Diagnostic Tools in Ocular Allergy

Andrea Leonardi[1], Serge Doan[2], Jean Luc Fauquert[3], Banu Bozkurt[4], Pia Allegri[5], Farid Marmouz[6], Carmen Rondon[7], Monica Jedrzejczak[8], Peter Hellings[9], Luis Delgado[10], Virginia Calder[11]

1 Department of Neuroscience, Ophthalmology Unit, University of Padua, Italy
2 Service d'Ophtalmologie, Hôpital Bichat and Fondation A. de Rothschild, Paris, France
3 CHU Estaing, Unité d'Allergologie de l'Enfant CHU Estaing 63003 Clermont-Ferrand Cedex1, France
4 Selcuk University, Faculty of Medicine, Department of Ophthalmology, Konya, Turkey
5 Ocular Inflammatory Diseases Referral Center, Allergic Conjunctivitis Unit, Rapallo Hospital Ophthalmology Department, Genova, Italy
6 Pole sanitaire du Vexin, 27140 Gisors & 1 Rue Thiers 95300 Pontoise, France
7 Allergy Unit, Regional University Hospital of Malaga, IBIMA, UMA, Malaga, Spain
8 Department of Immunology, Rheumatology, and Allergy, Medical University of Lodz, Poland
9 University Hospitals Leuven, Belgium
10 Laboratory of Immunology, Basic and Clinical Immunology Unit, and Center for Research in Health Technologies and Information Systems (CINTESIS), Faculty of Medicine, University of Porto, Porto, Portugal
11 Department of Ocular Biology & Therapeutics, UCL Institute of Ophthalmology, London, UK

Abstract: 190
Words: 4728

Short Title: Ocular Allergy Diagnosis
Source of Funding: supported by EAACI BoO and ExCom TF budget 2014-16
Final Approval by EAACI ExCom on 5 December 2016
The number of Authors exceeds the suggested maximum 8 because of the need for a wide consensus from different European Countries and specialties.

Corresponding author:
Andrea Leonardi, MD
Department of Neuroscience, Ophthalmology Unit, University of Padua
via Giustiniani 2
35128 Padua, Italy
Fax: +39-049-875 5168
email: andrea.leonardi@unipd.it

Abstract

Ocular allergy (OA) includes a group of common and less frequent hypersensitivity disorders frequently misdiagnosed and not properly managed. The diagnosis of OA is usually based on clinical history and signs and symptoms, with the support of *in vivo* and *in vitro* tests when identification of the specific allergen is required. To date, no specific test is available for the diagnosis of the whole spectrum of the different forms of OA. The lack of recommendations on diagnosis of OA is considered a medical need not only for allergists but also for ophthalmologists.
This position paper aims to provide a comprehensive overview of the currently available tools for diagnosing OA to promote a common nomenclature and procedures to be used by different specialists. Questionnaires, sign and symptom grading scales, tests and potential biomarkers for OA are reviewed. We also identified several unmet needs in the diagnostic tools to generate interest, increase understanding and inspire further investigations. Tools, recommendations and algorithms for the diagnosis of OA are proposed for use by both allergists and ophthalmologists. Several unmet needs in the diagnostic tools should be further improved by specific clinical research in OA.

Key words:
Allergic conjunctivitis; biomarkers; diagnosis; ocular allergy; quality of life.

Introduction and Current Classification of Ocular Allergy

The term allergic conjunctivitis (AC) or ocular allergy (OA) refers to a collection of ocular surface disorders that affects the eyelid and conjunctiva. IgE- and non-IgE-mediated hypersensitivity disorders include intermittent/seasonal and persistent/perennial allergic conjunctivitis (SAC and PAC according to the historical nomenclature and classification), the diseases vernal and atopic keratoconjunctivitis (VKC and AKC) and contact blepharoconjunctivitis (CBC) (1) (Table 1). These clinical subtypes are diagnosed and managed by ophthalmologists, allergists, pediatricians and rhinologists usually considering clinical history and signs and symptoms, aided by *in vivo* and *in vitro* tests when identification of the specific allergic sensitization is required (1). While clinical characteristics can provide a relatively convincing diagnosis, in their initial or chronic stages there can be some confusion as to which form of allergy is present. At times, pseudo-allergic forms, with clinical manifestations similar to allergy but with a non-allergic equivocal pathogenesis, are difficult to distinguish from OA forms. In fact, several ocular surface diseases, including tear film dysfunction, blepharitis, infections, toxic and mechanical conjunctivitis, may mimic the clinical pictures of OA (1). To date, there is no specific clinical and laboratory evaluation suitable for the diagnosis and monitoring of OA. Ancillary tests, such as skin prick test and the identification of serum-specific IgE, can be useful for diagnosis and management, however it is well known that the results may not correlate with the ocular disease triggers.
As in many other diseases, accurate, rapid diagnosis and, in particular, the paucity of guidelines on OA diagnosis, represent major unmet needs (2). This EAACI Task Force aims to provide a comprehensive overview of the currently available tools to make recommendations concerning the diagnosis of OA in daily clinical practice.

Methods

A systematic review of the literature was performed ~~in 2015-16~~ in PubMed and Science Direct databases, using the following key words: Allergic Conjunctivitis or Ocular Allergy [AND] Diagnosis workup, Investigations, Imaging, Questionnaire / QoL, Scoring/ Grading, Instruments / Specific tools, Clinical, Visual function/acuity, Ocular sampling/Tears, Tear Function, Biomarkers, Psychological impact. Confounding diagnosis of terms OA (MESH): not allergic hypersensitivity such as Stevens Johnson syndrome, graft versus host disease, were eliminated using a filter: NOT *(Stevens OR retina OR uveitis OR optic nerve)*.
Manual searches of the reference lists of selected studies were performed and relevant studies identified. Experts were contacted to suggest other studies not previously revealed from the database search. Studies were considered if they included human subjects, irrespective of age and race, and addressed diagnostic procedures, diagnostic utilities, regardless of the type of OA. No time or language limitations were established. Papers were selected according to the information provided on the title and abstract. Each topic was reviewed by two independent experts, and finally included and analyzed by the whole panel. Evidence to support each point was reviewed and a consensus decision was made for each chapter. As evidence regarding a diagnostic procedure was limited, some of the

recommendations were based on consensus-driven proposals by the task force working group.

1. Patient's clinical history

Rationale

The patient's medical history is the first crucial step in the diagnosis of OA, especially in the differential diagnosis of "Red Eye", one of the most common ophthalmic conditions (3). A well-performed medical history may help to understand the etiology of the conjunctivitis.
The medical history should cover types of symptoms (itching, burning, photophobia, discharge, visual changes, pain), whether there is unilateral or bilateral eye involvement, duration of symptoms, presence of allergies or systemic diseases, previous treatments, family history, environmental and occupational exposures, use of contact lenses and any type of ocular medication and surgery (1).

