

Nasal and ocular challenges



Amber N. Pepper, MD, and Dennis K. Ledford, MD Tampa, Fla

Nasal and ocular challenges facilitate the evaluation of subjective and objective responses to defined allergen or irritant exposure. Nasal and ocular allergen challenges are the gold standard to diagnose allergic rhinitis and conjunctivitis, respectively, and aid in the evaluation of novel therapies in clinical trials. Additionally, nasal and ocular allergen challenges might help identify medically relevant allergens in clinical practice. Nonspecific or irritant challenges evaluate mucosal hyperreactivity. Direct mucosal challenges, which can be performed in an office or research setting, expose the participant to higher allergen doses than common in the natural environment. Park studies and environmental chambers, which are most practical in clinical trials, more closely simulate natural allergen exposure. International consensus guidelines for nasal and ocular challenges do not exist. Therefore the positivity criteria, methodologies, and extract or allergen preparations used in challenges vary in the literature. Regardless of these limitations, nasal and ocular challenges are helpful clinical and research tools for nasal and ocular diseases. (J Allergy Clin Immunol 2018;141:1570-7.)

Key words: Nasal challenge, ocular challenge, allergen challenge, provocation test, allergic rhinitis, allergic conjunctivitis, local allergic rhinitis, nonallergic rhinitis

Nasal and ocular challenges permit quantification of changes in upper airway resistance, local inflammatory mediators, symptoms, and signs in response to defined allergen or irritant exposure. Nasal allergen challenges (NACs) and ocular allergen challenges (OACs) are the gold standard to diagnose nasal and ocular allergy, respectively.¹ Clinically, both NACs and OACs can help optimize allergen immunotherapy or environmental

Abbreviations used

AERD:	Aspirin-exacerbated respiratory disease
FENO:	Fraction of exhaled nitric oxide
LAR:	Local allergic rhinitis
NAC:	Nasal allergen challenge
NSAID:	Nonsteroidal anti-inflammatory drug
OAC:	Ocular allergen challenge
PNIF:	Peak nasal inspiratory flow
TNSS:	Total Nasal Symptom Score
VAS:	Visual analog scale

prevention measures in polysensitized subjects by confirming the most clinically relevant allergen or allergens.²⁻⁴ Such challenges also enable the assessment of novel treatments for allergic rhinitis and conjunctivitis in clinical trials.³⁻⁵ This review discusses the methodologies and relative advantages and disadvantages of nasal and ocular challenges with a focus on allergen challenges.

International consensus guidelines for NACs and OACs are not available, and thus the methodologies, positivity criteria, and allergen or extract concentrations used vary. Table I^{1,3,5-8} outlines a representative protocol for mucosal NACs and OACs, but other protocols are in the literature and used in various clinical and research settings. Direct mucosal challenges are performed by directly applying the allergen or extract to nasal or ocular mucosal surfaces.⁴ Challenge methods using inhalation of airborne allergens, such as outside in a park or inside a chamber, better reflect natural exposure (Table II).^{2,3,5} Environmental chambers or units provide an enclosed space in which the allergen content can be more closely monitored.

Allergen exposure during challenges is often graded (multiple doses to establish a dose threshold) but can also be a single dose (symptom or outcome severity).^{4,6} For graded challenges, incremental doses are delivered by using a series of increasingly concentrated solutions of allergen or extract or, alternatively, an increasing quantity of allergen measured by mass units or number of pollen grains.^{1,3,6} Most challenges replicate acute high-dose allergen exposure. Chronic low-dose allergen exposure, which is typical in the natural environment, is more difficult to simulate.^{2,5}

Medications, such as antihistamines, topical corticosteroids, and possibly leukotriene modifiers, which interfere with challenge tests, should be discontinued before NACs and OACs (Table III).^{3,5-7} It is best to perform challenges when subjects are asymptomatic or minimally symptomatic.^{3,6} NACs and OACs to seasonal allergens should be performed ideally outside of their respective seasons because of priming, the variable effects of natural environmental exposure modifying the threshold dose, and symptom severity.^{2,9} Priming leads to enhanced responses, which can be allergen specific (eg, heightened response to grass pollen

From the Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida Morsani College of Medicine and James A. Haley Veterans' Affairs Hospital.

