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POSITION PAPER

Conjunctival allergen provocation test: guidelines for daily practice

J.-L. Fauquert[1], M. Jedrzejczak-Czechowicz[2], C. Rondon[3], V. Calder[4], D. Silva[5], B. K. Kvenshagen[6], I. Callebaut[7], P. Allegri[8], N. Santos[5], S. Doan[9], D. Perez Formigo[10], F. Chiambaretta[11], L. Delgado[12] & A. Leonardi[13] on behalf of the Interest Group on Ocular Allergy (IGOA) from the European Academy of Allergy and Clinical Immunology

[1]Unité d'Allergologie de l'enfant, CHU Estaing, Clermont-Ferrand Cedex 1, France; [2]Department of Immunology, Rheumatology, and Allergy, Medical University of Lodz, Lodz, Poland; [3]Allergy Unit, Regional University Hospital of Malaga, IBIMA, UMA, Malaga, Spain; [4]Department of Ocular Biology & Therapeutics (ORBIT), UCL Institute of Ophthalmology, London, UK; [5]Serviço de Imunoalergologia, Centro Hospitalar São João, E.P.E., Porto, Portugal; [6]Pediatric Department, Oestfold Hospital Trust, Fredrikstad, Norway; [7]Clinical Immunology, Department of Microbiology and Immunology, Catholic University of Leuven, Leuven, Belgium; [8]Allergic Conjunctivitis Unit, Ocular Inflammatory Diseases Referral Center, Rapallo Hospital Ophthalmological Dep., Genova, Italy; [9]Service d'Ophtalmologie, Hôpital Bichat and Fondation A. de Rothschild, Paris, France; [10]Servicio de Oftalmologia, Hospital Universitario de Torrejon, Madrid, Spain; [11]Service d'Ophtalmologie, CHU Montpied, Clermont-Ferrand Cedex 1, France; [12]Department of Immunology, Faculty of Medicine, University of Porto, Porto, Portugal; [13]Ophthalmology Unit, Department of Neuroscience, University of Padua, Padua, Italy

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Correspondence
Jean-Luc Fauquert, Unité d'Allergologie de l'enfant, Pole pédiatrique, Centre Hospitalier Universitaire (CHU Estaing), 1 Place Lucie Aubrac, 63003 Clermont-Ferrand Cedex 1, France.
Tel.: 00 33 473 750 299
Fax: 00 33 473 750 004
E-mail: jlfauquert@chu-clermontferrand.fr

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Abstract

Conjunctival allergen provocation test (CAPT) reproduces the events occurring by instilling an allergen on the ocular surface. This paper is the compilation of a task force focussed on practical aspects of this technique based on the analysis of 131 papers. Main mechanisms involved are reviewed. Indications are diagnosing the allergen(s)-triggering symptoms in IgE-mediated ocular allergy in seasonal, acute or perennial forms of allergic conjunctivitis, especially when the relevance of the allergen is not obvious or in polysensitized patients. Contraindications are limited to ongoing systemic severe pathology, asthma and eye diseases. CAPT should be delayed if receiving systemic steroids or antihistamines. Local treatment should be interrupted according to the half-life of each drug. Prerequisites are as follows: obtaining informed consent; evidencing of an allergen by skin prick tests and/or serum-specific IgE dosages; being able to deal with an unlikely event such as acute asthma exacerbation, urticaria or anaphylaxis, or an exacerbation of allergic conjunctivitis. Allergen extracts should be diluted locally prior to administration. Positive criteria are based on itching or quoted according to a composite score. An alternative scoring is based on itching. CAPT remains underused in daily practice, although it is a safe and simple procedure which can provide valuable clinical information.

The conjunctival allergen provocation test (CAPT), also known as conjunctival allergen challenge (CAC), is a conjunctival provocation test (CPT) used to evaluate the inflammatory effects on the external ocular surface after the topical application of an allergen in a presumed sensitized patient. The aim was to objectively evaluate the reactivity to specific allergens at the mucosal surface (1).

As stated in a recent Position Paper on Ocular Allergy, CAPT is a method for investigating the ocular surface IgE-mediated hypersensitivity disorders. It is used to determine or confirm which allergen(s) triggers the ocular symptoms, using the eye as a model to evidence a specific reactivity to allergen(s) (2). Conjunctival allergen provocation test is also a tool for investigating allergic inflammation mechanisms and biomarkers of the ocular surface, as well as its treatments. Recently, it has been used as a surrogate test of mucosal reactivity in other allergic diseases, namely rhinitis, asthma, food and latex allergy (3–5).

Conjunctival allergen provocation test has been extensively used in investigational settings. However, despite the fact that it is a safe, simple and fast tool to assess ocular or other IgE-mediated allergic diseases, it is clearly underused in daily clinical practice. Members of the EAACI Interest Group on Ocular Allergy formed a Task Force to make recommendations concerning CAPT in daily practice.