Signs and symptoms

Ocular *itching* is the hallmark subjective symptom of OA. It indicates the release of histamine from conjunctival mast cells and the activation of H1 receptors on nerve endings (1). Eyelid itching frequently occurs in blepharitis patients especially if related to eyelash infestation.
Ocular *redness* (*hyperemia*) is the primary sign of OA due to conjunctival vasodilatation, which is often diffuse. Localized hyperemia is suggestive of subconjunctival hemorrhage or episcleritis. Photographic scales can be helpful in minimizing subjective observer variability.
Tearing or "watery eyes", is a non-specific consequence of the lacrimal gland response-reflex to several stimuli involving conjunctival and nasal sensory nerve endings. Tearing is associated with OA but also other forms of ocular surface diseases such as infectious and mechanical conjunctivitis, and with evaporative dry eye.
Edema is easy to observe when limited to eyelid swelling but can also involve the conjunctiva, resulting in *chemosis*. This symptom is easy to observe if intense. When mild or moderate, slit lamp examination is required.
Pain is not a typical symptom of OA, but indicates a corneal involvement in VKC and AKC.
Allergic patients may report mild *visual disturbances,* blurring or normal vision. Visual impairment is a sign of corneal involvement in VKC and AKC.
Discharge is usually watery or serous; mucopurulent or purulent discharge suggests infectious conjunctivitis; bilateral discharge upon wakening is a sign of blepharitis; sticky mucous discharge and tearing, especially if associated with severe photophobia, are significant indicators of VKC and AKC.
Bilateral involvement is typical for OA. Asymmetrical forms are possible. In viral conjunctivitis, the symptoms are often unilateral at onset and become bilateral after one or two days.
Time of onset of the first symptoms of SAC and PAC is during adolescence and young adulthood (80% of patients are younger than 30 years old) and infrequently in older

patients (4). In boys VKC begins rarely before the age of 3 or after puberty, and usually subsides after puberty (5). It is observed more commonly in warm climates (6). In AKC, symptoms may appear at 30-50 years of age, sometimes years after being diagnosed with atopic dermatitis or asthma (7). Overlaps or evolutions from VKC to AKC may occur.

In intermittent OA/SAC, the onset and *duration of symptoms* are limited to the pollen season or to the local seasonal variability of specific allergens including mites. Symptoms recur every season but are self-limiting (8). In persistent OA/PAC, symptoms are usually mild but persistent, exacerbating after increased or chronic exposure to allergens such as house dust mites, animal dander, occupational allergens (9, 10), or to non-specific irritating factors. PAC and AKC may also present with seasonal exacerbations. VKC worsens in the spring and summer. If severe, symptoms can be observed all year round. In CBC, attention should be made to substances applied into or around the eye (medications, cosmetics, etc.) (9). If suspected, a work-related allergy has to be confirmed by worsening of symptoms in the workplace (1, 10). In CBC the delay between the exposure to the allergen and the occurrence of symptoms is usually longer. Some non-specific factors like smoke, pollution or wind can increase symptoms of OA (11). In addition, food or food additives may influence OA.

Many patients have a history of *co-morbidity* with other allergic diseases. Conjunctival symptoms are present in 30-71% of patients with allergic rhinitis (AR) (11). AC alone has been estimated in 6-30% of the general population and in up to 30% of children, alone or in association with AR (12). Up to 40-75% of VKC patients suffer from other allergic diseases (12). AKC is present in up to 40% of atopic dermatitis patients and the co-morbidity with atopic dermatitis and asthma is around 90% (13).

It is always necessary to consider patients' activity limitations, sleep problems, coexisting nose, respiratory and cutaneous problems but also practical and emotional aspects.

Recommendations

- Accurate medical and personal history is fundamental for diagnosis.
- Consider different causes of red eye.
- Always ask for ocular signs and symptoms in other allergic co-morbidities.
- Investigate triggers for signs and symptoms.
- Refer to an ophthalmologist especially in cases of severe symptoms, unilateral redness, ocular pain, visual disturbance or long term use of topical drugs

Unmet needs

- Primary care awareness in OA.
- Standardized questionnaire to be used by both ophthalmologists and allergists

2. Clinical Ocular Examination

Rationale

Diagnosis of OA relies on the combination of a suggestive medical history and conjunctivitis signs (Figure 1 and Table 1). Some relevant clinical signs can be assessed by non-ophthalmologists (Table 2) although most of them are not specific

for allergy and may be present in any type of conjunctivitis. Some specific signs of VKC/AKC may be visualized by macroscopic examination: superior tarsal conjunctival giant papillae (visible after lid eversion) and Trantas dots (white/gray inflammatory infiltrates at the limbus) (Figure 2). Lid eczema is a specific marker for AKC. Ophthalmological examination reveals conjunctival papillary hypertrophy, follicles, scarring, blepharitis (lid margin inflammation), meibomian gland dysfunction (MGD) (Figure 3) and tear instability, an important sign of dry eye. Corneal involvement, found only in VKC and AKC, is a marker of severity. Superficial corneal epitheliopathy, neovascularization and scars are non-specific, whereas shield ulcers and plaques are much more suggestive of severe VKC and AKC (Figure 4).

Techniques

Observation, by daylight or direct light, of the face, lids, lid margin, palpebral and bulbar conjunctiva is the first approach. To evaluate the superior palpebral conjunctiva, use lid eversion. More accurate ocular examination requires the use of a slit lamp. Corneal and conjunctival epitheliopathy are assessed by fluorescein staining usually under slit lamp evaluation (Figure 4).

Recommendations

- Always look at the eyelid skin and the lid margin.
- When slit-lamp is not available, look at the eye using natural light.
- Examination by an ophthalmologist is required in atypical or severe cases.

3. Scoring OA and quality of Life

Rationale and Definitions

Different scales and criteria have been used, highlighting the difficulties encountered in grading acute and chronic manifestations. Main indications for scoring signs and symptoms include evaluating the disease severity, assessing the response to conjunctival and nasal provocation tests (CPT and NPT), and the efficacy of therapeutic agents.

Health-related quality-of-life (QoL) has been defined as “the functional effects of an illness and its resulting therapy upon a patient, as perceived by the patient”. OA may significantly impact activities like reading, computer or tablet use, recreation, games, sports, television, movies and electronics. These disturbances generate worries, anxiety and some psychological discomfort for the patient and family, and impact on daily tasks, work, leisure, sleep and mood. To date, no specific QoL questionnaire has been validated for OA, except for VKC. In terms of important patient-reported outcomes, the ocular component of AR impacts patient QoL in meaningful ways (14).

Instruments

Grading Signs and Symptoms

The visual analogue scale (VAS), mainly used in clinical trials (15), is a useful semi-quantitative method to express the intensity of symptoms by the patients. The self-recording is performed on a 100mm scale without marked intervals by indicating the most severe symptoms on the far right and the absence of symptoms on the far left.

The severity of subjective symptoms may also range from 0 to 10.

Grading questionnaires
The score may be based on the duration of symptoms: 0= no symptom, 1= "some of the time", 2= "half of the time", 3 = "most of the time", 4 = "all the time". The QoL in VKC children (QUICK) questionnaire (16), uses a three-point scale: 1= never, 2= sometimes, 3= always. The total sum score obtained determines the status of the disease.

Ocular Severity scores
The "Severity Index" (SI)-system is based on the patient's subjective assessment of the severity of each particular symptom and the grade of severity of objective findings: 0= no signs or symptoms, 1= mild, 2= moderate, 3= severe. The SI is calculated as the total score of individual signs and symptoms (Appendix 1). In 2012 (1, 17), we proposed a severity classification of OA based on ARIA guideline criteria (17) (Table 3). For severe and chronic forms (VKC, AKC) a 3 grade score for corneal ulcer assessment has been used - grade 1: ulcers that extend the beyond the epithelial basal membrane and yet have a transparent base; grade 2: ulcers with opaque base and partially filled with inflammatory debris; grade 3: ulcers filled with debris (plaque) remaining above the surrounding epithelium.

Scores for evaluating therapy
The recent recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis (RC) (18) suggested: a homogeneous terminology for nasal and conjunctival symptoms using six organ-related categories (itchy nose, sneezing, runny nose, blocked nose, itchy/red eyes, watery eyes) in the daily symptom score (dSS); a stepwise use of rescue medication added in the daily medication score (dMS); a scoring system for a combined symptom and medication score (CSMS), based on an equal weight of the dSS and of the dMS. This scoring system follows the EMA guideline, the World Allergy Organization (WAO) recommendations (19, 20).