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Corresponding author: Dennis K. Ledford, MD, James A. Haley Veterans' Affairs Medical Center, Division of Allergy/Immunology, Department of Internal Medicine, 13000 Bruce B. Downs Blvd, Suite 111D, Tampa, FL 33612. E-mail: dledford@health.usf.edu.

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TABLE I. Example protocols for graded NACs and OACs using allergen solutions or extracts

	Nasal ⁵⁻⁸	Ocular ^{1,3}
Control application (completed before allergen application)	Diluent, applied to contralateral, bilateral, or ipsilateral nares	Diluent (usually normal saline), applied to contralateral eye
Allergen extract or solution application	Unilateral or bilateral Apply to or direct toward the anterior section of inferior turbinate	Unilateral Apply to inferior-lateral quadrant of bulbar conjunctiva
Allergen extract or solution concentration (single dose or graded, graded preferred to assess for dose response; standardized allergen extracts preferred)	Starting dose: 1/100th of the concentration that produces a positive skin prick test response or the concentration that produces a 3-mm wheal on skin prick tests ⁵⁻⁷ or 1:10,000 (wt/vol) if negative skin test result ⁷	Starting dose: 3- to 4-log fold less than skin prick/puncture concentration (approximately 1:10,000-1:100,000 [wt/vol]) ¹ or 3 IR/mL ³
Graded challenge	Increasing doses by 3- to 10-fold every 15-60 minutes until positive response ⁸ or 1:1000 (wt/vol) tested without response ⁷	Increasing doses by 2- to 10-fold every 15-60 minutes until positive response or 1:1000 (wt/vol) or 100 IR/mL tested without response ^{1,3}
Selected positivity criteria ^{3,6,27,29} (compared with diluent control; measured at baseline and before each increasing dose)	Symptom score: increase by $\geq 30\%$ of the maximum possible score PNIF: decrease by $\geq 40\%$ Rhinomanometry: increase in airflow resistance by $\geq 100\%$ Acoustic rhinometry: decreased nasal cavity volume/minimum cross-sectional area by $\geq 25\%$	TOSS score: ≥ 5 points or redness + itching score > 2 points

This table contains an example protocol for nasal and ocular challenge using allergen extract solutions. Several possible protocols with various starting and ending allergen concentrations exist for NACs and OACs. Positivity criteria are not standardized, and other criteria are in the literature and used for research and clinical practice.

IR, Index of reactivity; TOSS, Total Ocular Symptom Score.

TABLE II. Methods of allergen exposure in NACs and OACs

Methods of allergen exposure	Advantages	Disadvantages
Nasal challenge ^{2,5}		
Direct application of allergen or extract into nasal cavity (eg, sprays, drops, powders, and discs)	Simple to perform Standardized dose	Allergen exposure likely does not mimic natural exposure (acute high dose vs chronic low dose)
Park or field study	Mimics natural allergen exposure during season	Multiple possible confounders given uncontrolled environment Allergen exposure can vary from season to season Difficult to control for variable priming from current seasonal exposure, unless subjects live remotely Single-day exposure does not reflect repeated exposure during season
Environmental chamber	More consistently simulates natural allergen exposure Controls for possible confounders that might be present in park study (standardized allergen dose and controlled environment)	Costly, availability limited
Ocular challenge ³		
Direct application of allergen or extract	Simple to perform Standardized dose Other applications: food or latex allergy evaluation	Allergen exposure likely does not mimic natural exposure (acute high dose vs chronic low dose)
Environmental chamber	Simulates natural allergen exposure Standardized dose	Costly, availability limited

challenge during the grass season) or nonspecific (eg, increase in response to other allergens or irritants).^{2,5} Likewise, inflammation, such as after a viral respiratory tract infection, can result in nonspecifically increased responses. Thus, as discussed further below, challenges should not be performed in temporal proximity to infections.^{3,6,7}

