



Collection of nasal secretions and tears and their use in allergology

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Purpose of review

The identification of immunological markers in nasal secretions and tears is becoming essential in the study of allergic diseases. The collection procedure of nasal and ocular secretions directly influences the results, thus it is of paramount importance to validate and standardize the sampling process.

Recent findings

Current techniques for nasal secretions sampling are mainly based on three principles: collection of spontaneous secretions, nasal washings, and absorption. Collection of spontaneous secretions is appropriate in subjects with nasal hypersecretion, whereas in healthy individuals the collected volume is frequently insufficient. Nasal washings are associated with an unpredictable, high dilution and concentrations of markers often fall below detection limits of immunological assays. Absorption seem to provide the best compromise between sufficient sample amounts and detectability of inflammatory mediators and immunoglobulin E. Tear samples can be obtained by glass capillary tubes, filter paper strips and ophthalmic sponges. Volumes are however small or highly diluted through reflex tearing.

Summary

Secretions reflect the local inflammatory activity and provide valuable information about the immunological reaction to allergens at the target organ. There is increasing evidence of the potential clinical role of their analysis, for diagnosis, and monitoring of allergic rhino-conjunctivitis. Appropriate collection and processing is very important and requires special attention.

Keywords

allergic conjunctivitis, allergic rhinitis, immunoglobulin E, nasal secretions, tears

INTRODUCTION

The analysis of human secretions, such as nasal fluid and tears, provide valuable information on pathophysiological aspects of allergic diseases [1–3]. There is increasing evidence of its potential role in allergology diagnostics [4,5] and in immunotherapy monitoring [6,7]. However, testing nasal fluids (and tears) still remains a research domain and is neither included in practice guidelines nor common in clinical practice. Major concerns are the inconsistent data on biomarker concentrations as their low concentration in secretions [8]. Several techniques have been described for the collection of nasal secretions and tears. Standardization is needed, because the sampling method has an important influence on the results obtained [9]. The present review aims to get an overview on the most common methods of nasal secretions and tears sampling and on testing of their content in immunoglobulin E (IgE) and other mediators. Advantages and limitations in their use for the

diagnosis of allergic rhinitis and conjunctivitis will be considered.

NASAL SECRETION PRODUCTION AND COMPOSITION

Nasal secretions derive from four major sources: seromucous submucosal glands, goblet cells, trans-epithelial transport of ion and water, and plasma

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Curr Opin Allergy Clin Immunol 2018, 18:1–9

DOI:10.1097/ACI.0000000000000412

KEY POINTS

- Analysis of nasal secretions and tears is valuable for research quests, diagnostic, and therapy monitoring of allergic diseases.
- Appropriate collection and processing of secretions is important because of its influence on the results, but there is still no standard method.
- Collection of nasal secretions with absorptive devices seems to offer the best compromise between sample volume and IgE levels.
- Aspiration of tears by glass capillary tube requires time and may be uncomfortable for patients, but with precise handling is so far the best method to avoid tear reflex.

transudate [10]. Their volume is augmented by fluid from the lacrimal glands through the nasolacrimal duct and condensed water at the mucosal surface during expiration [11]. The mucus blanket laying on the respiratory epithelium exists as a bilayer fluid, with a surface gel phase and an aqueous periciliary sol layer [12]. It contains 95% water, 2% mucin, 1% electrolytes, 1% lipids, 1% of other proteins, such as albumin, immunoglobulins, lysozyme, kallikrein, and lactoferrin [13]. Albumin represents about 15% of total protein in resting secretions, suggesting that transudation occurs under normal conditions [14]. Albumin secretion increases under inflammatory condition, reflecting increased vascular permeability [15]. The mucus glycoproteins, secreted principally from submucosal glands, give the mucus its characteristic viscoelastic properties [16]. Critical functions of human respiratory secretions are protection of the mucosa and essential host-defence, mainly through proteins secreted by serous cells in the submucosal glands [12].

NASAL SECRETIONS IN PATIENTS WITH ALLERGIC RHINITIS

Allergic Rhinitis is a very common IgE-mediated inflammatory disease caused by mucosal exposure to inhalant allergens [17]. The interaction of the inhaled allergen with the mastcell-bound IgE initiate a cascade of biochemical reactions, cellular activation, and recruitment of inflammatory mediators responsible for end organ response such as watery rhinorrhea [18]. Other characteristic symptoms include sneezing, nasal congestion, and itching [19]. The exploration of inflammatory cells and mediators in nasal secretions helps the understanding and monitoring of mucosal activity [20]. Specific

secretory proteins and cellular activation markers may be used to measure the presence of inflammatory cells and their activity [21]. Eosinophil cationic protein (ECP) is a well standardized marker protein for the activity of eosinophilic nasal mucosal inflammation [22,23]. Myeloperoxidase (MPO) and Elastase has been used in many studies to reflect neutrophil activation [24]. Mast cell activity is marked notably by histamine, and by the more mast-cell-specific tryptase [25]. Cytokines play a dominant role in the pathophysiology of nasal diseases and can be determined in nasal secretions [26].

