and cystic fibrosis.[E10] Nasal NO values are typically higher than fractional exhaled NO values from the lung. The mean nasal NO value for healthy children was 660 parts per billion in 1 study.[E11] Therefore, nasal NO measurement has no clinical value in most cases due to its lack of reproducibility.

Nasal biopsies

Biopsies can be taken from the nasal mucosa, usually from the head of the inferior turbinate, using topical anesthesia and nasal biopsy forceps.[E12] The analysis of nasal biopsies before and after allergen challenge, mostly for research purposes, has allowed obtaining crucial information about pathophysiological mechanisms of the nasal allergic response.[E13,E14] There is some correlation regarding cell and mediator measurements when obtained by nasal lavage or biopsies[E15] or when comparing nasal biopsies and brushing.[E16] However, nasal biopsies allow obtaining information directly from deeper areas of the tissue when compared to nasal brushing or nasal lavage, especially with repetitive allergen challenges.[E17]

APPLICATION OF NAC IN DIFFERENT SETTINGS AND THE REAL-WORLD CHALLENGES FOR PERFORMING NAC

NAC in research and clinical trials

There are ample opportunities to enhance the understanding and utilization of NACs. International consensus guidelines for nasal and ocular challenges do not exist. Therefore, the positive criteria, methodologies, and extract or allergen preparations used in challenges vary in the literature.[E2] A number of clinical trials using NAC are being performed in many countries. The goals of these trials vary. Some are investigating inflammatory mediator responses, and others are exploring the effects of pharmacologic interventions. Many are procedural and studying the methodology of NAC. A variety of methods for challenges exist including direct mucosal challenges and environmental challenges (park or field studies).[E2] Comparative studies would be useful to define the most appropriate methodology to use in the clinical setting. Research is also needed to better define the most appropriate dilution or dose needed to properly conduct direct nasal challenges with each allergen. Further research would also be helpful to better define the best method for measuring outcomes in clinical trials. Symptoms scores appear to be the most useful and expeditious way of measuring responses in a practice setting. The TNSS and VAS are commonly used subjective assessment tools.[E3,E6,E18-E20] However, other clinical tools such as PNIF, acoustic rhinometry, and RNM may prove useful. Although further research is needed to identify nasal mucosa cells and inflammatory mediators evoked by NAC, a tremendous amount of work using NACs has contributed to major scientific findings for more than four decades, improving our understanding of allergic airway diseases.

Environmental challenges

Environmental chambers can be used for allergen challenge in a controlled setting. Environmental chamber allergen exposure is the most similar way to natural allergen exposure, in opposition to NAC that most likely does not mimic natural exposure. Therefore, it is ideal to use allergen chamber provocation in a clinical trial of new drug or treatment for the treatment of AR. In comparison to

NAC, chambers can be mobile or stationary and allow a group of people to receive a single allergen challenge or multiple simultaneous allergen challenges.[E21] Studies using chambers can achieve similar conclusions with fewer participants in less time because of decreased variability.[E22] However, chambers are too expensive to use even in a research setting and are not realistic in a clinical setting, in contrast with NAC, which is simple to perform and cheaper. The reproducibility and calibration between different chambers is not established.[E21,E22] Therefore, multicenter studies are difficult to conduct using chambers and are primarily used in single-center clinical trials.[E23]

Park or field studies are able to evaluate the effects of natural allergen exposure outdoors during the pollen season. However, these studies may be influenced by several variables that can cause fluctuations in pollen counts (weather variations, geographic location) that might impact in the reproducibility of the results.[E24] Also, pollen exposures may be influenced by lifestyle choices.[E22,E24] Multicenter studies are challenging due to the short period of time where the challenges can be performed.[E24]

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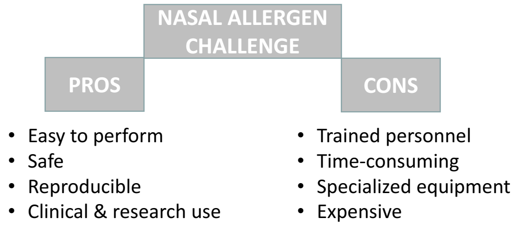

FIG E1. Pros and cons of NAC.

TABLE E1. Selected nasal secretion mediators, cytokines, markers, and cells

Measured mediators, cytokines, markers, and cells	Examples	Comments
Inflammatory cells	Eosinophils, neutrophils, basophils, mast cells, lymphocytes	High concentrations of eosinophils present in the allergic response (AR or LAR) Basophils present in LPR of AR
Immunoglobulins	Total IgE and sIgE	Elevated in AR and possibly LAR
Cytokines (T_H2-cell associated)	IL-4, IL-5, IL-13	Elevated in AR, predominantly LPR
Chemokines	IL-8, eotaxin	IL-8 increased in EPR of AR
Eosinophil mediators	Major basic protein, eosinophilic cationic protein (ECP)	Elevated in AR, predominantly LPR
Mast cell mediators	Histamine, tryptase, prostaglandin D_2, cysteinyl leukotrienes	Elevated in EPR of AR Histamine also elevated in LPR likely due to basophil, rather than mast cell, origin
Markers of glandular secretion	Lactoferrin	Parallels the increase in secretion weights bilaterally in AR after NAC Upregulated in serum in AR after NAC
Neuropeptides	Substance P, vasoactive intestinal peptide	Substance P elevated in AR and NAR
Plasma leakage mediators	Albumin	Used in standardization of nasal lavage samples
Exhaled gas	Nasal exhaled nitric oxide (FENO)	Objective measure of eosinophilic inflammation May be affected by smoking, sinus patency, and other factors

This table is not an all-inclusive list. Other measurable mediators are reported in the literature. Adapted from Pepper and Ledford.[E23]
EPR, Early phase response; *LPR*, late phase response.