NASAL CHALLENGES

NAC is used to identify clinically relevant allergens and confirm nasal allergy, evaluate the effectiveness of therapeutic interventions for rhinitis, and safely diagnose occupational rhinitis and possibly asthma.^{1,2,4,6} NAC is also essential in the diagnosis of local allergic rhinitis (LAR), although the existence

TABLE III. Common medications to hold before NACs and OACs

Medication	Approximate duration to hold	
	NAC ^{6,7}	OAC ³
Topical antihistamines	4-5 d	2-3 d
Topical corticosteroids	2-3 d	2-3 d
Topical mast cell stabilizers	7-21 d	2-3 d
Topical cyclosporine	No recommendation	7 d
Topical NSAIDs	7 d	7 d
Systemic antihistamines*	7 d	7 d
Systemic corticosteroids	14-21 d	14 d
Systemic NSAIDs	7 d	No recommendation
Leukotriene modifiers	No recommendation	21 d
Topical and systemic decongestants	2 d	No recommendation
Tricyclic antidepressants	14-21 d	No recommendation
Clonidine and other central-acting antihypertensives	21 d	No recommendation

This table is not an exhaustive list of all medications that can interfere with challenge results. Any medication with the potential to influence challenge results should be held for an appropriate amount of time based on its half-life.

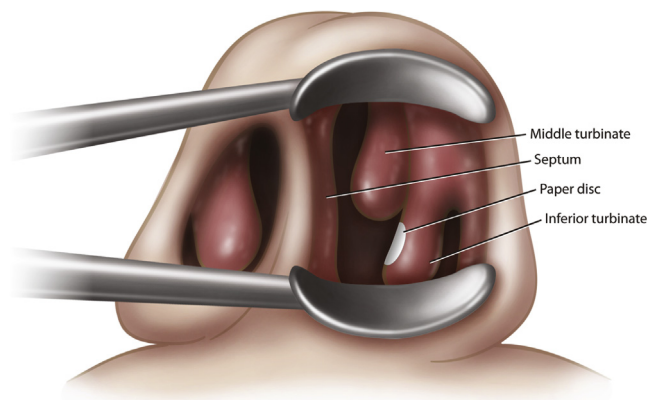
*Some systemic antihistamines should be held for longer durations. Ketotifen should be held for 3 weeks.

TABLE IV. Nonspecific or irritant nasal challenges

Nonspecific nasal challenges ^{2,12,13}	Comments
Cold dry air	Measures nasal hyperreactivity Simple, well tolerated
Hyperosmolar solutions (mannitol, hypertonic saline)	Stimulates release of epithelial cell mediators ¹³ Mannitol induces burning sensation
Hypotonic distilled water	Measures nasal hyperreactivity Minimal side effects
Histamine	Direct stimulation of histamine receptors and simulation of EPR in patients with allergic rhinitis and LAR Induces contralateral reflex response
Methacholine	Stimulates glandular secretion Does not induce contralateral reflex response
Adenosine monophosphate	Stimulates primed mast cell degranulation Good correlation with specific allergen challenge
Capsaicin	Measures nasal hyperreactivity
Exercise	Difficult to quantify stimulus and control conditions

EPR, Early-phase response.

of this entity is controversial.^{10,11} LAR is the occurrence of localized nasal allergic sensitivity with signs and symptoms after allergen exposure but without detectable levels of systemic specific IgE.¹⁰ In addition to allergens, nonspecific nasal challenges with irritants and physical triggers, such as cold dry air and capsaicin, might help quantify mucosal hyperreactivity in patients with allergic and nonallergic rhinitis (Table IV).^{2,12,13} Nasal challenges with aspirin or other nonsteroidal anti-inflammatory drugs

**FIG 1.** Placement of a paper disc containing allergen on the inferior turbinate for a NAC.**FIG 2.** Photo of the Global Allergy and Asthma European Network (GA²LEN) mobile chamber: one example of an environmental chamber. Photo courtesy of Thomas B. Casale, MD, Torsten Zuberbier, MD, PhD, and the GA²LEN Coordinating Office.