Ig are the principle-specific mediators of host-defence in nasal secretions [12]. Immunoglobulin A (IgA) is the predominant immunoglobulin present on nasal mucosal surfaces, in which it acts as an active barrier against inhaled antigens and pathogens [27]. Secretory IgA (sIgA) is produced by plasma cells locally in the nasal mucosa and can be used as a marker of glandular secretions [28]. It takes part in the mucosal immunity of target organs of allergic diseases, such as the nasal airways [29]. Immunoglobulin G (IgG) is another major nasal protein that mediates specific antimicrobial functions [30]. It increases in secretions as a consequence of increased vascular permeability [30]. Different subclasses (IgG1–IgG4) have different roles in relation to the antigen [31]. IgG4 is associated with tolerance to aeroallergens, and is used as parameter of allergen immunotherapy monitoring [32].

It is of great interest to measure IgE, key effector element in the allergic reaction, at the mucosal level. Local IgE production in nasal mucosa has been investigated with the analysis of nasal secretions since early years [33]. It was suggested that systemic IgE levels resulted from a surplus of the IgE produced locally in the nasal mucosa [34]. Evidence has been proved that the nasal mucosa is an active site for local somatic hypermutation, clonal expansion, and class switch recombination, crucial steps for the maturation of a B-cell into an IgE-secreting plasma-cell [35–38]. Measurements of local IgE may show high levels independently of serum IgE levels and atopic status [39]. Increased attention to the concept of a form of allergic rhinitis with local (nasal) but not systemic evidence of IgE sensitization [40–42] has raised the debate about the need of a new diagnostic algorithm, with a major role of antigen-specific IgE (sIgE) detection in nasal secretions [5,43,44]. IgE plays an important role also in other nasal diseases: increased levels of the antibody have been found in nasal polyposis [45,46]. The role of IgE detection in nasal secretions is gaining importance, thus it is still challenging because IgE is the least abundant Ig isotype, present at low concentrations in human fluids, in nanogram range [47]. Furthermore, several techniques are

Table 1. Common techniques for nasal secretions sampling

Spontaneous secretions
Nose blowing
Aspiration
Nasal washings
Spray
Irrigation
Pool
Absorption
Cotton wool
Filter paper
Rubber foam
Cellulose sponges: sinus packs, filter discs

currently used for the collection of nasal secretions but there is no standardized method [9].

SAMPLING METHODS OF NASAL SECRETIONS

Current sampling techniques are quite heterogeneous and they are mainly based on the following principles: collection of spontaneous secretions, nasal washings and the use of different absorption devices (Table 1). They all reflect different compromises between the need to obtain a sufficient sample volume and the desire to avoid artefacts of the procedure [8]. The sampling method has an important impact on the level of any substance detected in nasal secretions [9]. The comparison of the outcomes of different studies is thus difficult.

Spontaneous secretions

The amount of spontaneously secreted nasal fluid is often insufficient in healthy individuals under normal conditions [8]. On the contrary, patients with nasal mucosa diseases frequently present nasal hypersecretion and rhinorrhea. In this case, spontaneous secretion are a specimen simple to collect and offering valid results [23].

Nose blowing

Blowing the nose is a simple way to collect nasal secretions and can be performed with a plastic or an aluminum handkerchief in which each nostril should be blown separately. With the help of a Pasteur pipette the fluid can be recovered and transferred into an Eppendorf tube containing a fixed amount of buffer for dilution. Arguments for this method might be that it is quick and easy to

perform, it allows a fixed dilution, and it does not irritate the mucosa. However, the quantities of nasal fluid obtained is very variable, and insufficient sample size for analysis is often observed, especially in healthy individuals [8].

Aspiration

Aspiration techniques were designed to optimize the collection of spontaneous secreted nasal fluid. The microsuction device is a quick method with minimal irritation of the mucosa [48]. The direct aspiration system consists in an outside siliconized metal aspiration tube connected through a metal cap on one side to a tube linked to a vacuum pump and on the other to a disposable collection tube for the secretions [48]. Phosphate buffered saline (PBS) is added to the collected sample after aspiration. Though, the method is limited when too little secretion is present in the nasal cavity [48]. Another major concern is that the dilution factor may differ between the samples and it may be limited for IgE evaluation whose concentration is often under the detection level [49]. Technologic advances in laboratory analysis with the development of highly sensitive immunological assays have partly limited the problem [50,51].

Recently, the aspiration properties of a device originally designed for the collection of middle ear effusion in otitis media, were used to obtain nasal secretions [52]. Nasal secretions were obtained easily and safely, and the characteristic of the aspirator, with its thin tip and the rapid preparation, makes it applicable to daily clinical practice and also to children [52]. With this sampling method, sIgE to Japanese Cedar Pollen was detected in nasal secretions with an immunoassay in most of the symptomatic subjects and in more than half asymptomatic but sensitized patients [52].