QoL evaluation and questionnaires
Generic QoL questionnaires assessing the global health status, like the Short Form 36 and 20 (SF-36 and SF-20), the EuroQoL (EQ-5D), and the Sickness Impact Profile (SIP) (21) or the KINDL questionnaire in children (22), are the most frequently used.

QoL in OA
In the absence of specific questionnaires for OA, those validated for RC can be used: the Juniper's questionnaire (RQLQ) (23); its shortened version (mini-RQLQ) (24); specific questionnaires for adolescents and children (25, 26). The RQLQ includes 28 items divided amongst 8 domains. The mini-RQLQ has been reduced to 14 items and 5 domains: activity limitations, practical problems, nasal symptoms, ocular symptoms and other symptoms. OA is addressed in 5 symptoms in the RQLQ and 4 in the mini-RQLQ: need to rub nose/eyes, itchy eyes, sore eyes, watery eyes, and swollen eyes. Interestingly, the analysis of 4 clinical trials revealed that the highest scoring items were the "need to rub eye/nose" and "itchy eyes" (24). In a large

cohort of 1009 rhinitis patients, the presence of ocular symptoms statistically increased the RQLQ score by +0.5 (27). The smallest improvement in RQLQ score considered worthwhile by a patient affected by grass-pollen allergic RC was consistently estimated as 1 (28).
VKC and AKC should probably be considered separately, because the impact on QoL is often intense. The QUICK questionnaire, developed for VKC (16), contains 16 items pooled in 2 domains: symptoms; daily activities. In contrast to children with RC (26), daily activities (going to the swimming pool, practicing sports, meeting friends) were significantly impacted by VKC.

Impact on economic costs
SAC patients experienced QoL reductions in general health and specific aspects of vision and productivity, but also a significant economic cost to treat their condition when assessed by the Health Economic and Demographic Questionnaire (HEDQ)(29). This aspect must be taken into account for both private and public health care modalities when attempting to ascribe a total cost to a medical condition.

Recommendations

- Score signs and symptoms for diagnosis and monitoring.
- Use VAS system to evaluate patients' perspective.
- Assess the OA impact on QoL.
- Use EuroQoL (EQ-5D) to gain basic information on general QoL.
- In the absence of a specific questionnaire on OA, use the mini-RQLQ.
- QUICK is the only specific and validated questionnaire for VKC

Unmet needs

- Correlation among currently available severity scoring systems is unknown.
- Specific QoL questionnaires should be developed to study the impact of OA on vision, general and psychological condition in different ages.
- An ideal tool could measure disease, symptoms perception and treatment efficacy by the patient.

4. Allergy testing in OA

Rationale and definitions

During the process of diagnosing OA, the allergen may be considered as either the main trigger of symptoms, closely associated with non-allergenic factors, or unrelated to clinical symptoms. The relationship between allergen sensitivity and allergen exposure is easy to assess, particularly in SAC. Allergen investigations may be unnecessary in cases where symptoms resolve with symptomatic treatment, or when symptoms occur after an obvious allergen exposure. If symptoms persist or recur regardless of treatment, allergen identification is required. In the majority of cases, skin prick tests (SPT) are unanimously considered the gold standard and the first-line approach for identifying IgE-mediated sensitization, due to their efficiency, safety and relatively low cost (30). Biological assays such as specific IgE measurement, are used to confirm sensitization in specific cases. Nevertheless, polysensitization (e.g a positive specific IgE to two or more allergens) is often found in patients with allergic rhinitis (31). Once sensitization is demonstrated, its

relevance for the patient can be investigated by the more specific conjunctival allergen provocation test (CAPT), particularly when polyallergy (e.g. clinical response to 2 or more allergens) is suspected or when clinical history suggests OA but SPT and IgE are negative or inconclusive (1) (31-33). Procedures, indications and limitations of CAPT have been recently described (32).
When medical history suggests contact allergy, non-IgE mediated test are relevant, especially in cases of CBC (10).

Techniques
The SPT should be performed with airway allergens, and be read according to rigorous methodology, according to EAACI recommendations (34, 35). Indications for intradermal tests are limited to selected cases and allergens.
Serum allergen-specific IgE measurements detect IgE sensitization over 0.1 IU/mL, although low levels (< 0.35 IU/mL) are less likely to be clinically relevant, are less sensitive than SPT (31, 36), and are not mandatory when SPT correlates with clinical history. Local sensitization may be suspected in OA, therefore microarray measurements of specific IgE in tears may be helpful (37). Comparing serum and tear total IgE levels could be considered an indirect method for evidencing local allergy (37).
The Component Resolved Diagnosis (CRD), based on the pure allergen molecules (microarray or recombinant allergen-specific IgE detection), may be helpful in polysensitized or polyallergic patients. It can identify IgE antibody responses to cross-reactive allergens present in foods and pollens and identify genuine primary sensitization, which is not possible with the use of allergen extracts. However, it is still rarely used in routine patient testing (38, 39).
The CAPT evaluates the inflammatory effects on the conjunctiva after topical allergen application (1, 32) triggering the same signs (redness, chemosis and lid swelling) and symptoms (itching and tearing) as those of a natural allergen exposure. Contraindications are limited to uncontrolled allergy and chronic diseases. It requires an ophthalmic examination to rule out any ocular inflammation and a controlled protocol based on positivity criteria. Itching and redness are the main criteria to achieve a positive response when scored 2+ (Appendix 1) (40). The IGOA TF on CAPT recommends the use of total ocular symptom score (TOSS; range 0-13) considered positive when it reaches 5 (32). Both early and late allergic responses can be monitored. Since CAPT may induce nasal symptoms, scoring them is useful. However, there is a lack of a standardized method for evaluating the combined ocular and nasal symptoms. Non-specific challenge tests are only used for research purposes.
Patch tests aim to explore non-IgE-mediated allergy (41). Haptens involved may be preservatives or additives in eye drops, cosmetics, or professional allergens.

Recommendations
- If SPT, IgE levels and allergen exposure history are in concordance, the involvement of an allergen can be assumed in SAC and PAC.
- CAPT should be considered in polysensitized or suspected polyallergic patients.
- CAPT is recommended when medical history suggests an allergen sensitization but SPT are negative.
- In VKC and AKC, allergen-specific triggering is less frequently involved.

- Patch tests are required in CBC.

Unmet needs

- Standardized methods for evaluating the ocular and nasal symptoms in response to CPT or NPT.
- Standardized specific IgE analysis for small tear volumes.

5. Visual Function

Rationale and Definition

OA is not considered as a sight-threatening disorder. However, the clinical presentation ranges from mild forms not (or only slightly) influencing visual acuity (VA) to severe diseases (VKC and AKC), which affect the ocular structures, VA and QoL. Complications (corneal impairment, irregular astigmatism and scars, keratoconus, cataract, steroid-induced glaucoma) result in visual impairment (42). Visual disturbances are also caused by symptoms (intense itching, watering, foreign body sensation, mucus discharge, eyelid pseudo-ptosis, ocular pain and photophobia). Few population-based data on VA impairment in OA are available since VA rarely represents a primary or secondary outcome in clinical trials.

Techniques

VA is measured by asking the subject to discriminate letters of known visual angle (Snellen and derived optotypes) or letters of equal recognition difficulty. The log of the minimal angle of resolution at a given distance and at high contrast (ETDRS and similar) uses an eye chart (optical instruments or computerized tests) at standard conditions. Since VA sometimes is difficult to measure in active VKC and AKC, QoL can be quantified as a surrogate criterion (43), but improves depending on the severity of corneal involvement (44). All VKC and AKC patients should routinely undergo topographic corneal assessment because of the higher incidence of keratoconus (45, 46).