This Task Force aimed firstly at providing an updated review of CAPT regarding various points such as mechanisms, indications, methods and practical aspects. The second purpose was to make recommendations for CAPT performance and evaluation in daily clinical practice. Nonspecific and chamber provocation tests are also CPTs challenge. They will not be reviewed in the present document (6, 7).

Methods

A systematic review of the literature was performed in PubMed and Science Direct databases (using the key words 'Conjunctival provocation test', 'conjunctival allergen challenge', 'ocular challenge test'). Hand searches of the reference lists of selected studies were performed and relevant studies identified. Experts were contacted to suggest relevant studies not previously encountered in the database search. Studies were considered if they included human subjects, irrespective of age and race, and addressed conjunctival challenge procedures, diagnostic utility or safety issues, irrespectively of the type of challenge performed. No time or language limitations were established. Papers were selected according to the information provided on the title and abstract for the covered topics of the review: indications and contraindications, prerequisites, practical aspects, positivity criteria and safety. Before challenging the ocular surface with an allergen, the physician should be aware of the main mechanisms involved. Thus, the Task Force group decided to describe these mechanisms before dealing with the other aspects of CAPT.

From the 1185 retrieved papers, each topic was reviewed by two independent experts, and finally, 131 papers were included and analysed. Evidence to support each point was reviewed, and a consensus decision was made for each of the above chapters. As the evidence approaching the diagnostic procedure and supporting the use of CAPT was scarce, some of the recommendations of the diagnostic procedures were based on consensus-driven proposals from the Task Force working group. The quality of the evidence was assessed using the Scottish Intercollegiate Guidelines Network (SIGN) classification and rated accordingly to SIGN levels of evidence (8–10). For each main topic, whenever possible, a grade of recommendation was done and a good practice recommendation performed.

Mechanisms

CAPT and inflammatory cells

The ocular reaction to a specific CAPT is a typical IgE-mast cell-dependent cascade of events that occur in subjects previously sensitized to the allergen. A positive CAPT triggers the same symptoms (itching and tearing) and signs (redness, chemosis and lid swelling) as those of a natural exposure to the allergen. This immediate response, also named early-phase reaction (EPR), usually gradually subsides within 20 min. In addition to EPR, even when the incrementation has been stopped, a late-phase reaction (LPR) may occasionally occur within 24 h, depending on the allergen dose and patient sensitivity (11, 12).

Engagement of IgE and its high-affinity receptor activates mast cells, leading to degranulation and immediate release of mediators such as histamine, tryptase, chymase and cytokines (13). Simultaneously, downstream signalling leads to the release of newly formed mediators. Mast cell degranulation, in addition to the histamine-mediated vasodilation, induces vascular endothelial cell activation and thus expression of chemokines and adhesion molecules, initiating the recruitment phase of inflammatory cells to the conjunctival mucosa. This LPR is the basis for clinical inflammation that is consistent with signs and symptoms of perennial and chronic conjunctivitis (14). During that phase, eosinophils are activated. They are a key source of inflammatory mediators, including major basic protein and eosinophil cationic protein (ECP), which causes cellular disaggregation, epithelial desquamation and toxicity. Eosinophil-associated corneal damage (epitheliopathy and ulcers) occurs only in severe chronic allergic conditions but not in seasonal (SAC) and perennial allergic conjunctivitis (PAC). T-helper lymphocytes (CD4+) are present in inflamed conjunctival tissues and may be found as well within the LPR cellular infiltrate (15).

The human conjunctiva is supplied with sensory and autonomic sympathetic and parasympathetic nerve fibres, forming a plexus within the stroma and surrounding the base of epithelial cells, and expressing adrenergic and cholinergic muscarinic receptors along its epithelium layer (16). Neuronal stimulation generates allergy symptoms (itching, ocular irritation, sneezing), and allergic inflammation activates local neuronal activity (17). There is some evidence for a relationship between positive nasal provocation tests and ocular reactions (18).

Specific CAPT performed in grass-sensitive patients caused persisting inflammatory changes in conjunctival scrapings and tear fluids with a significant accumulation of different inflammatory cells depending on the time of observation (neutrophils: 20 min; eosinophils: 6 h; neutrophils, eosinophils and lymphocytes: 12–24 h after provocation). Increasing the dose of allergen resulted in a dose-dependent recruitment of inflammatory cells. In addition with late-phase histological changes, challenge with high doses of allergen induces clinical symptoms 6–10 h after provocation (19). The induction of CD54/ICAM-1 expression on conjunctival epithelium after CAPT is an immediate event, concomitant with the local inflammatory infiltrate (15). Therefore, the conjunctival epithelium is more than a bystander in the allergic reaction, but it is an active participant interacting with the inflammatory infiltrate. It may also be modulated by specific treatments (20, 21).