Nasal washing

The most commonly dilution technique applied is nasal washings. In literature, different variations of nasal lavages has been described, with differences in terms of application, instilled volume, and recollection device. This method overcomes the problem of sample volume, always providing a large quantity of fluid. Major concern of those techniques is the unknown dilution factor of the secretions by the washing fluid [53]. Partly it is because of the fact that unpredictable fractions of the washing solution may be swallowed, absorbed, or get lost. That results in great variability of the analyte concentrations, which often fall below the lower detection limit of the assays, resulting in high numbers of missing values [26,54].

Nasal irrigation

In the classical nasal lavage, an irrigation fluid (about 10 ml of buffer saline) is applied into the nose and the solution, mixed with a sample of epithelial lining fluid, is recovered by allowing it to run from the nasal cavity into a Petri dish [34]. Alternatively, a catheter can be inserted in the nose to recover the fluid [14,55]. This method seems to be not the most appropriate to detect IgE in nasal secretions, that is the immunoglobulin isotype present at lowest concentrations in humans. The high dilution factor of nasal irrigation may explain the very low nasal secretions concentrations of IgE and IgE binding activity for grass pollen described with this methodology in patients with seasonal allergic rhinitis [34].

Nasal spray

A spray pump or a syringe can be used to deliver small portions of isotonic or hypertonic sodium chloride. This method allows to keep the volume of washing fluid considerably smaller. The volume instilled with the dispenser in each nostril ranges in different studies from 1 to 5 ml NaCl 0.9–1.8% [56,57]. The patient has to sit with his head extended 45° from the horizontal during instillation and keep the position. The diluted mucus is recollected into a vessel after a prefixed time of incubation. This method produced secretions with levels of IgE high enough to be detected in standard immunological assays [4,44,56].

Nasal pool

Nasal pool is a variation of the 'classical' nasal washing [58,59]. The 'nasal pool' device is a soft plastic container with a nasal adapter. The adapter exists in different sizes and is also applicable for children. By gently compressing the device, 10 ml of saline are instilled in each nostril of the subject sitting in a flexed neck position. Challenge – agents, in form of standardized be added to the lavage fluid if a concomitant mucosal provocation is wished. The washing fluid is maintained in contact with the mucosal surface of the nasal cavity for up to 15–20 min in adults. When the pressure is released, the fluid returns into the container. The technique is atraumatic, requires little cooperation from the patient and is easy to handle [60]. When compared to the nasal spray method [57], this modified method gave more reproducible ECP measurement in patients with rhinitis [61] and a higher total number of inflammatory cells in both normal individuals and patients with rhinitis [61]. This may be because of the higher exposure time and the larger

volume instilled by the nasal pool method, suggesting that these two factors are relevant for the results. A limitation of the technique is again the unknown, high dilution factor, that may reduce the concentrations of sIgE antibodies in the collected nasal fluids below the detection limits of the standard tests [40].

The dilution factor issue

The unknown dilution factor is the main problem of the lavage techniques and should be always measured. Plasma albumin passes freely into extravascular spaces and is passively ultra-filtered into nasal secretions [18]. Therefore, albumin was described as a valuable 'endogenous' marker of dilution of nasal secretions [62]. The relative concentration of any analyte can be referred to albumin concentration and expressed as an index [62]. However, the use of the ratio analyte-to-albumin only allows relative but not absolute comparisons [62]. The levels of total protein are also used to correct for the unknown dilution and allow comparison of levels of substances between individuals [48,49,53,63]. To calculate the actual concentration of a substance 'exogenous' markers may be more effective. Among other markers, inulin [55] and lithium chloride [64] have been tested. These substances, not physiologically present in nasal secretions, are added in fixed concentrations to the washing solution, and then detected in the recovered lavage fluid to calculate the dilution factor. The most promising marker seems to be lithium chloride, outperforming inulin and other substances because of its harmless, easy, and low-cost application [64]. However, the application of markers to determine the dilution factor increases the complexity of the procedure and no standard marker has yet been set [8].

Absorption

The use of a sampler with absorptive properties placed within the nasal cavity is another concept of nasal secretion collection. Different materials have been tested for this purpose: cotton [65], filter paper [33], rubber foam [26,66], and cellular sponges of different sizes and forms [54,67]. Absorption method is based on capillary suction, recovering mainly the aqueous sol phase of the bilayer fluid on the nasal mucosa [8]. Absorbent materials are inserted in both nasal cavities on the floor between the septum and inferior turbinate and left in place for 5–10 min (Fig. 1) [54]. After removal, rapid sealing and centrifugation is needed to extract the absorbed fluid and avoid drying [8]. Sufficient amounts of undiluted nasal secretions have been

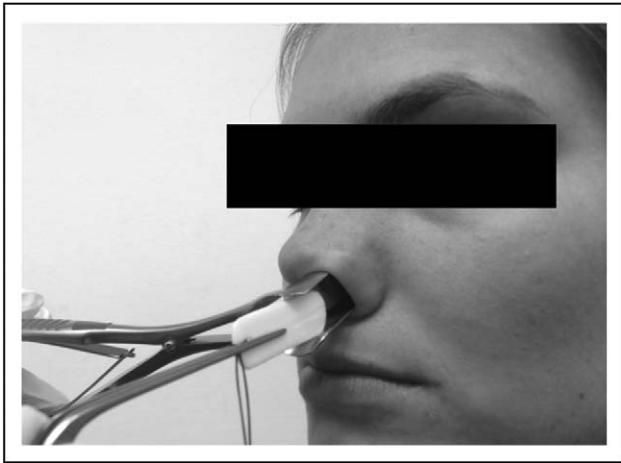


FIGURE 1. Insertion of sinus pack in the nasal cavity.