Recommendations

- Consider visual acuity assessment in daily practice
- VKC and AKC subjects should undergo topographic corneal examination to rule out keratoconus

6. Ocular surface evaluation (Tear film function)

Rational and Definition

Ocular allergy and dry eye syndrome are the most common ocular surface inflammatory disorders. One does not preclude the coexistence of the other (47). Chronic OA is causative of dry eye, which is classified as hypo-secretory (reduced tear production) or evaporative (due to low tear lipid layer thickness related to MGD) (48). Tear film instability was found in atopic children not only affected by allergic conjunctivitis but also rhinitis and asthma (49). Simple tear film-evaluating tests should be performed by ophthalmologists.

Techniques

Invasive breakup time (BUT): Insert a pre-soaked fluorescein strip in the inferior conjunctival fornix, and remove when tears are stained. Use blue cobalt light +/- yellow filter for observation. After a few blinks, measure time between the last complete blink and tear film break (when black striae appear in the pre-corneal tear film). Repeat measure 3 times. Mean values below 10 seconds reflect tear instability (48).

Schirmer test: Insert a nitrocellulose Schirmer strip into the inferior conjunctival fornix, at the external third of the inferior lid. Measure the length of wetted strip within 5 minutes. A test without anesthesia is recommended as being more reproducible. Values below 5 mm reflect aqueous deficient dry eye (48).

Tear osmolarity can be measured by an osmolarimeter. The Tearlab® device provides an immediate result after automated collection of a few nano liters. Values beyond 312 mOsm/L and inter-ocular variability of >8mOsm/L are abnormal (48).

Tear lipid layer thickness can be measured by interferometers.

Recommendations

- Consider tear film-evaluating tests since dry eye and OA can coexist

7. Ocular Sampling and Tear biomarkers

Rational and definition

Tear specimens can be easily obtained from the ocular surface, making cytology and assays for mediators, potential diagnostic tools. The tear fluid is an extremely complex biological mixture containing cells, proteins/peptides, electrolytes, lipids, and small molecule metabolites, which can be measured for diagnostic, prognostic and experimental purposes. Tear protein analysis has been limited to a few analytical techniques depending on antibody availability and the small sample size. With increasing proteomic applications, tears show great potential as a source of biomarkers in the development of clinical assays for various human diseases, including OA.

Technique

Different methods for tear collection can be used: capillary tube, filter paper, ophthalmic sponges and eye washes (50). Aspiration of tears by glass capillary tubes can yield volumes of 20-50 µl. Tear collection is tedious, time-consuming, uncomfortable for patients and may provoke the production of reflex tears. Tears can be recovered from Schirmer's strips (51), cellulose or polyurethane sponges, porous polyester or cellulose acetate rods placed in the lower tear meniscus and held for a fixed period of time (52, 53). For the eye-wash technique, a fixed volume of saline is instilled into the inferior fornix and the fluid collected by a capillary tube (54), however, it is impossible to determine the original tear volume and the dilution coefficient.

Cytology can be performed using tears, conjunctival scrapings, impression cytology or biopsy. For tear cytology, only a few microlitres of tears are needed. Conjunctival scrapings, performed with a spatula or brush, allow for the collection of epithelial

and inflammatory cells (if present). Pre-colored slides or rapid dyes can be used for a quick response. Impression cytology refers to the application of a cellulose acetate filter on the ocular surface to remove the superficial epithelial layers (55).
Conjunctival biopsy (performed under topical anesthesia) is required when a neoplastic pathology or autoimmune disease is suspected. Samples can be used for immunostaining, flow cytometry, RNA or DNA extraction, all depending on the number of cells collected.
In OA, neutrophils, eosinophils and lymphocytes can be found in tears with different cellular profiles in acute and chronic phases, or after specific CPT and NPT (56, 57). T-cells, B-cells and CD4:CD8 T-cell ratios were found to be higher in AKC tears than controls (58), whilst increased Th2 cells have been found in VKC tears (59).
Tear specimens contain various enzymes and hydrolases, thus proper storage is important to prevent sample loss and/or variable results (60, 61). Total and specific IgE, several mediators, cytokines, chemokines, growth factors, angiogenic modulators, proteases, enzymes and inhibitors have been identified in cell-free tear fluids using methods such as ELISA, RIA, multiplex bead arrays, membrane-bound antibody array and proteomic techniques (see review (62)). Increased tear levels of IgE, tryptase, histamine and eosinophil cationic protein (ECP) have been considered biomarkers of OA (62, 63). Proteomic analysis of tear fluid has proven to be a promising approach to gain more information about the pathogenesis of diseases and lead to new diagnostic possibilities (64, 65).
Excluding assays for total IgE and MMP-9 specifically designed for tears, no local tests have been standardized for clinical use and probably no single factor or test can be considered as a specific disease marker. More likely, a combination of them may be required to indicate a single disease phenotype, activity phase, or therapeutic effect.

Recommendations

- Collect tear samples independently from both eyes because of possible asymmetrical conditions.
- Prefer capillary tube collection since cells and mediators bind to the strips or sponges.
- The presence of one eosinophil by cytology is highly indicative of OA, whereas their absence does not exclude it.
- Tear collection and storage can influence protein detection

Unmet need

- An assay kit for detecting a panel of tear mediators should be validated.

8. Imaging and emerging additional tests

Rational

In-vivo imaging technologies have been widely used in the diagnosis, assessment of clinical severity and follow-up of ocular surface disorders. The application of these technologies may help to quantify the extent of inflammation and evaluate the efficacy of anti-allergic agents.

Definition and Techniques

In-vivo Confocal Microscopy *(IVCM)* is a real-time, non-invasive imaging technology, which enables microstructural analysis of the cornea in more physiological conditions at a cellular level (66). IVCM creates a point source of light focused by an objective lens on the tissue. Compared to traditional slit lamp imaging, IVCM provides a higher magnification and depth of view, but examines only a limited area. With the recent in-vivo laser scanning CM (LSCM), it is possible to study the microscopic anatomy of semi-opaque structures, such as the conjunctiva and meibomian glands (MG).
Morphological changes of superficial epithelial cells and nerves, increased presence of activated keratocytes, inflammatory cells, and Langerhans cells in the corneal stroma were shown in VKC and AKC by LSCM (67, 68) (Figure 5).
Following CPT, IVCM can be used to track and score the progress of the acute and late-phase allergic inflammation, capturing video images useful to study cell dynamics (scale 0-4; patent pending) (69).
IVCM may become useful for evaluating the efficacy of anti-allergic medications. In VCK and AKC, topical cyclosporine A (CsA) significant reduced the density of inflammatory cells (70, 71).

Meibography is a non-invasive technique developed to observe MG structure. Using laser confocal meibography, shortening, distortion and dropout of MG may be observed in PAC (72, 73).

Lipid layer Interferometry measures tear film stability and analyzes the depth of the lipid layer. Advanced tear instability and thickening of the tear film lipid layer were found in 80% of SAC patients (74).

Photography: Conjunctival hyperemia can be evaluated on the basis of digital photos using a modified grading scale or by using different digital image analysis software (75).

Optical coherence tomography (OCT) is an adjunctive, non-invasive, diagnostic method that can help the diagnosis of ocular surface lesions (76). However, it is not routinely used in OA.

Recommendations

- Use new imaging techniques to more accurately assess the degree of inflammation in diagnosis, follow-up and treatment.