CAPT and inflammatory mediators

Measuring mediator levels in tears before and after challenge is also a tool to demonstrate the conjunctival response or the efficacy of a treatment. Immediately after CAPT, tear levels of histamine, tryptase, and prostaglandin D2, but not ECP, increase significantly (15, 22). Six hours after challenge (LPR), a second significant peak of histamine in the tears can be found in the challenged eye, without a parallel rise in tryptase levels; at this time, tear ECP increases significantly as an active seasonal reaction (23–25). A significant increase in IFN-γ, IL-6 and IL-10 was shown in tears 48 h after CAPT in atopic keratoconjunctivitis (AKC), suggesting that a LPR can be induced by the allergen in this condition (26). Tear levels of neuropeptides – substance P, calcitonin gene-related peptide, neuropeptide Y and vaso-intestinal peptide – have also been shown to significantly increase immediately after CAPT reaction. They may participate in modulating the allergic response at the ocular surface (27).

Indications and contraindications of the CAPT

Indications

Conjunctival allergen provocation test has been used as a practical tool to diagnose which allergen(s) triggers symptoms in IgE-mediated ocular allergy (Table 1). Routine procedures in ocular allergy diagnosis involve the medical history, skin prick tests (SPT) and specific IgE measurement. However, positive SPT and elevated specific IgE dosages may solely account for sensitization to a specific allergen. Conjunctival allergen provocation test is known as the only way to confirm the conjunctival specific response to a clinically suspected allergen, in SAC and, particularly, in PAC (28). It may also confirm the diagnosis when an unusual allergen is suspected (29, 30). In a specific paper focused on conjunctivitis due to mite allergy, Bertel et al. included 30 patients affected by PAC and sensitized to mites, 21 patients also affected by ocular allergy but without mite sensitization and nine asymptomatic patients. In this population, CAPT had 90% diagnostic sensitivity and 100% specificity, compared to 70% and 76% for SPT (31). Moreover, CAPT is useful in selected cases of vernal keratoconjunctivitis (VKC) (32) and AKC (26). Conjunctival allergen provocation test is particularly helpful in clarifying the connection between symptoms and exposure, especially in cases of multiple sensitizations (33), in doubtful cases (34), or when discrepancies between clinical history and allergen sensitization data occur, because systemic sensitization may exist without clinical allergy, and local symptoms may occur without evidence of systemic sensitization (35). Conjunctival allergen provocation test is particularly indicated when sensitization is not concordant with medical history, when a patient is multisensitized or when previous tests are negative or contradictory despite a medical history strongly suggesting a specific allergen to be involved in the ocular pathology. In SAC and PAC, the detection of the most relevant allergen is fundamental before initiating an allergen immunotherapy. Conjunctival allergen provocation test has been used as a follow-up tool after specific allergen immunotherapy (36). In addition the use of CAPT has been proposed as an effective parameter to predict allergic rhinoconjunctivitis symptoms during the season in patients treated with preseasonal sublingual immunotherapy tablets (37). If the patient is not fully controlled, because of great variations in pollen exposure, or in case of multi-allergy, a CAPT may be useful to assess the potential effect of current specific allergen immunotherapy (38). In addition, CAPT model studies have been pivotal to FDA in evaluating the anti-allergic properties of topical drugs for allergic conjunctivitis (39, 40) or the potential gains of using two combined ocular drugs (41).

Although SPT remain the gold standard in terms of defining allergic sensitization, CAPT has been used as a surrogate of the ocular mucosal sensitivity/tolerance to an allergen. Conjunctival allergen provocation test has proven helpful in diagnosing occupational allergies (42, 43) and has been suggested as a diagnosis tool in latex allergy (44). An interesting application in food allergy diagnosis has been reported, as sensitization without allergy is more frequent with food than with respiratory allergens (45). A study showed that, in a

Table 1 Indications for conjunctival allergen provocation tests in daily practice

Evaluation of	Indication	Potency	Level of evidence	Grade
Allergen-triggering factors in ocular allergy	SAC	+	2++/2+	B
	PAC	+++	2++/2+	B
	VKC, AKC (selected cases)	++	2	C
Doubtful cases	Discrepancy between ocular medical history and allergen sensitizations	++	3	D
	Polysensitized patients	+	2−	C
	Evaluation of anti-allergic properties of topical drugs	+	1+	B
Surrogate of mucosal sensitivity to/tolerance of an allergen	Occupational allergy (e.g. latex)	±		
	Follow-up of allergy immunotherapy	+	1++	A
	Food allergy	±	3	D

SAC, seasonal allergic conjunctivitis; PAC, perennial allergic conjunctivitis; VKC, vernal keratoconjunctivitis; AKC, atopic keratoconjunctivitis.

population of 174 children suspected of food allergy to milk, egg, peanut and fish, a negative CAPT indicates no clinical food allergy, irrespectively from the value of specific IgE. Conversely, a convincing positive CAPT was consistent with clinical IgE-mediated food allergy (46). Moreover, CAPT has been included in the evaluation of some patients affected by respiratory allergic diseases in specific environments (34, 42).