demonstrated with cotton wool samplers [8] and rubber-foam samplers [26] with no need of PBS addition. Processing the fluid collected with cellular sponges, such as sinus packs and filter disks, require a small amount of physiologic solution to mobilize the nasal secretions out of the absorbent material [54]. This procedure can be performed with fixed dilution or with fixed volume [68[•]]. The latter requires the calculation of dilution factor to correct the concentrations measured [54].

When compared to other sampling methods, absorption demonstrated a superior detectability and reproducibility for analyzing local inflammatory mediators [8,26,66,69]. The procedure requires minimum cooperation from the patient [26]. Another argument for the method is the possibility of a clear differentiation between nasal sides [54]. However, the sponge may cause little irritation of the mucosa and possibly produce artefacts [54]. Sponges of bigger size, like sinus packs and rubber-foam samplers, guarantee sufficient amount of absorbed secretions but may not be applicable in case of severe nasal polyposis and anterior septal deviation [66]. In these cases, filter paper strips are more suitable, though the volume collected with this material is often insufficient because of contact with a limited area of the nasal mucosa [66]. Patients should be allowed to freely breathe through the nose during sampling time. However, the nasal air flow is hindered by most of the absorbent samplers [66]. Inserting a plastic tube in the center of the RFS could improve both air flowing and sampler handling [66].

DETECTION OF IMMUNOGLOBULIN E *IN SITU*: NASAL TEST

To overcome the main problems of nasal secretions sampling, some authors have developed an alternative

method in which the test device is put in direct contact with the nasal mucosa, the 'Nasal Test' [70]. In this procedure, the solid phase coupled with allergens is placed directly on nasal mucosa behind the internal ostium and kept in place for 5 min [70]. Allergen-IgE antibody incubation is so performed *in situ*, in the same conditions in which IgE-mediated reaction takes place [70]. Compared to the 3 h incubation time needed in collected nasal secretions to reach the maximum value, the Nasal Test is described as a time saving procedure in which the maximum value is reached within 5 min incubation [70]. Good sensitivity, specificity, and reproducibility of the method are reported [70,71]. The Nasal Test seems to be suitable and well accepted even by children [70,71]. However, there are also limitations to be considered: only few studies have been performed with this method [70,71]. Furthermore, the allergens come into contact with the mucosa of the patient. The Nasal Test is therefore at the same time a provocation test and might provoke a reaction. The safety of the procedure has thus to be carefully investigated.

An overview of the principal advantages and disadvantages of each method for nasal secretions collection is shown in Table 2.

IMMUNOLOGICAL ASSAYS FOR ANTIBODY DETECTION

The most common immunological detection assays for biomarkers in serum can detect analytes also in nasal secretions [56,72,73]. However, they require large volumes and the detection level is not always sensitive enough for molecules present at low concentration such as IgE [68[•]]. Recently, our group pioneered the use of microarray biochips for measurement of sIgE against a large number of different allergen molecules [68[•]]. The results validated the allergen microarray chip as a high sensitive and specific detection assay for sIgE in nasal secretions. It combines the advantages of being a multiplex test requiring relative little sample volumes [68[•]].

TEARS IN PATIENTS WITH OCULAR ALLERGY

Ocular and nasal symptoms commonly occur in combination in IgE-mediated response to allergens, in form of an allergic rhino-conjunctivitis [74]. Allergic conjunctivitis also exists as an independent symptom complex, characterized by ocular itching, hyperemia, and lacrimation, with similar but distinct pathophysiological characteristics to allergic rhinitis [75]. The detection of tears specific immunologic and inflammatory markers may provide additional information about local allergic processes

Table 2. Comparison of different techniques for nasal secretions collection

Method	Advantage	Disadvantage
Nose blowing [8,23]	Quick Easy to perform Nonirritative Fixed Dilution	Often insufficient sample Subject-cooperation is needed
Aspiration [48,49]	Quick Easy to perform Nonirritative	Often insufficient sample Technically difficult
Nasal washing [14,26,34,53–59]	Easy to perform Large quantity of fluid	Dilution of mediators and cytokines Variable recovery of fluid Subject-cooperation is needed
Absorption devices [8,26,54,65–67,68 [■]]	No/limited dilution of mediators Best results in literature	May irritate the nose At times insufficient sample Non applicable if: nasal polyposis, septal deviation
Nasal Test [70,71]	Collection and incubation in one step Time-saving	Not many data available Allergic reaction may occur

and be useful for the diagnosis or monitoring of allergic conjunctivitis [76–78].

IgE sensitization in patients with allergic diseases may be limited at a local, conjunctival level [76]. Measurement of total and allergen-sIgE in tear fluid can help to diagnose IgE-mediated allergic conjunctivitis also in the absence of systemic sensitization [76].