(NSAIDs) might help in the diagnosis of aspirin-exacerbated respiratory disease (AERD).

Methods of provocateur exposure

Purified allergen or pollen is generally used for environmental chamber challenges, whereas extracts containing multiple allergens eluted from a single allergen source are typically used in direct mucosal NACs and OACs.^{2,3,9,14} However, extract preparations and units of measure differ depending on the source country and standardization status of the extract. Glycerinated extracts, which are common in the United States, might be too viscous for sprays and irritate the mucosa if not properly diluted.⁶ Lyophilized extracts are available and commonly used in Europe.³

Direct mucosal challenges. For direct mucosal challenges, allergen extracts are applied directly to the nasal mucosa through sprays, aerosols, drops, powders, or impregnated paper discs containing known allergen concentrations (Fig 1 and Tables I and II).^{4,6} Dry pollen grains can also be applied directly to the nasal mucosa.¹ For extracts, a challenge with diluent is performed first to exclude nonspecific or irritant responses.² A commonly used NAC control is the contralateral nasal airway, but this



FIG 3. Photo of the Environmental Exposure Unit (Kingston General Hospital and Queen's University, Kingston, Ontario, Canada): one example of an environmental chamber. Photo courtesy of Thomas B. Casale, MD, and Anne K. Ellis, MD, MSc.

comparison might be obfuscated by contralateral neurogenic reflex responses after a positive ipsilateral challenge result.² These direct application methods allow for controlled exposure to a specific allergen concentration and can be performed easily in the clinical or research setting but usually use doses much greater than those found with natural exposure.

Direct mucosal nasal challenges with aspirin or other NSAIDs are used to diagnose AERD in patients with severe asthma or other contraindications to oral or bronchial aspirin challenge.^{4,15} Nasal challenges are also used before oral aspirin challenge to help improve safety but are contraindicated in patients with severe nasal polyposis.^{15,16} Most protocols use soluble lysine aspirin, which is not available in the United States.^{15,16} Nasal challenge with ketorolac solution, an alternative NSAID, is used in the United States.¹⁵⁻²⁰ Initiating the challenge with nasal mucosal application can decrease the time required for the systemic challenge and may reduce the risk for a severe asthma response.^{16,20} Nasal ketorolac challenge, followed by oral aspirin challenge, may lessen the total required duration from 3 to 1.5 days.^{16,20} Lung function measurements, including FEV₁ and peak expiratory flow, should be monitored along with the upper airway outcome measures discussed below.¹⁵ If the nasal challenge result is negative, a subsequent oral aspirin challenge is needed to exclude AERD.¹⁵

Environmental challenges. Park or field studies examine the effects of natural allergen exposure outdoors during the pollen season. Despite being the traditional method for assessing natural allergen exposure in clinical trials, park studies are potentially affected by several confounding variables that can cause

fluctuations in pollen counts.^{5,21} Pollen counts change with time (from one season to another or within a season), geographic location, and weather variations, which might compromise the reliability and reproducibility of park studies.^{14,22} Furthermore, individual pollen exposures are influenced by lifestyle choices.^{14,21,22} The coordination of multicenter park studies can be challenging because, as inherent to their design, these studies must be conducted within a narrow window of time during the pollen season.²²

Environmental chambers or units permit airborne allergen or irritant exposure in a controlled setting (Figs 2 and 3).^{9,14,22,23} Nasal and ocular symptoms in the natural ragweed season correlate highly with chamber exposure to short ragweed pollen in one study.²⁴ However, in another study of grass pollen, a correlation between natural and chamber exposure was present only at the 24-hour time point after a 4-hour challenge.²⁵ Chambers can be stationary or mobile and allow for a single allergen challenge or multiple simultaneous challenges because of advances in airflow technology.^{9,14,23} The Global Allergy and Asthma European Network (GA²LEN) chamber allows for 9 patients to be challenged concurrently, each with different allergens, if necessary (Fig 2). It can also be transported by a truck equipped with a crane.¹⁴ Studies using chambers can achieve similar conclusions with fewer participants in less time because of decreased variability.⁹ However, chambers are expensive and relatively scarce, and the reproducibility and calibration between different chambers is not established, making multicenter studies difficult to conduct.^{5,9,14} Thus these devices are primarily used in single-center clinical trials.^{2,5}