Temporary and definitive contraindications

Conjunctival allergen provocation test is a well-established and safe procedure to evidence an IgE-mediated response to different environmental allergens, thus improving the diagnosis and monitoring the management of allergies (44, 47) (Table 2). It should be performed outside exposure period (pollen season particularly) and without any interacting treatment or drug, to ensure a reliable outcome and the patient's safety (48, 49). To avoid complications, CAPT must be performed by well-trained and experienced staff. Before the procedure, the physician must investigate any concomitant eye or systemic disorder as well as potential adverse effects of any anti-allergic treatment needed (18). Conjunctival allergen provocation test should be performed in fully asymptomatic patients (32, 33) and avoided in subjects with any other ocular disorder, including inflammation/infection of the conjunctiva, cornea or iris, and in cases of severe dry eye syndrome (36, 39). No previous ocular surgery over the past 6 months is recommended (39), and contact lenses must be removed 72 h before (47, 48). As CAPT is an *in vivo* diagnosis procedure, the application of the allergen is not appropriate for pregnant or lactating women, as well as patients affected by uncontrolled diseases, particularly uncontrolled asthma, and severe systemic diseases such as autoimmune, heart and vascular diseases (e.g. uncontrolled hypertension, in case adrenaline is needed to treat an adverse event), hyperthyroidism, severe liver or renal insufficiencies and ongoing malignancies (15, 51, 52). Conjunctival allergen provocation test is not recommended in patients suspected of allergy to drugs used for the CAPT procedure (e.g. topical antihistamines or benzalkonium containing eyedrops) (53). Moreover, CAPT is not recommended in ocular surface diseases where IgE-mediated hypersensitivity is not involved: sicca syndrome, blepharitis, blepharo-conjunctivitis, urban eye syndrome, giant papillary conjunctivitis following intolerance to contact lenses or foreign bodies (32, 33, 54).

Prerequisites to perform a CAPT

Informed consent

Detailed information of the challenge benefits and risks should be provided to the patient. Informed consent and signature should be collected before CAPT. A version suitable for adults and children should be provided according to European recommendations and national regulations (55, 56) (Table 3).

Defining the candidate allergen(s) for CAPT

Candidate allergen(s) should be determined before performing CAPT either in patients already sensitized to a specific allergen or in patients suspected of local specific reactivity. Allergens are suspected according to the symptomatic period and the patient potential allergen exposures. Sensitization criteria to one allergen should be searched by the specialist in one of the following cases: positive SPT according to published references (or elevated serum-specific IgE levels) (57). Conjunctival allergen provocation test is particularly indicated, when sensitization is not concordant with medical history, when a patient is multisensitized or when previous tests are negative or contradictory despite a medical history strongly suggesting a specific allergen to be involved in the ocular pathology. In selected cases of allergic keratoconjunctivitis, indirect evidence for the involvement of an allergen may be considered, such as eosinophils in tears, local production of total IgE, highlighted by quantitative determination of IgE in tears (58), raised ECP tear values comparatively to serum level (59).

Drug discontinuation

Drugs that might influence the response after allergen instillation should be interrupted for an adequate time (Table 4) (60, 61). The half-life of each drug should be taken into

Table 2 Temporary and definitive contraindications of conjunctival provocation test

Contraindications	Clinical reason	References	Level of evidence	Grade
Temporary	Allergen exposure period	32, 33	2++	B
	Intake of drug that could interfere with the allergen response	19	2+	C
	Any other ocular disorder	36, 39	2+	C
	Any ocular surgery (<6 months)	50	2+	C
	Current use of contact lenses	18, 47, 48	2+	C
	Pregnancy or lactation	15, 51, 52	4	D
Definitive	Uncontrolled diseases, particularly asthma and severe systemic diseases	15, 51, 52	4	D
	Hypersensitivity to drugs used during or after conjunctival allergen provocation test	18	2+	C
	Non-IgE-mediated ocular surface disease	32, 33, 54	2++	B

account, as well as interindividual variations (32). Moreover, use of over-the-counter medication should be taken into account (50).

Medical environment

Prior to a CAPT, the patient must be asymptomatic, without any local and systemic inflammation (2, 32, 44). His ocular surface should adapt to the local environmental conditions. A baseline ophthalmologic examination should rule out inflammation of the ocular surface before scheduling a CAPT (47).

As in other 'in vivo' allergy diagnostic tests, the medical structure should be able to deal with an asthma exacerbation or acute urticaria/anaphylaxis. The presence of an ophthalmologist or an allergist is mandatory on location except for severe forms of ocular allergy, which requires the presence of both (to detect a mild chemosis or any other sign of ocular allergy that could only be assessed by slit-lamp examination) (32, 33, 44). Local and systemic antihistamines, corticosteroids, as well as bronchodilators and adrenaline (e.g. auto-injection devices), should be available. In case of a positive CAPT, instillation of antihistamines will be systematically performed and monitoring will be prolonged for 2 h or until symptoms subside. Instillation of topical corticosteroids should be considered. Given the potential late reactions, the patient will be monitored for 24 h (available contact with the medical team), and oral plus topical antihistamines should be systematically prescribed.