TEAR COLLECTION

Different methods and techniques allow to sample tears from the ocular surface: the microcapillary tube method [79,80[■]] and the use of filter paper [81–83] or ophthalmic sponges [84] are the most common (Table 3). The sampling procedures are uncomplicated and do not need local anesthesia, but the

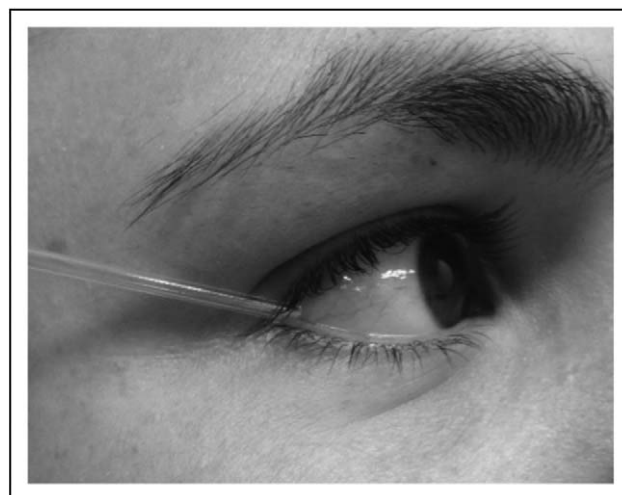
recovered volumes are small or highly diluted through tear reflex provoked by touching the conjunctiva [85]. Reflex tears and basal tears are different in composition [86]. Obtaining reproducible and unaltered samples with the minimum discomfort of the patient is thus a challenge. The ideal collection technique should be well tolerated, minimal invasive, quick, and easy to handle, and guarantee good extraction and detection of biomarkers with the current used immunological assays.

Microcapillary tube method

Aspiration of ocular fluid by glass capillary tubes or pipettes is performed from the external canthus of the eye (Fig. 2). Widely used and validated in

Table 3. Comparison of different techniques for tears collection

Method	Advantage	Disadvantage
Schirmer test strips [81–83]	Quick Easy to perform Suitable for clinical routine	Reflex tearing Strong irritation
Microcapillary tube [3,79,80 [■]]	Large volume Rare reflex tearing if precise handling	Technically difficult Time-consuming Inappropriate for children or anxious patients
Ophthalmic sponges [84,85]	Quick Easy to perform Standard volume Good acceptance	Reflex tearing Problematic mediators-extraction

**FIGURE 2.** Sampling tear fluid with capillary tube.

literature, it is considered to be the best method for avoiding reflex tearing [3]. However, handling needs experience and precision and is time-consuming [84]. Therefore, it is not always practicable in field studies or clinical routine, especially if pediatric populations are involved [84]. Furthermore, it may result in an inappropriate method for anxious or uncooperative patients [84].

Schirmer's test strips

Filter paper strips commonly used for the Schirmer's test in the diagnosis of dry eye are an easy and quick method for tears collection, suitable also in clinical routine. The paper strip is placed in the lower fornix of each eye and left in place until it has been wetted to the control line [81]. However, the strip may cause strong irritation and tear reflex often occurs causing unwanted dilution of analytes [3].

Ophthalmic sponges

Tear fluid can be alternatively collected from the external canthus of the eye by ophthalmic sponges, which seem to be well tolerated by patients [85]. Another advantage is that sponges allow standardization of the collected tear volume [84]. Sponges of different materials and various extraction buffers were combined and tested in order to optimize the tear collection method and overcome the most common limitations, making it however difficult to compare the results [84,85,87]. Another major concern is that some cytokines bind tightly to the sponges causing difficulties during the extraction procedure [84,88]. Tear reflection may occur also with this method, causing unwanted dilution [85].

CONCLUSION

Appropriate collection and processing of nasal and ocular secretions is necessary to obtain valid and comparable results allowing an accurate evaluation of allergic diseases at local level. An ideal sampling method should allow to obtain a sufficient sample volume without need of high dilution, be easy to handle and suitable for clinical routine, require minimal cooperation from the subject, be not too invasive and not irritate or traumatize the target organ, have a high detectability with common detection assays, be reproducible, and keep the artefacts at the minimum. For nasal secretions, absorption methods seems to be up to date advantageous, giving the best results in terms of detectability and reproducibility in

the analysis of immunoglobulins and local inflammatory mediators [8,26,66,69]. Collecting tears with microcapillary tube method is still considered the best method to obtain reproducible and unaltered samples [3].