TABLE V. TNSSs and TOSSs

Symptom	Scale	Interpretation
TNSS^{2,5,27}		
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	
TOSS^{3,26}		
Itching	0-4	0 = None
Redness	0-3	1 = Mild
Chemosis	0-3	2 = Moderate
Tearing	0-3	3 = Severe
Sum total	0-13	4 = Incapacitating
Lid swelling (if present)	0-3	

The TNSS is the sum total of sneezing, rhinorrhea, congestion, and itching. A change in TNSS of 30% or greater of the maximum total score is clinically important.²⁷ The TOSS is the summation of itching, redness, chemosis, and tearing. Lid swelling can be assessed separately. A TOSS of 5 or greater or redness plus itching sum total of greater than 2 is a positive response.³

TOSS, Total Ocular Symptom Score.

Evaluating outcomes

Nasal challenges are assessed with subjective and objective measures. The Total Nasal Symptom Score (TNSS) and visual analog scale (VAS) are commonly used subjective assessment tools (Table V).^{2,3,5,26,27} The TNSS is the sum of sneezing, rhinorrhea, congestion, and nasal itching, each scored on a scale from 0 (none) to 3 (severe).^{2,5,28,29} In the VAS subjects place a mark on a 100-mm (10-cm) line to indicate the severity of individual or overall symptoms on a scale from 0 (none) to 100 (severe).^{2,5} Ocular symptoms not assessed by the TNSS are included in some scoring methods.⁶

Nasal resistance measurements, sneeze counts, nasal fraction of exhaled nitric oxide (FENO) measurements, analysis of nasal secretions, and mucosal biopsies are objective assessments of nasal challenge response.^{1,2} Peak nasal inspiratory flow (PNIF), rhinomanometry, and acoustic rhinometry measure nasal patency, and each has advantages and disadvantages. PNIF is simple, noninvasive, and well tolerated, facilitating repetitive measurements (Fig 4).^{2,5} However, PNIF is effort dependent, and comparisons between subjects are less informative.² Rhinomanometry and acoustic rhinometry are more complex measurements of nasal resistance or patency. Rhinomanometry measures transnasal pressure, either anteriorly or posteriorly, depending on the location of sensor placement in the nasal cavity. It is also classified as active or passive, contingent on the subject using a specified breathing pattern or breathing spontaneously.^{2,5} Acoustic rhinometry uses transmitted sound waves to measure the minimal cross-sectional area of the nasal airway (Fig 5). It is less dependent on subject effort and correlates with the size of the inferior turbinate.^{2,5} Correlations with subjective nasal symptoms are variable for rhinomanometry and acoustic rhinometry, but PNIF correlates with subjective nasal congestion.^{2,5} Nasal FENO values are increased in allergic rhinitis and eosinophilic nasal polyposis. However, reproducibility is inconsistent, and measurements might be affected by variations in sinus patency.^{4,30} Sinus obstruction decreases measured nasal FENO levels because nitric oxide is primarily produced by the mucosal epithelium of the paranasal sinuses.³⁰

Nasal secretions and, in research settings, mucosal biopsy specimens contain inflammatory mediators, markers, and cells,



FIG 4. Example of a PNIF meter with facemask attached (Clement Clarke International, Harlow, Essex, United Kingdom).