Table 3 Informed consent. Main items for an extensive explanation. These recommendations should be developed and adapted to the usual practice of the physician and to national regulations.

Title of chapter	Topic	Explanations
Basic information: What is	Atopic background	Medical history
	Allergenic sensitization	Skin prick test and specific IgE quantification
	Allergic reaction	Eye-related reaction
Conjunctival allergen provocation test	Aim	To prove the allergic reaction
	Indications	Established by the physician (ophthalmologist or allergist)
	Prerequisites	Discontinuation of ongoing treatments
	Expected and unexpected effects	Mainly local transient effects
	Safety measures	Making anti-allergic drugs available
Practical aspects: how does it work?	Baseline examination	Ocular examination
	Practical protocol	Incremental increase in drops
Consequences	Meaning and therapeutic consequences	Avoidance measures and/or allergen-specific immunotherapy
	Signed consent	Adapted to age

These recommendations should be developed and adapted to the usual practice of the physician and to national regulations.

Practical aspects of the CAPT

As in other provocation tests the quality of allergen extracts is especially important. Mixtures of different allergens should be avoided. The extracts provided should be standardized. Only lyophilized extracts meet these requirements. Available allergens and unit standards differ according to manufacturers and countries. Cost problems induced important reduction of allergen portfolio for conjunctival challenge. Nevertheless, availability of a wide range of high-quality extracts is required (62). The major allergens should be quantified to avoid discrepancies. The allergen extract for CAPT should be diluted in diluent or saline and prepared according to the manufacturer's recommendations. Phenolic and glycerinated solutes should be avoided. After dilution, the stability of the solution is guaranteed for 6–24 h (depending on the extract source used) and test dilutions should be prepared at room temperature to avoid nonspecific reactions (47) (Fig. 1).

Table 4 Drug discontinuation before conjunctival allergen provocation test

Route	Medication	Published recommendations	References	Task Force recommendations	Level of evidence	Grade
Local	Antihistamines	3 days to 4 weeks	11, 44, 47, 78, 80	2 days	2+	C
	Mast cell stabilizers	3 days	11, 44, 78	2 days	2+/2−	C
	NSAIDs	1 week	11, 44, 47	1 week	2+	C
	Corticosteroids	1–4 weeks	11, 24, 44, 47, 78, 80	2 days	2+/2−/4	D/E
	Cyclosporine	1 month		1 week	4	E
Systemic	Antihistamines	5 days to 4 weeks	11, 24, 44, 46, 47, 80	1 week*	2++/2+	B
	Corticosteroids	2–4 weeks	11, 18, 24, 46, 47, 80	2 weeks	2++/2+	B
	Antileukotrienes	3 weeks	11, 44	3 weeks	2+/2−	C

NSAIDS, nonsteroidal anti-inflammatory drugs.
*Except Ketotifen 3 weeks.

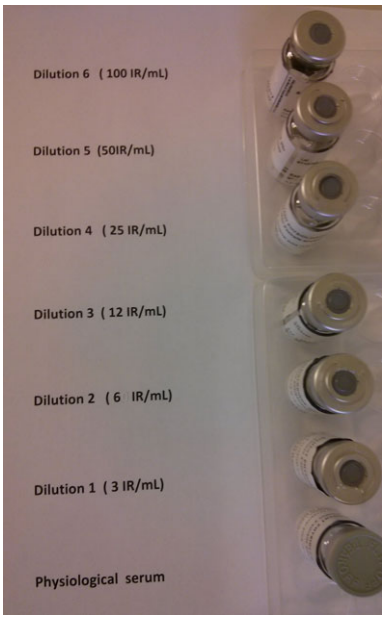

Figure 1 Dilutions.

The principles of CAPT were established in 1990 by Abelson (63, 64). Eyedrops (20–40 µl, to avoid overflow) should be instilled in the inferior-external quadrant of the bulbar conjunctiva (Figs 2 and 3). Before instillation of the allergen extract, the left eye is used as control – one drop of saline (NaCl 0.9%) is expected not to induce any inflammatory reaction. Anderson et al. (65) recommend nasolacrimal duct occlusion during allergen instillation to minimize the absorption of the challenge solution through the nasal mucosa, and consequently to reduce the risk of an adverse event. The interval between two allergen instillations should be at least

Figure 2 Pipetting.

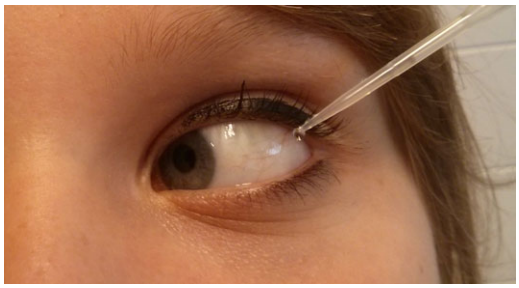

Figure 3 Instilling.