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bachert C, Becker W, Ganzer U. The role of nasal secretions in allergic disease of the nose. *Arch Otorhinolaryngol* 1989; 246:173–182.
2. Togias A, Naclerio RM, Proud D, *et al.* Mediator release during nasal provocation. A model to investigate the pathophysiology of rhinitis. *Am J Med* 1985; 79(6A):26–33.
3. Leonardi A. Allergy and allergic mediators in tears. *Exp Eye Res* 2013; 117: 106–117.
4. Miadonna A, Leggieri E, Tedeschi A, Zanussi C. Clinical significance of specific IgE determination on nasal secretion. *Clin Allergy* 1983; 13: 155–164.
5. Rondon C, Campo P, Togias A, *et al.* Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol* 2012; 129: 1460–1467.
6. Platts-Mills TA, von Maur RK, Ishizaka K, *et al.* IgA and IgG antiragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 1976; 57: 1041–1050.
7. Scadding GW, Eifan AO, Lao-Araya M, *et al.* Effect of grass pollen immunotherapy on clinical and local immune response to nasal allergen challenge. *Allergy* 2015; 70:689–696.
8. Klimek L, Rasp G. Norm values for eosinophil cationic protein in nasal secretions: influence of specimen collection. *Clin Exp Allergy* 1999; 29: 367–374.
9. Maremmani C, Fattori B, De Ciccio M, *et al.* Electrophoretic pattern of physiological human nasal secretions. *Rhinology* 1996; 34:147–150.
10. Kaliner MA. Human nasal respiratory secretions and host defense. *Am Rev Respir Dis* 1991; 144(3 Pt 2):S52–S56.
11. Dahl R, Mygind N. Anatomy, physiology and function of the nasal cavities in health and disease. *Adv Drug Deliv Rev* 1998; 29(1–2):3–12.
12. Kaliner MA. Human nasal host defense and sinusitis. *J Allergy Clin Immunol* 1992; 90(3 Pt 2):424–430.
13. Kaliner M, Marom Z, Patow C, Shelhamer J. Human respiratory mucus. *J Allergy Clin Immunol* 1984; 73:318–323.
14. Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine-induced nasal secretions. *Am Rev Respir Dis* 1988; 138:413–420.
15. Raphael GD, Meredith SD, Baraniuk JN, *et al.* The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis* 1989; 139:791–800.
16. Baraniuk JN, Kaliner M. Neuropeptides and nasal secretion. *Am J Physiol* 1991; 261(4 Pt 1):L223–L235.
17. Naclerio RM. Allergic rhinitis. *N Engl J Med* 1991; 325:860–869.
18. Raphael GD, Igarashi Y, White MV, Kaliner MA. The pathophysiology of rhinitis. V. Sources of protein in allergen-induced nasal secretions. *J Allergy Clin Immunol* 1991; 88:33–42.
19. Norman PS. Allergic rhinitis. *J Allergy Clin Immunol* 1985; 75:531–548.
20. Wilson SJ, Lau L, Howarth PH. Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998; 28:220–227.
21. Venge P. Soluble markers of allergic inflammation. *Allergy* 1994; 49:1–8.
22. Venge P. Monitoring the allergic inflammation. *Allergy* 2004; 59:26–32.