9. Diagnostic algorithm

The proposed diagnostic algorithm (Figure 1) is based on the clinical expertise of the authors and relevant recommendations from the literature (1, 3, 13, 33, 77). The diagnosis of a suspected OA is based on medical history and the presence of suggestive signs and symptoms. In case of symptoms unusually not suggestive of OA, the differential diagnosis can be made, with the assistance of an ophthalmologist, considering the wide spectrum of disorders affecting the ocular surface. If signs and symptoms are suggestive of OA, primary skin or *in vitro* testing in addition to the

clinical characteristic of each ocular disease (Tables 1 and 2), will improve the diagnosis. If primary diagnostic tools are uncertain or negative, secondary, more specific, local diagnostic tests may help to either define a specific ocular hypersensitivity disease or lead to a proper differential diagnosis.

10. Concluding Remarks

Characteristics of each diagnostic tool are summarized in Table 4. Specific questionnaires on medical history and QoL for OA are still needed and the correlation among currently available severity scoring systems is largely unknown. Ophthalmologists should refer their patients to the allergist any time there is a suspect of a specific sensitization and/or allergic co-morbidity. On the other hand, allergists should refer to the ophthalmologists all patients with severe ocular signs and symptoms, when ocular symptoms do not correlate with a specific sensitization or to rule out other non-allergic eye disorders. It is still not clear why, in some cases, the eye may be the only affected organ, or why some patients suffer from severe untreatable or chronic forms of OA only. Coordinated clinical and experimental studies are required to identify specific molecular and cellular mechanisms associated with each subtype of OA for developing new diagnostic tools and therapies.

Acknowledgments.

a. Funding/Support: This work was done under the approval of EAACI with a TF budget 2014-16

b. Conflicts of Interest:
Andrea Leonardi: No Conflicts of Interest.
Serge Doan: No Conflicts of Interest
Jean Luc Fauquert: No Conflicts of Interest
Banu Bozkurt: No Conflicts of Interest
Pia Allegri: No Conflicts of Interest
Farid Marmouz: No Conflicts of Interest
Carmen Rondon: No Conflicts of Interest
Monica Jedrzejczak: No Conflicts of Interest
Peter Hellings: No Conflicts of Interest
Luis Delgado: No Conflicts of Interest
Virginia Calder: No Conflicts of Interest

c. Contributions to Authors in each of these areas: Andrea Leonardi (AL), Serge Doan (SD) , Jean Luc Fauquert (JLF), Banu Bozkurt (BB), Pia Allegri (PA), Farid Marmouz (FM), Carmen Rondon (CR), Monica Jedrzejczak (MJ), Peter Hellings (PH), Luis Delgado (LD), Virginia Calder (VC)
Conseption and Design: AL, JLF, LD, VC
Analysis and interpretation: AL, PB, FC, ADS, ET, SC, AJC, BZ
Writing the article: AL, SD, JLF, BB, PA, FM, CR, MJ
Critical revision of the article: LD, PH
Final approval of the article: AL, SD, JLF, BB, LD, VC
Data Collection: AL, SD, JLF, BB, PA, FM, CR, MJ
Provision of materials, patients, or resources: AL, SD, JLF, BB, PA, FM, CR, MJ
Statistical expertise: none
Obtaining funding: AL
Literature search: AL, SD, JLF, BB, PA, FM, CR, MJ
Administrative, technical or logistical support: AL, JLF, VC, LD
d. Statement about Conformity with Author Information: none
e. Other Acknowledgments: none

Tables

Table 1. Clinical features of major ocular allergy syndromes, including the underlying hypersensitivity mechanism and ophthalmological presentation (adapted from Leonardi et al, Allergy 2012).

	SAC	PAC	VKC	AKC	GPC	CBC
Presentation	Intermittent	Persistent	Persistent ± intermittent exacerbations	Chronic	Persistent	Chronic ± intermittent exacerbations
Allergic Mechanism	IgE-mediated	IgE-mediated	IgE and non-IgE-mediated	IgE- and non-IgE-mediated	Non allergic	Non-IgE-mediated
Background	Atopic	Atopic	Childhood ± atopic	Adult atopic	Atopic or non-atopic	Non-atopic
Eyelids	**NA**	± Palpebral edema	Eyelid edema	Eczema + meibomitis blepharitis	**NA**	Erythema, eczema
Conjunctiva	Follicles &/or papillae	Follicles &/or papillae	Giant papillae	Papillae ± fibrosis	Giant papillae	± Hyperemia Follicles
Limbus	**NA**	**NA**	± Thickened + Tranta's dots	± Thickened ± Tranta's dots	Hyperemia	**NA**
Cornea	**NA**	**NA**	SPK ± Ulcer ± Vernal plaque	SPK Ulcer, Plaque, Opacities, neo-vascularization	Rare	**NA**

SAC=seasonal allergic conjunctivitis; PAC=perennial allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=atopic keratoconjunctivitis; GPC=giant papillary conjunctivitis; CBC=contact blepharoconjunctivitis; SPK= superficial punctate keratitis; NA=not affected.

Table 2. Relevant signs related in clinical ocular examination

Sign	Sign of Severity	Positive association with OA	Type of OA	Differential diagnosis
Signs assessable by a non ophthalmologist				
Conjunctival redness	N	+	All	Non allergic conjunctivitis, (epi)scleritis, keratitis, uveitis
Conjunctival giant papillae	Y	++++	VKC, AKC	GPC
Limbal inflammation	Y	++++	VKC, AKC	Limbal tumor
Chemosis, lid edema	N	+	All	Non allergic conjunctivitis
Mucus discharge	N	++	All, especially VKC, AKC	Infection, severe dry eye, GPC
Lid eczema	N	++++	AKC, CBC, VKC	Seborrhoeic dermatitis, psoriasis, lid molluscum
Blepharitis	N	(Only in AKC)	All	Rosacea, seborrhoeic dermatitis
Signs only assessable by an ophthalmologist				
Conjunctival papillae	N	+++	All	Bacterial conjunctivitis, rosacea, dry eye
Conjunctival follicles	N		All, especially CBC	Viral or chlamydial conjunctivitis, Parinaud's oculoglandular syndrome
Superficial punctate keratopathy, corneal scars, pannus	Y		VKC, AKC	Non allergic keratitis
Corneal shield ulcer or plaque	Y	++++	VKC, AKC	None
Tear instability	N		PAC > AKC, VKC	Rosacea, non allergic tear instability

OA= Ocular allergy; SAC=seasonal allergic conjunctivitis; PAC= perennial allergic conjunctivitis; VKC= vernal keratoconjunctivitis; AKC= atopic keratoconjunctivitis; GPC= giant papillary conjunctivitis; CBC= contact blepharo conjunctivitis; Y=yes; N=no.

Table 3. Grading of symptoms and severity of hypersensitivity disorders at the ocular surface based on ARIA criteria.

A. Persistence of symptoms 1- "Intermittent" means that the symptoms (itching and redness) are present: • Less than 4 days a week • Or for less than 4 weeks 2- "Persistent" means that the symptoms (itching and redness) are present: • More than 4 days a week • And for more than 4 weeks
B. Severity of symptoms 1- "Mild" means that none of the following items are present: • Vision disturbance • Impairment of daily activities, leisure and/or sport • Impairment of school or work • Troublesome symptoms 2- "Moderate" means that one of the following items are present: • Vision disturbance • Impairment of daily activities, leisure and/or sport • Impairment of school or work • Troublesome symptoms 3- "Severe" means that two or more of the following items are present: • Vision disturbance • Impairment of daily activities, leisure and/or sport • Impairment of school or work • Troublesome symptoms

The items: vision disturbance, impairment of daily activities/ leisure/ sport, impairment of school or work activities, troublesome symptoms, are quoted "yes" or "no". Accordingly the number of items affected, the disease is considered "mild" (0 items affected), "moderate" (1 item) or "severe" (2-4 items).