15 min (28, 66). Dilutions below or equal to 1 IR/ml are not recommended because no reaction occurs. For dilutions of more than 100 IR/ml, the specificity of the response decreases. A 10-time increment scale (0.1, 1, 10, 100 IR/ml) generates an intense reaction as from instillation of 10 IR/ml. Increments according to a factor of 2 (for example, 3, 6, 12, 25, 50, to 100 IR/ml) are more appropriate and recommended by the Task Force (59). The instillation of allergen drops should be stopped when a positive response occurs. The interval between two successive CAPT should be at least 1 week. According to Abelson (39), there is no difference between reactions to CAPTs at that interval, whereas Leonardi observed in multisensitized patients a decreased response during a second CAPT performed with the same allergen 1 week after (54). Performing in a single session a first test on the right eye, then a second test with another allergen on the left eye is controversial (28). As with prick-to-prick tests, natural food allergens have been used in clinical research to test conjunctival reactivity as a surrogate of an IgE-mediated allergic response at the mucosal level (46).

Positivity criteria

The response to CAPT is commonly evaluated by a clinical assessment of signs and symptoms and is mostly associated with EPR. However, the use of subjective plus objective criteria is essential to assess the reproducibility of the test, and the monitoring of both EPR and LPR. The intensity of the reaction is related to the allergen dose and to the individual sensitivity. Clinical scoring systems have therefore been suggested for the assessment of the objective clinical response to specific allergens.

The existing methods for CAPT evaluation have not been consistently defined by international guidelines (12). Although itching and redness are the hallmarks of positive response to CAPT recognized by the FDA (63), most studies also recommend the evaluation of secondary ocular signs and symptoms (47). Four clinical criteria of positivity – ocular itching, redness, tearing and chemosis – were proposed in 1990 (64) to standardize the clinical response after challenge (Table 5). Therefore, they might also be used for CAPTs in clinical practice. Ocular itching and redness, the clinical hallmarks of the allergic conjunctival reaction, are therefore considered as primary outcomes in the

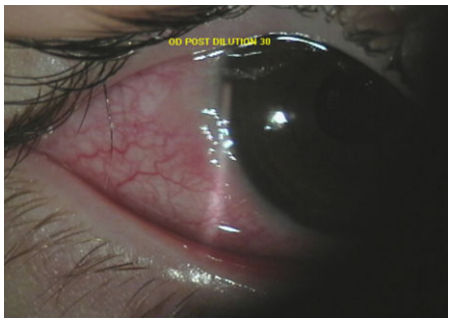

Figure 4 Mild chemosis.

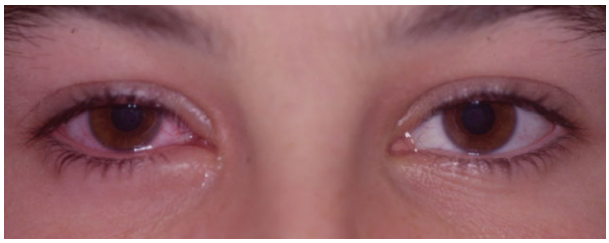

Figure 5 Positive Conjunctival Allergen Provocation Test (mild reaction on the right eye; Dermatophagoides Pteronyssinus).

interpretation of CAPT. Tearing, conjunctival chemosis and lid swelling do not occur in all positive CAPT responses and thus have been considered as secondary outcomes (Fig. 4). Itching (I) is the main criteria. It is the first to occur, 3–5 min after allergen exposure (32), increasing to peak after 10–15 min and beginning to decrease after 20 min (67). Itching intensity can be scored according to a 0- to 4-point scale. A visual analogue scale has been used in clinical studies (28) and may also be useful in daily practice as an alternative method to score the intensity of itching. Ocular redness (R) or hyperaemia is a primary sign of the conjunctival response. It appears 5 min after allergen exposure, reaching peak intensity after 20 min, and beginning to subside after about 30 min (40) (Fig. 5). It must be estimated by the physician to observe the vascular responses at ciliary, episcleral and conjunctival levels, and can be more precisely scored by slit-lamp examination (64). Conjunctival hyperaemia photographic scales can be very useful to minimize observer subjective variability (32). The reproducibility of the redness has been studied after repeated CAPT: the decrease in the redness was estimated to be from 1% to 3%, possibly as a result of local desensitization (68). As far as secondary outcomes are concerned, tearing (T) or watery eyes, and chemosis (C) must be rated by the physician (Table 5). When swelling involves the lid, a scoring could be added. Nasal symptoms (rhinorrhea, nasal pruritus, nasal congestion, and ear and palate pruritus) may also occur minutes after CAPT, and a nasal scoring system after CAPT has also been suggested (15). In daily practice, the clinical scoring should be rated before and 15 min after each instillation of eye drop and reported on a table. A total ocular symptoms score (TOSS) (range: 0–13) is obtained by adding the value of each criterion: it is considered positive over a cumulative score of 5 (Fig. 5). If TOSS is below 5, the test is considered negative and the next increasingly concentrated doses are successively applied until a positive response occurs, or until the maximal dose is reached. A simplified protocol based on itching score