23. Niehaus MD, Gwaltney JM Jr, Hendley JO, *et al.* Lactoferrin and eosinophilic cationic protein in nasal secretions of patients with experimental rhinovirus colds, natural colds, and presumed acute community-acquired bacterial sinusitis. *J Clin Microbiol* 2000; 38:3100–3102.
 24. Venge P. Eosinophil and neutrophil granulocytes. *Allergy* 1993; 48(17 Suppl):39–47; discussion 48–9.
 25. Rasp G, Hochstrasser K. Tryptase in nasal fluid is a useful marker of allergic rhinitis. *Allergy* 1993; 48:72–74.
 26. Riechelmann H, Deuschle T, Friemel E, *et al.* Biological markers in nasal secretions. *Eur Respir J* 2003; 21:600–605.
 27. Russell MW, Mestecky J. Induction of the mucosal immune response. *Rev Infect Dis* 1988; 10(Suppl 2):S440–S446.
 28. Raphael GD, Jeney EV, Baraniuk JN, *et al.* Pathophysiology of rhinitis. Lactoferrin and lysozyme in nasal secretions. *J Clin Invest* 1989; 84: 1528–1535.
 29. Carlier FM, Sibille Y, Pilette C. The epithelial barrier and immunoglobulin A system in allergy. *Clin Exp Allergy* 2016; 46:1372–1388.
 30. Meredith SD, Raphael GD, Baraniuk JN, *et al.* The pathophysiology of rhinitis. III. The control of IgG secretion. *J Allergy Clin Immunol* 1989; 84(6 Pt 1):920–930.
 31. Fokkens WJ, Scheeren RA. Upper airway defence mechanisms. *Paediatr Respir Rev* 2000; 1:336–341.
 32. James LK, Till SJ. Potential mechanisms for IgG4 inhibition of immediate hypersensitivity reactions. *Curr Allergy Asthma Rep* 2016; 16:23.
 33. Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 1975; 2:148–150.
 34. Platts-Mills TA. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 1979; 122:2218–2225.
 35. KleinJan A, Godthelp T, van Toornenbergen AW, Fokkens WJ. Allergen binding to specific IgE in the nasal mucosa of allergic patients. *J Allergy Clin Immunol* 1997; 99:515–521.
 36. KleinJan A, McEuen AR, Dijkstra MD, *et al.* Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation. *J Allergy Clin Immunol* 2000; 106: 677–686.
 37. Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J Immunol* 2003; 171:5602–5610.
 38. Takhar P, Smurthwaite L, Coker HA, *et al.* Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol* 2005; 174:5024–5032.
 39. Powe DG, Bonnin AJ, Jones NS. 'Entropy': local allergy paradigm. *Clin Exp Allergy* 2010; 40:987–997.
 40. Rondon C, Romero JJ, López S, *et al.* Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis. *J Allergy Clin Immunol* 2007; 119:899–905.
 41. Rondon C, Doña I, López S, *et al.* Seasonal idiopathic rhinitis with local inflammatory response and specific IgE in absence of systemic response. *Allergy* 2008; 63:1352–1358.
 42. Arasi S, Pajno GB, Lau S, *et al.* Local allergic rhinitis: a critical reappraisal from a paediatric perspective. *Paediatr Allergy Immunol* 2016; 27:569–573.
 43. Rondon C, Campo P, Galindo L, *et al.* Prevalence and clinical relevance of local allergic rhinitis. *Allergy* 2012; 67:1282–1288.
 44. Zicari AM, Occasi F, Di Fraia M, *et al.* Local allergic rhinitis in children: novel diagnostic features and potential biomarkers. *Am J Rhinol Allergy* 2016; 30:329–334.
 45. Wise SK, Ahn CN, Schlosser RJ. Localized immunoglobulin E expression in allergic rhinitis and nasal polyposis. *Curr Opin Otolaryngol Head Neck Surg* 2009; 17:216–222.
 46. Gevaert P, Nouri-Aria KT, Wu H, *et al.* Local receptor revision and class switching to IgE in chronic rhinosinusitis with nasal polyps. *Allergy* 2013; 68:55–63.
 47. Dullaers M, De Bruyne R, Ramadan F, *et al.* The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 2012; 129:635–645.
 48. Biewenga J, Stoop AE, Baker HE, *et al.* Nasal secretions from patients with polyps and healthy individuals, collected with a new aspiration system: evaluation of total protein and immunoglobulin concentrations. *Ann Clin Biochem* 1991; 28(Pt 3):260–266.
 49. Swart SJ, van der Baan S, Steenbergen JJ, *et al.* Immunoglobulin concentrations in nasal secretions differ between patients with an IgE-mediated rhinopathy and a non-IgE-mediated rhinopathy. *J Allergy Clin Immunol* 1991; 88:612–619.
 50. Ono T, Kawamura M, Arai S, Nariuchi H. A highly sensitive quantitative immunochromatography assay for antigen-specific IgE. *J Immunol Methods* 2003; 272(1–2):211–218.
 51. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol* 2010; 125(2 Suppl 2):S284–S296.
 52. Sakaida H, Masuda S, Takeuchi K. Measurement of Japanese cedar pollen-specific IgE in nasal secretions. *Allergol Int* 2014; 63:467–473.
 53. Heikinen T, Shenoy M, Goldblum RM, *et al.* Quantification of cytokines and inflammatory mediators in samples of nasopharyngeal secretions with unknown dilution. *Paediatr Res* 1999; 45:230–234.
 54. Watelet JB, Gevaert P, Holtappels G, *et al.* Collection of nasal secretions for immunological analysis. *Eur Arch Otorhinolaryngol* 2004; 261:242–246.
 55. Cohen AB, Goldberg S, London RL. Immunoglobulins in nasal secretions of infants. *Clin Exp Immunol* 1970; 6:753–760.
 56. Merrett TG, Houri M, Mayer AL, Merrett J. Measurement of specific IgE antibodies in nasal secretion—evidence for local production. *Clin Allergy* 1976; 6:69–73.
 57. Naclerio RM, Meier HL, Kagey-Sobotka A, *et al.* Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983; 128:597–602.
 58. Greiff L, Meyer P, Svensson C, *et al.* The 'nasal pool'-device for challenge and lavage of the nasal mucosa in children: histamine-induced plasma exudation responses. *Pediatr Allergy Immunol* 1997; 8:137–142.