Table 4. Main characteristics of diagnostic tools in ocular allergy

	Aims	Methods /Instruments	Recommendations
Clinical History	Evaluation of: -Symptoms and severity -Co-morbidities and general -Medical condition -Medical/surgical history -Exposure to allergens/ irritants/ non specific triggers -Visual tasks	-Talk to patients and parents -Questionnaires	•Accurate medical and personal history •Red eye differential diagnosis •Always ask for ocular signs and symptoms in other allergic co-morbidities •Investigate triggers for signs and symptoms •Refer to an ophthalmologist especially in case of ocular pain, visual disturbance or severe symptoms (red eye DD)
Clinical Ocular Examination	-To assess specific and non specific signs	-Observation with and without the slit lamp	•Always look at the eyelid skin and the lid margin •If you don't have a slit lamp, look at the eye using natural light when possible •Look for severity signs •Examination by an ophthalmologist may be required in atypical or severe cases
Scores	Evaluation of: -severity of the disease -CPT and NPT response -efficacy of treatments	-Grading signs and symptoms -Severity index	•Scoring signs and symptoms is recommended for diagnosis and monitoring • Use the VAS system to gain patients' perspective
QoL	-To assess the effects of disease on daily functioning, work, leisure and school perceived by patient	-Questionnaires	•Consider an assessment of impact of OA on QoL, however a specific questionnaire is missing •Use EuroQoL (EQ-5D) to gain basic information on general QoL •In the absence of a specific questionnaire on ocular allergy the miniRQLQ can be used •The QUICK is the only specific and validated questionnaire for VKC
Allergy Tests	-Evaluation of the specific sensitization state	-Skin prick tests -Blood analysis with allergen-specific IgE -Patch tests	•When an IgE allergic disease is suspected •When contact blepharitis or blepharo-conjunctivitis is suspected
Conjunctival Allergen Provocation Test	-Evaluation of the conjunctival response to specific allergens	-Provocation by topical allergen at fixed dilutions	•In case of negative SPT /sIgE •In case of polysensitization / polyallergy •To evaluate desensitization
Visual function	-To assess best visual acuity	-Optotypes	•Consider visual acuity assessment as a primary outcome in daily practice •VKC and AKC subjects should undergo topographic corneal examination to rule out keratoconus
Tear film function	-Evaluate tear film stability, quantity and dynamic	-BUT -Fluorescein staining -Schirmer test -Tear osmolarity -Meibomian glands	•In all patients with chronic OA •When adverse environment cause symptoms •Consider tear film-evaluating tests since dry eye and OA can coexist
Ocular sampling	-Obtain tear fluids, cells, tissues for analysis	-Tear collection Capillary tubes Schirmer strips Sponges -Scrapings -Brush -Impression cytology -Biopsy	•Collect samples independently from both eyes •Capillary tube collection is preferable •Use cytology for qualitative inflammation •Biopsy in case of autoimmune diseases and unilateral/malignant disease
Tear Biomarkers	-Disease biomarkers -Severity biomarkers -Prognostic biomarkers	-ELISA -RIA -Omics	•In experimental and clinical studies •Diagnostic tools in severe cases
Imaging	-To evaluate corneal cells, nerves and stroma -Blood cell dynamics in conjunctiva -Meibomian glands	-Confocal microscopy -Meibography -Photography	•In evaluating ocular surface changes •In experimental and clinical studies

Figure legend

Figure 1: Algorithm for the diagnosis of Ocular Allergy. The presence of signs and symptoms suggesting ocular allergy (OA) or the presence of signs and symptoms usually not associated with OA together with an accurate medical history is the first step in the diagnostic algorithm. In case of unusual signs and symptoms, the differential diagnosis can be made considering the wide spectrum of disorders affecting the ocular surface after consulting an ophthalmologist. When signs and symptoms suggest OA, primary skin or *in vitro* testing in addition to the clinical characteristic of each ocular disease (Tables 1 and 2) will consent to define a specific ocular allergic disorder. If primary diagnostic tools are uncertain or negative, secondary, more specific, local diagnostic test may help to either define a specific ocular hypersensitivity disease or suggest a proper differential diagnosis.

Figure 2. A: giant papillae on the tarsal conjunctiva in a VKC patient (tarsal form of VKC). B: Limbal Trantas dots in a VKC patient (limbal form of VKC).

Figure 3. A: diffuse small papillae at the upper tarsal conjunctiva with reticular sub-epithelial fibrosis. B: follicles at the lower tarsal and fornix conjunctiva. C: anterior blepharitis in a child. Note the hyperemia and crusts on the anterior lid margin. D: anterior and posterior blepharitis (Meibomian gland disease) with crusts on the eyelashes, meibomian gland obstruction, neovascularization and keratinization of the lid margin, conjunctival redness and peripheral corneal involvement.

Figure 4. A: Diffuse superficial (epithelial) punctate keratitis in a tarsal VKC patient highlighted by the fluorescein staining (yellow dots) and the blue light on the slit lamp. B: Corneal shield ulcer in a VKC patient.

Figure 5. Corneal confocal microscopy in a patient affected by VKC. A: sub-basal corneal nerve plexus with adjacent dendritic cells. B: anterior corneal stroma with highly reflecting cells corresponding to activated keratocytes.

Appendix

Appendix 1.
Scoring system in ocular allergy (including CAPT)

Itching
0 = none
1 = mild (intermittent itching sensation)
2 = moderate (continual awareness but without the desire to rub)
3 = severe (continual awareness with the desire to rub the eyes)
4 = incapacitating itching (subject insists on rubbing eyes).

Redness
0 = none
1 = mild (perhaps localized within some quadrant)
2 = moderate (more marked and diffuse reddening in the quadrants)
3 = severe (very marked and diffuse reddening in the quadrants).

Tearing
0 = none
1 = mild (slightly humid eye)
2 = moderate (some tears, blows nose occasionally)
3 = severe (profuse tearing, tears rolling down cheeks)

Chemosis
0 = none
1 = mild (detectable with slit lamp, conjunctiva raised from sclera)
2 = moderate (visually evident, raised conjunctiva, especially at the limbal area)
3 = severe (ballooning of conjunctiva)