Table 5 Conjunctival allergen provocation test protocol with increments (×2) and positivity criteria

		Criterion	Left eye	Right eye					
	Total ocular symptom score	Dilution	Saline	1×	2×	4×	8×	16×	32×
0	None	Itching (I)							
1	Intermittent itching sensation								
2	Continual awareness but without the desire to rub								
3	Continual awareness with the desire to rub the eyes								
4	Subject insists on rubbing eyes								
0	None	Redness (R)							
1	Perhaps localized within some quadrant								
2	More marked and diffuse reddening in the quadrants								
3	Very marked and diffuse reddening in the quadrants								
0	None	Tearing (T)							
1	Slightly humid eye								
2	Some tears, blows nose occasionally								
3	Profuse tearing, tears rolling down cheeks								
0	None	Chemosis (C)							
1	Detectable with slit lamp, conjunctiva raised from sclera								
2	Visually evident, raised conjunctiva, especially in the limbal area								
3	Ballooning of conjunctiva								
	Total ocular symptom score (positive if ≥5)								

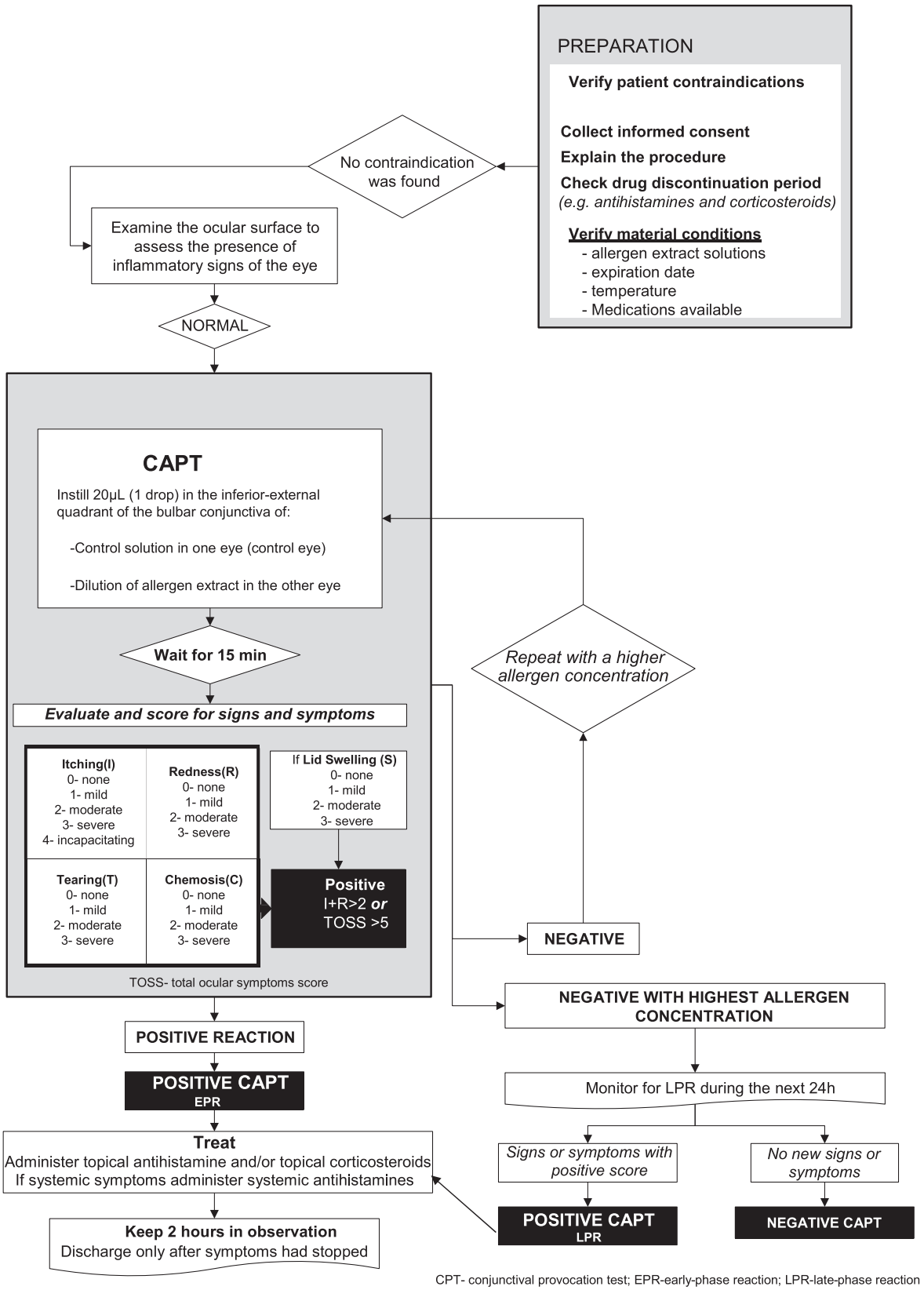

Figure 6 Flowchart of CAPT in daily practice.