 59. Grunberg K, Timmers MC, Smits HH, *et al.* Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp Allergy* 1997; 27:36–45.
 60. Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990; 20: 253–259.
 61. Belda J, Parameswaran K, Keith PK, Hargreave FE. Repeatability and validity of cell and fluid-phase measurements in nasal fluid: a comparison of two methods of nasal lavage. *Clin Exp Allergy* 2001; 31:1111–1115.
 62. Mathews KP. Calculation of secretory antibodies and immunoglobulins. *J Allergy Clin Immunol* 1981; 68:46–50.
 63. Reisacher WR. Mucosal brush biopsy testing of the inferior turbinate to detect local, antigen-specific immunoglobulin E. *Int Forum Allergy Rhinol* 2012; 2:69–74.
 64. Linder A, Ronquist G, Deuschl H. Random distribution of exogenous lithium in nasal secretion and its application in substance determination. *Acta Otolaryngol* 1983; 96(3–4):287–293.
 65. Eichner H, Behbehani AA, Hochstrasser K. [Diagnostic value of nasal secretions, current state: normal values. 1]. *Laryngol Rhinol Otol (Stuttg)* 1983; 62:561–565.
 66. Lü FX, Esch RE. Novel nasal secretion collection method for the analysis of allergen specific antibodies and inflammatory biomarkers. *J Immunol Methods* 2010; 356(1–2):6–17.
 67. Kristiansen BE. Collection of mucosal secretion by synthetic discs for quantitation of secretory IgA and bacteria. *J Immunol Methods* 1984; 73:251–257.
 68. Berings M, Arasi S, De Ruyck N, *et al.* Reliable mite-specific IgE testing in nasal secretions by means of allergen microarray. *J Allergy Clin Immunol* 2017; 140:301–303.e8.
- This is the first study to use an allergen microarray chip for specific IgE detection in nasal secretions of allergic rhinitis patients.
69. Alam R, Sim TC, Hilsmeier K, Grant JA. Development of a new technique for recovery of cytokines from inflammatory sites in situ. *J Immunol Methods* 1992; 155:25–29.
 70. Marcucci F, Sensi L. A new method for IgE detection in nasal mucosa. *Clin Exp Allergy* 1989; 19:157–162.
 71. Fuiano N, Fusilli S, Incorvaia C. A role for measurement of nasal IgE antibodies in diagnosis of Alternaria-induced rhinitis in children. *Allergol Immunopathol (Madr)* 2012; 40:71–74.
 72. Kim JH, Yoon MG, Seo DH, *et al.* Detection of allergen specific antibodies from nasal secretion of allergic rhinitis patients. *Allergy Asthma Immunol Res* 2016; 8:329–337.
 73. Huttunen K, Tiitonen K, Roponen M, *et al.* The effect of assay type and sample matrix on detected cytokine concentrations in human blood serum and nasal lavage fluid. *J Pharm Biomed Anal* 2014; 96:151–155.
 74. Bielory L. Allergic conjunctivitis and the impact of allergic rhinitis. *Curr Allergy Asthma Rep* 2010; 10:122–134.
 75. Watelet JB, McGill JI, Pawankar R, *et al.* Allergic rhinitis and conjunctivitis: update on pathophysiology. In: Pawankar R, Holgate ST, Rosenwasser LJ, editors. *Allergy frontiers: clinical manifestations*. Springer: Tokyo, Japan; 2009pp. 1–23.
 76. Leonardi A, Battista MC, Gismondi M, *et al.* Antigen sensitivity evaluated by tear-specific and serum-specific IgE, skin tests, and conjunctival and nasal provocation tests in patients with ocular allergic disease. *Eye (Lond)* 1993; 7(Pt 3):461–464.
 77. Leonardi A, Borghesan F, Faggian D, *et al.* Tear and serum soluble leukocyte activation markers in conjunctival allergic diseases. *Am J Ophthalmol* 2000; 129:151–158.
 78. Cook EB. Tear cytokines in acute and chronic ocular allergic inflammation. *Curr Opin Allergy Clin Immunol* 2004; 4:441–445.
 79. Nomura K, Takamura E. Tear IgE concentrations in allergic conjunctivitis. *Eye (Lond)* 1998; 12(Pt 2):296–298.
 80. Leonardi A, Borghesan F, Faggian D, Plebani M. Microarray-based IgE detection in tears of patients with vernal keratoconjunctivitis. *Paediatr Allergy Immunol* 2015; 26:641–645.
- This study identifies local allergic sensitisation measuring specific IgE in tears with a multiplex allergen microarray.
81. Mimura T, Usui T, Mori M. Immunochromatographic assay for measurement of total IgE in tears, nasal mucus, and saliva of patients with allergic rhinoconjunctivitis. *J Asthma* 2010; 47:1153–1160.
 82. Mimura T, Usui T, Yamagami S, *et al.* Relationship between total tear IgE and specific serum IgE in autumnal allergic conjunctivitis. *Cornea* 2013; 32:14–19.

83. Mimura T, Yamagami S, Noma H, *et al.* Specific IgE for wheat in tear fluid of patients with allergic conjunctivitis. *Cutan Ocul Toxicol* 2015; 34: 25–34.
84. Inic-Kanada A, Nussbaumer A, Montanaro J, *et al.* Comparison of ophthalmic sponges and extraction buffers for quantifying cytokine profiles in tears using Luminex technology. *Mol Vis* 2012; 18:2717–2725.
85. Esmaeelpour M, Cai J, Watts P, *et al.* Tear sample collection using cellulose acetate absorbent filters. *Ophthalmic Physiol Opt* 2008; 28:577–583.
86. Filik J, Stone N. Analysis of human tear fluid by Raman spectroscopy. *Anal Chim Acta* 2008; 616:177–184.
87. Lopez-Cisternas J, Castillo-Díaz J, Traipe-Castro L, López-Solis RO. Use of polyurethane minisponges to collect human tear fluid. *Cornea* 2006; 25: 312–318.
88. Rohan LC, Edwards RP, Kelly LA, *et al.* Optimization of the weck-Cel collection method for quantitation of cytokines in mucosal secretions. *Clin Diagn Lab Immunol* 2000; 7:45–48.