FIG 5. An examiner (right) performing nasal acoustic rhinometry on a subject (left). Photo courtesy of Thomas B. Casale, MD.

which can be analyzed and quantified before, during, and after challenge.^{2,5} Nasal lavage, scrapings, brushings, blown secretions, and application of absorptive matrixes are less invasive than biopsies.^{1,2,4,5} Depending on the timing of collection, secretions can contain early- or late-phase inflammatory mediators and cells (Table VI).^{1-3,5,31} 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.^{2,5}

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. Both scrapings and brushings are more invasive than lavage but less than biopsy.^{2,5}

Obtaining blown secretions, including nasal cytology, is minimally invasive, but the sample size is highly effort dependent and might be inadequate in many subjects.⁵ Collection of secretions with a matrix, such as foam or filter paper, corrects for the dilution of lavage fluid.^{2,5,6} However, the material used, nasal placement, and mucosal contact time vary among studies, making comparisons and standardization difficult.⁵

OCULAR CHALLENGES

OACs share many similarities to NACs and traditionally have been used to help diagnose ocular allergy and in some cases

TABLE VI. Selected nasal and ocular secretion mediators, cytokines, markers, and cells

Measured mediators, cytokines, markers, and cells	Examples	Comments	
		Nasal ^{1,2,5}	Ocular ^{3,31}
Inflammatory cells	Eosinophils, neutrophils, basophils, mast cells, lymphocytes	High concentrations of eosinophils present in allergic response (AR or LAR) Basophils present in LPR of patients with AR	High concentrations of eosinophils present in AC; however, neutrophils and other inflammatory cells possibly present as well
Immunoglobulins	Total and specific IgE	Increased in patients with AR and possibly LAR	Increased in patients with AC
Cytokines (T _H 2 cell associated)	IL-4, IL-5, IL-13	Increased in patients with AR, predominantly LPR	Increased in patients with AC
Chemokines	IL-8, eotaxin	IL-8 increased in EPR of patients with AR	Eotaxin increased in patients with AC
Eosinophil mediators	Major basic protein, eosinophilic cationic protein	Increased in patients with AR, predominantly LPR	Eosinophilic cationic protein increased in patients with AC
Mast cell mediators	Histamine, tryptase, prostaglandin D ₂ , cysteinyl leukotrienes	Increased in EPR of patients with AR Histamine also elevated in LPR, likely because of basophil, rather than mast cell, origin	Increased in patients with AC Histamine can also be increased in patients with nonallergic conjunctivitis
Neuropeptides	Substance P, vasoactive intestinal peptide	Substance P increased in AR and nonallergic rhinitis	Increased in patients with AC and nonallergic conjunctivitis
Plasma leakage mediators	Albumin	Used in standardization of nasal lavage samples	
Exhaled gas	FENO	Objective measure of eosinophilic inflammation Might be affected by smoking, sinus patency, and other factors	

This table is not an all-inclusive list. Other measureable mediators are reported in the literature.
AC, Allergic conjunctivitis; AR, allergic rhinitis; EPR, early-phase response; LPR, late-phase response.

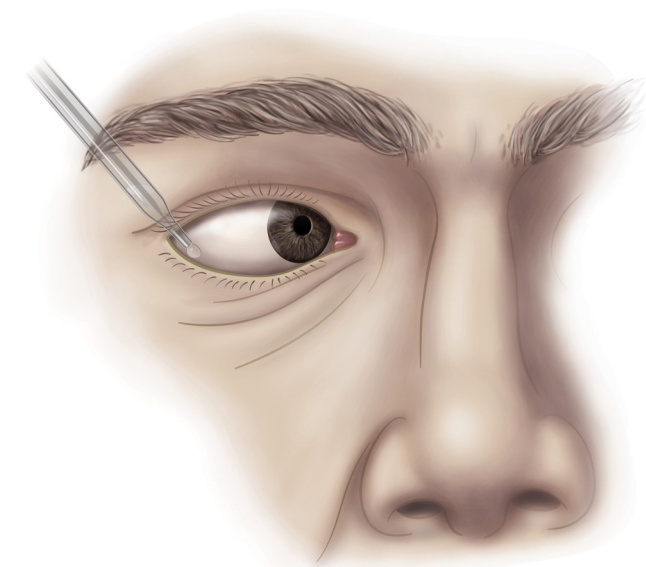


FIG 6. Application of an allergen extract or solution to the inferior lateral area of the bulbar conjunctiva in an OAC. Adapted from Fauquert et al.³