References

Uncategorized References

1. Leonardi A, Bogacka E, Fauquert JL, Kowalski ML, Groblewska A, Jedrzejczak-Czechowicz M, et al. Ocular allergy: recognizing and diagnosing hypersensitivity disorders of the ocular surface. Allergy. 2012;67(11):1327-37.
2. Papadopoulos NG, Agache I, Bavbek S, Bilo BM, Braido F, Cardona V, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. Clinical and translational allergy. 2012;2(1):21.
3. Cronau H, Kankanala RR, Mauger T. Diagnosis and management of red eye in primary care. American family physician. 2010;81(2):137-44.
4. Dart JK, Buckley RJ, Monnickendan M, Prasad J. Perennial allergic conjunctivitis: definition, clinical characteristics and prevalence. A comparison with seasonal allergic conjunctivitis. Trans Ophthalmol Soc U K. 1986;105 (Pt 5):513-20.
5. Lambiase A, Minchiotti S, Leonardi A, Secchi AG, Rolando M, Calabria G, et al. Prospective, multicenter demographic and epidemiological study on vernal keratoconjunctivitis: a glimpse of ocular surface in Italian population. Ophthalmic Epidemiol. 2009;16(1):38-41.
6. Leonardi A. Vernal keratoconjunctivitis: pathogenesis and treatment. Prog Retin Eye Res. 2002;21(3):319-39.
7. Foster CS, Calonge M. Atopic keratoconjunctivitis. Ophthalmology. 1990;97(8):992-1000.
8. Cortes-Morales G, Velasco-Medina AA, Arroyo-Cruz ME, Velazquez-Samano G. [Frequency of sensitization to aeroallergens in patients with seasonal and perennial allergic conjunctivitis]. Revista alergia Mexico. 2014;61(3):141-6.
9. Tagliaferri A, Love TE, Szczotka-Flynn LB. Risk factors for contact lens-induced papillary conjunctivitis associated with silicone hydrogel contact lens wear. Eye Contact Lens. 2014;40(3):117-22.
10. Kari O, Saari KM. Diagnostics and new developments in the treatment of ocular allergies. Curr Allergy Asthma Rep. 2012;12(3):232-9.
11. Leonardi A, Piliego F, Castegnaro A, Lazzarini D, La Gloria Valerio A, Mattana P, et al. Allergic conjunctivitis: a cross-sectional study. Clin Exp Allergy. 2015;45(6):1118-25.
12. Leonardi A, Castegnaro A, Valerio AL, Lazzarini D. Epidemiology of allergic conjunctivitis: clinical appearance and treatment patterns in a population-based study. Curr Opin Allergy Clin Immunol. 2015;15(5):482-8.
13. Bogacka E, Gorski P, Groblewska A, Misiuk-Hojlo M, Jedrzejczak-Czechowicz M, Palczynski C, et al. [Polish agreement in ocular allergy diagnostics and treatment]. Klinika oczna. 2008;110(4-6):219-26.
14. Stull DE, Schaefer M, Crespi S, Sandor DW. Relative strength of relationships of nasal congestion and ocular symptoms with sleep, mood and productivity. Curr Med Res Opin. 2009;25(7):1785-92.
15. Senti G, Vavricka BM, Graf N, Johansen P, Wuthrich B, Kundig TM. Evaluation of visual analog scales for the assessment of symptom severity in allergic rhinoconjunctivitis. Ann Allergy Asthma Immunol. 2007;98(2):134-8.
16. Sacchetti M, Baiardini I, Lambiase A, Aronni S, Fassio O, Gramiccioni C, et al. Development and testing of the quality of life in children with vernal keratoconjunctivitis questionnaire. Am J Ophthalmol. 2007;144(4):557-63.

17. Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop G, World Health O. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108(5 Suppl):S147-334.
18. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. Allergy. 2014;69(7):854-67.
19. Canonica GW, Baena-Cagnani CE, Bousquet J, Bousquet PJ, Lockey RF, Malling HJ, et al. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. Allergy. 2007;62(3):317-24.
20. Hafner D, Reich K, Zschocke I, Lotzin A, Meyer H, Kettner J, et al. Prospective validation of the "rhino conjunctivitis allergy-control-SCORE(c)" (RC-ACS(c)). Clinical and translational allergy. 2012;2(1):17.
21. Bergner M, Bobbitt RA, Carter WB, Gilson BS. The Sickness Impact Profile: development and final revision of a health status measure. Medical care. 1981;19(8):787-805.
22. Ravens-Sieberer U, Bullinger M. Assessing health-related quality of life in chronically ill children with the German KINDL: first psychometric and content analytical results. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 1998;7(5):399-407.
23. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clin Exp Allergy. 1991;21(1):77-83.
24. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN. Development and validation of the mini Rhinoconjunctivitis Quality of Life Questionnaire. Clin Exp Allergy. 2000;30(1):132-40.
25. Juniper EF, Guyatt GH, Dolovich J. Assessment of quality of life in adolescents with allergic rhinoconjunctivitis: development and testing of a questionnaire for clinical trials. J Allergy Clin Immunol. 1994;93(2):413-23.
26. Juniper EF, Howland WC, Roberts NB, Thompson AK, King DR. Measuring quality of life in children with rhinoconjunctivitis. J Allergy Clin Immunol. 1998;101(2 Pt 1):163-70.
27. Virchow JC, Kay S, Demoly P, Mullol J, Canonica W, Higgins V. Impact of ocular symptoms on quality of life (QoL), work productivity and resource utilisation in allergic rhinitis patients--an observational, cross sectional study in four countries in Europe. J Med Econ. 2011;14(3):305-14.
28. Devillier P, Chassany O, Vicaut E, de Beaumont O, Robin B, Dreyfus JF, et al. The minimally important difference in the Rhinoconjunctivitis Total Symptom Score in grass-pollen-induced allergic rhinoconjunctivitis. Allergy. 2014;69(12):1689-95.
29. Smith AF, Pitt AD, Rodruiguez AE, Alio JL, Marti N, Teus M, et al. The economic and quality of life impact of seasonal allergic conjunctivitis in a Spanish setting. Ophthalmic Epidemiol. 2005;12(4):233-42.
30. Scadding G, Hellings P, Alobid I, Bachert C, Fokkens W, van Wijk RG, et al. Diagnostic tools in Rhinology EAACI position paper. Clinical and translational allergy. 2011;1(1):2.
31. Migueres M, Davila I, Frati F, Azpeitia A, Jeanpetit Y, Lheritier-Barrand M, et al. Types of sensitization to aeroallergens: definitions, prevalences and impact on the diagnosis and treatment of allergic respiratory disease. Clin Transl Allergy. 2014;4:16.

32. Fauquert JL, Jedrzejczak-Czechowicz M, Rondon C, Calder V, Silva D, Kvenshagen BK, et al. Conjunctival allergen provocation test : guidelines for daily practice. Allergy. 2017;72(1):43-54.
33. Agache I, Bilo M, Braunstahl GJ, Delgado L, Demoly P, Eigenmann P, et al. In vivo diagnosis of allergic diseases--allergen provocation tests. Allergy. 2015;70(4):355-65.
34. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, et al. Practical guide to skin prick tests in allergy to aeroallergens. Allergy. 2012;67(1):18-24.
35. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clinical and translational allergy. 2013;3(1):3.
36. Chinoy B, Yee E, Bahna SL. Skin testing versus radioallergosorbent testing for indoor allergens. Clinical and molecular allergy : CMA. 2005;3(1):4.
37. Leonardi A, Borghesan F, Faggian D, Plebani M. Microarray based IgE detection in tears of patients with vernal keratoconjunctivitis. Pediatr Allergy Immunol. 2015.
38. Vieira T, Lopes C, Pereira AM, Araujo L, Moreira A, Delgado L. Microarray based IgE detection in poly-sensitized allergic patients with suspected food allergy - an approach in four clinical cases. Allergol Immunopathol (Madr). 2012;40(3):172-80.
39. Hamilton RG. Microarray Technology Applied to Human Allergic Disease. Microarrays (Basel). 2017;6(1).
40. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. Arch Ophthalmol. 1990;108(1):84-8.
41. Brasch J, Becker D, Aberer W, Bircher A, Kranke B, Jung K, et al. Guideline contact dermatitis: S1-Guidelines of the German Contact Allergy Group (DKG) of the German Dermatology Society (DDG), the Information Network of Dermatological Clinics (IVDK), the German Society for Allergology and Clinical Immunology (DGAKI), the Working Group for Occupational and Environmental Dermatology (ABD) of the DDG, the Medical Association of German Allergologists (AeDA), the Professional Association of German Dermatologists (BVDD) and the DDG. Allergo J Int. 2014;23(4):126-38.
42. Leonardi A, Bonini S. Is visual function affected in severe ocular allergies? Curr Opin Allergy Clin Immunol. 2013;13(5):558-62.
43. Brown MM, Brown GC, Sharma S, Busbee B, Brown H. Quality of life associated with unilateral and bilateral good vision. Ophthalmology. 2001;108(4):643-7; discussion 7-8.
44. Reddy JC, Basu S, Saboo US, Murthy SI, Vaddavalli PK, Sangwan VS. Management, clinical outcomes, and complications of shield ulcers in vernal keratoconjunctivitis. Am J Ophthalmol. 2013;155(3):550-9 e1.
45. Totan Y, Hepsen IF, Cekic O, Gunduz A, Aydin E. Incidence of keratoconus in subjects with vernal keratoconjunctivitis: a videokeratographic study. Ophthalmology. 2001;108(4):824-7.
46. Weed KH, MacEwen CJ, Giles T, Low J, McGhee CN. The Dundee University Scottish Keratoconus study: demographics, corneal signs, associated diseases, and eye rubbing. Eye (Lond). 2008;22(4):534-41.
47. Hom MM, Nguyen AL, Bielory L. Allergic conjunctivitis and dry eye syndrome. Ann Allergy Asthma Immunol. 2012;108(3):163-6.

48. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). The ocular surface. 2007;5(2):108-52.
49. Dogru M, Gunay M, Celik G, Aktas A. Evaluation of the tear film instability in children with allergic diseases. Cutan Ocul Toxicol. 2016;35(1):49-52.
50. Zhou L, Beuerman RW. Tear analysis in ocular surface diseases. Prog Retin Eye Res. 2012;31(6):527-50.
51. Stuchell RN, Feldman JJ, Farris RL, Mandel ID. The effect of collection technique on tear composition. Invest Ophthalmol Vis Sci. 1984;25(3):374-7.
52. Inic-Kanada A, Nussbaumer A, Montanaro J, Belij S, Schlacher S, Stein E, et al. Comparison of ophthalmic sponges and extraction buffers for quantifying cytokine profiles in tears using Luminex technology. Mol Vis. 2012;18:2717-25.
53. Lopez-Cisternas J, Castillo-Diaz J, Traipe-Castro L, Lopez-Solis RO. Use of polyurethane minisponges to collect human tear fluid. Cornea. 2006;25(3):312-8.
54. Markoulli M, Papas E, Petznick A, Holden B. Validation of the flush method as an alternative to basal or reflex tear collection. Curr Eye Res. 2011;36(3):198-207.
55. Singh R, Joseph A, Umapathy T, Tint NL, Dua HS. Impression cytology of the ocular surface. Br J Ophthalmol. 2005;89(12):1655-9.
56. Bonini S, Bonini S, Bucci MG, Berruto A, Adriani E, Balsano F, et al. Allergen dose response and late symptoms in a human model of ocular allergy. J Allergy Clin Immunol. 1990;86(6 Pt 1):869-76.
57. Pelikan Z. Cytological changes in tears during the secondary conjunctival response induced by nasal allergy. Br J Ophthalmol. 2012;96(7):941-8.
58. Avunduk AM, Avunduk MC, Tekelioglu Y. Analysis of tears in patients with atopic keratoconjunctivitis, using flow cytometry. Ophthalmic Res. 1998;30(1):44-8.
59. Leonardi A, DeFranchis G, Zancanaro F, Crivellari G, De Paoli M, Plebani M, et al. Identification of local Th2 and Th0 lymphocytes in vernal conjunctivitis by cytokine flow cytometry. Invest Ophthalmol Vis Sci. 1999;40(12):3036-40.
60. Sitaramamma T, Shivaji S, Rao GN. Effect of storage on protein concentration of tear samples. Curr Eye Res. 1998;17(10):1027-35.
61. Bjerrum KB, Prause JU. Collection and concentration of tear proteins studied by SDS gel electrophoresis. Presentation of a new method with special reference to dry eye patients. Graefes Arch Clin Exp Ophthalmol. 1994;232(7):402-5.
62. Leonardi A. Allergy and allergic mediators in tears. Exp Eye Res. 2013;117:106-17.
63. Bourcier T, Moldovan M, Goldschild M, Becquet F, Brignole F, Goguel A, et al. [Value of lacrymal IgE determination and conjunctival cytology in the diagnosis of chronic conjunctivitis]. J Fr Ophtalmol. 1998;21(3):209-13.
64. Pong JC, Chu CY, Chu KO, Poon TC, Ngai SM, Pang CP, et al. Identification of hemopexin in tear film. Anal Biochem. 2010;404(1):82-5.
65. Leonardi A, Palmigiano A, Mazzola EA, Messina A, Milazzo EM, Bortolotti M, et al. Identification of human tear fluid biomarkers in vernal keratoconjunctivitis using iTRAQ quantitative proteomics. Allergy. 2014;69(2):254-60.
66. Niederer RL, McGhee CN. Clinical in vivo confocal microscopy of the human cornea in health and disease. Prog Retin Eye Res. 2010;29(1):30-58.
67. Leonardi A, Lazzarini D, Bortolotti M, Piliego F, Midena E, Fregona I. Corneal Confocal Microscopy in Patients with Vernal Keratoconjunctivitis. Ophthalmology. 2011.

68. Hu Y, Matsumoto Y, Adan ES, Dogru M, Fukagawa K, Tsubota K, et al. Corneal in vivo confocal scanning laser microscopy in patients with atopic keratoconjunctivitis. Ophthalmology. 2008;115(11):2004-12.
69. Gomes PJ, Ousler GW, Welch DL, Smith LM, Coderre J, Abelson MB. Exacerbation of signs and symptoms of allergic conjunctivitis by a controlled adverse environment challenge in subjects with a history of dry eye and ocular allergy. Clin Ophthalmol. 2013;7:157-65.
70. Liu M, Gao H, Wang T, Wang S, Li S, Shi W. An essential role for dendritic cells in vernal keratoconjunctivitis: analysis by laser scanning confocal microscopy. Clin Exp Allergy. 2014;44(3):362-70.
71. Hu Y, Adan ES, Matsumoto Y, Dogru M, Fukagawa K, Takano Y, et al. Conjunctival in vivo confocal scanning laser microscopy in patients with atopic keratoconjunctivitis. Mol Vis. 2007;13:1379-89.
72. Arita R, Itoh K, Maeda S, Maeda K, Furuta A, Tomidokoro A, et al. Meibomian gland duct distortion in patients with perennial allergic conjunctivitis. Cornea. 2010;29(8):858-60.
73. Arita R, Itoh K, Maeda S, Maeda K, Tomidokoro A, Amano S. Association of contact lens-related allergic conjunctivitis with changes in the morphology of meibomian glands. Japanese journal of ophthalmology. 2012;56(1):14-9.
74. Suzuki S, Goto E, Dogru M, Asano-Kato N, Matsumoto Y, Hara Y, et al. Tear film lipid layer alterations in allergic conjunctivitis. Cornea. 2006;25(3):277-80.
75. Dogan S, Astvatsatourov A, Deserno TM, Bock F, Shah-Hosseini K, Michels A, et al. Objectifying the conjunctival provocation test: photography-based rating and digital analysis. Int Arch Allergy Immunol. 2014;163(1):59-68.
76. Shousha MA, Karp CL, Canto AP, Hodson K, Oellers P, Kao AA, et al. Diagnosis of ocular surface lesions using ultra-high-resolution optical coherence tomography. Ophthalmology. 2013;120(5):883-91.
77. Bielory L, Meltzer EO, Nichols KK, Melton R, Thomas RK, Bartlett JD. An algorithm for the management of allergic conjunctivitis. Allergy Asthma Proc. 2013;34(5):408-20.