rated 0–4 (positive over 2) has been proposed for ambulatory investigation of allergic conjunctivitis (32). An alternative classification of the response to the allergen extract based on qualitative criteria has been proposed, quoted level 0–4, with a positive threshold of 2 (47). Recently, photography-based rating were proposed (68–70). Digital images of the conjunctiva obtained by a high-resolution camera on a slit lamp have been suggested as an objective parameter to calculate the clinical response after CAPT. The redness has been assessed highly reproducible during CAPT in patients affected by rhinoconjunctivitis (71). The change of the optical density of the red fraction of the conjunctival image was considered a sensitive tool to measure the mucosal allergic reaction. Similarly, eyelid swelling was quantified with 3D imaging technology to offer a more precise assessment of eyelid swelling (73–75). In specialized centres, confocal microscopy can be used to visualize superficial conjunctival blood vessels and thus the allergic reaction (74).

Complementary positivity criteria

In research settings, variations of immunologic biomarkers in tears after CAPT (specific IgE, inflammatory mediators and cells) have been used to evidence a specific IgE-mediated immunologic response (75). Histamine is probably the most prominent and potent inflammatory mediator detected after CAPT (76). At baseline, tear histamine and tryptase levels are very low in nonactive allergic patients and nonallergic subjects. Significant increase in histamine in tears appears immediately after CAPT, as a result of massive mast cell degranulation (22, 75, 77). Significantly increased tryptase (76), TAME-esterase (*N*-tosyl L-arginine methyl esterase), prostaglandins, kinins and leukotrienes (21) are also detected during the EPR after ocular challenge. Six hours after CAPT, a second significant peak of histamine can be found without an increase in tryptase level (20, 22–24, 72). ECP, associated with eosinophil activation, is usually detected 6 h after ocular provocation, particularly in the most severe forms of allergic conjunctivitis (24, 25).

Tear cytology aims to evidence inflammatory cells involved in the EPR and LPR. It can be performed before, 30 min and hours after CAPT (20). In normal tear cytology, no inflammatory cells or rare neutrophils can be found. The presence of neutrophils, eosinophils, basophils and lymphocytes can be used as objective criteria to identify a positive conjunctival reaction. Conjunctival impression cytology can be collected to assess expression of inflammatory markers, such as CD54/ICAM-1, by the superficial conjunctival epithelial cells at baseline and after CAPT (72).

Safety

In many cases, symptoms resulting of CAPT are focused on the ocular surface. In isolated cases, peri-orbital oedema, rhinoconjunctivitis, urticaria and throat irritation have been reported, as well as wheezing in some asthmatic patients (63, 78). These symptoms were mostly transient (15, 48). Symptoms of LPR were rarely observed up to 24 h after a CAPT (12, 44, 48). One case of anaphylaxis has been reported (79). Consequently, if persistent or severe symptoms or systemic reaction occur, monitoring the patient should be continued for 24 h (28). Moreover, after a positive CAPT, the patient should stay on site for at least 2 h (28). The conjunctival reaction can be treated by eye wash solution, cold compresses and local symptomatic treatments (vasoconstrictors, antihistamines/mast cell stabilizers, topic corticosteroids) (40, 44, 47, 79, 80). Therefore, CAPT must only be performed in centres where side effects can be managed (75).

Conclusions and unmet needs

This position paper summarizes (Fig. 6) the current view on many of the practical aspects of CAPT, such as indications, methods, positive criteria and safety issues, regardless of the medical specialty setting involved. Therefore, in research settings or in pharmacological studies, some of the CAPT procedures can be modified. For daily practice, the scales to precisely collect the clinical signs and symptoms of ocular allergy should be validated, as well as the objective parameters to assess the clinical and biological consequences of mast cell activation on the ocular surface. The indications of CAPT for extraocular allergies and to phenotype its more severe and persistent forms (AKC and VKC) remain unclear, and need further investigation (81). The availability of reliable allergen sources for CAPT remains a major concern: standardization of units is required, and the definition of the major allergen content is still an unmet need. The possible use of recombinant allergens should be clarified in the future. As far as the practical aspects are concerned, CAPT remains a very simple method, although its safety profile might be assessed more thoroughly. Allergists should be much more involved and familiar with this technique in a closer collaboration with the ophthalmologist.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

This study is the contribution of a Task Force Group settled by the Interest Group on Ocular Allergy of the European Academy of Allergy and Clinical Immunology (EAACI). After selection of the papers to be studied, all the authors participate equally to the task of analysis, synthesis of writings. Each chapter, quoted 1–7, was then devoted to two contributors in charge of writing the synthesis. Then discussion of each chapter was assessed and validated by the entire group during a 2-day meeting. A. Leonardi and L. Delgado took an important contribution in the discussion. D. Silva and N. Santos focused on rating the quality of evidence. V. Calder looked over the accuracy of writings. The group was lead by J. L. Fauquert who conducted the discussion and writing.

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