differentiate sensitization from allergy.³ However, new applications are emerging. Studies suggest the utility of OAC in the diagnosis of food allergy, specifically a negative OAC result might help predict a negative oral food challenge result in children with increased *in vitro* specific IgE levels and mildly positive skin prick test responses.^{32,33} OACs also might be helpful in the

diagnosis of latex allergy, occupational allergies, and allergic rhinitis.³

Methods of provocateur exposure

Allergen extract drops are applied to the inferior lateral area of the bulbar conjunctiva (Fig 6). Dry allergen materials, such as pollen grains, are also applied in this area with an applicator.¹ The contralateral eye can serve as a control with the application of saline drops before allergen exposure (Table I).³ Occlusion of the nasolacrimal duct through gentle compression or plug placement during allergen application can prevent systemic absorption and improve safety.^{3,34} Similar to NACs, environmental chambers are another method of allergen challenge.³

Evaluating outcomes

OACs are usually assessed by using quantified scoring criteria, termed the Total Ocular Symptom Score.^{3,30} Ocular redness, tearing, and chemosis are assessed by the investigator, and pruritus is assessed by the subject on a scale from 0 (none) to 3 or 4 (severe or incapacitating, respectively; Table V). Pruritus can also be assessed by using the VAS.³ Additional scoring for eyelid swelling sometimes is added.³ Ocular redness quantification can be documented by using photographic scales. Inflammatory cell infiltration of the conjunctiva can be directly visualized and semi-quantified by using confocal microscopy.^{3,35}

As with nasal challenges, secretions (tears) can be collected and analyzed during and after OACs to assess inflammatory markers, mediators, and cells of the early- or late-phase allergic

response (Table VI).^{3,31} Tears are collected with microcapillary tubes, filter papers, or sponges.³¹ Similar to nasal challenges, each method has advantages and disadvantages, with some mediators binding better to filter paper and sponges than others.³¹ Tear measurements can be diluted unintentionally by irritation and tearing of the eye during collection.³¹ Microcapillary tubes are less likely to evoke reflex tearing.³¹

SAFETY AND CONTRAINDICATIONS

Both NACs and OACs are generally safe. However, a case of anaphylaxis has been reported with OACs, and thus NACs and OACs should be performed in a medical facility prepared to treat anaphylaxis.^{2,3} Subjects with restricted lung function, such as uncontrolled asthma or severe chronic obstructive pulmonary disease, should not undergo NACs or OACs.^{2,3,6,7} Other contraindications to nasal and ocular challenges include pregnancy and recent infection or surgery of the nose and eye, respectively.^{3,6} Nasal challenges should be avoided in subjects with severe nasal obstruction or septal deviation because results might be inaccurate.^{6,7} OACs are contraindicated in subjects with other non-IgE-mediated ocular disorders, including inflammatory diseases of the conjunctiva, iris, or cornea and some cases of severe dry eye syndrome.³ Contact lenses should be removed 72 hours before OAC.³

CONCLUSIONS

NACs and OACs are used commonly in clinical trials as the gold standard to detect nasal and ocular allergy and evaluate novel treatments. However, these challenges can also be used in clinical practice in special circumstances, such as identification of occupational allergy or recognition of LAR.³⁻⁵ A thorough history with appropriate *in vivo* and/or *in vitro* specific IgE tests is usually adequate to determine the most likely allergen or allergens responsible for nasal and/or ocular symptoms. However, when a clinically significant allergen is suspected but undetected, challenges can help guide decisions about immunotherapy and allergen avoidance. Furthermore, nonspecific or irritant challenges might be useful to assess hyperreactivity as a cause of symptoms. Direct mucosal challenges are administered often by using high doses of allergen and therefore may not replicate natural exposure. Park studies and environmental chambers better replicate natural exposure. Additionally, positivity criteria, methodologies, and extract or allergen preparations used in challenges are not uniform. Despite their limitations, NACs and OACs are useful research and diagnostic tools for nasal and ocular allergic diseases.

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