

Hyperosmolar conjunctival provocation for the evaluation of nonspecific hyperreactivity in healthy patients and patients with allergy

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Background: Tissue hyperreactivity of target organs to nonspecific stimuli is known to be an important factor in influencing the clinical picture of allergic disease.

Objective: To identify the sensitivity and specificity of a hyperosmolar conjunctival provocation test in predicting conjunctival hyperreactivity and to relate this reactivity to the presence of ocular discomfort in subjects with and without allergy.

Methods: In 50 healthy patients and 19 patients with allergic conjunctivitis during remission phase, symptoms of ocular discomfort triggered by nonspecific stimuli were identified and graded with a discomfort score. Subjects were then challenged with a glucose solution at increasing concentrations (from 10% to 50%). The glucose concentration that elicited 2+ conjunctival hyperemia was considered the provoking dose. The response to this hyperosmolar provocation in subjects with ocular discomfort was compared with that of asymptomatic subjects. Sensitivity and specificity of the test in predicting conjunctival hyperreactivity were analyzed.

Results: Six of 50 healthy subjects and 12 of 19 subjects with allergy complained of ocular discomfort after exposure to nonspecific stimuli. The hyperosmolar provocation test discriminated between subjects with and without ocular discomfort (mean provoking dose: $39.5\% \pm 5\%$ and $47.5\% \pm 5\%$ glucose, respectively; $P < .001$). Forty percent glucose was the optimal threshold dose that demonstrated the highest sensitivity and specificity for prediction of conjunctival hyperreactivity. Discomfort scores were significantly related to provoking dose values ($P < .05$).

Conclusion: This study provides a standardized procedure to detect nonspecific conjunctival hyperreactivity independent of underlying atopy.

Clinical implications: Hyperosmolar provocation test may be useful for identifying conjunctival hyperreactivity in subjects with and without allergy with a history of ocular discomfort. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Conjunctival hyperreactivity, conjunctival provocation test, ocular discomfort, nonspecific hyperreactivity, allergy

Ocular discomfort is a clinical condition frequently observed in ophthalmic practice. Typically, patients complain of conjunctival redness associated with mild, occasional, and transient symptoms of itching, tearing, burning, and photophobia after exposure to stimuli such as wind, smoke, light, and cold or warm air or water. Although signs and symptoms of ocular discomfort are common to several ocular surface diseases such as ocular allergy, blepharitis, and dry eye, they may also occur in healthy individuals without any evidence of ocular disease.¹⁻⁸ This condition has been referred to as *generic hyperreactivity of the conjunctiva to nonspecific stimuli*, as it is known to occur in other organs and mucosal tissues.

Nonspecific hyperreactivity is a well established clinical condition already described in the skin, nose, and lung. It is characterized by the occurrence of signs and symptoms after exposure to nonspecific stimuli such as dust particles, temperature changes, humidity, tobacco smoke, and so forth.⁹⁻¹³ The presence of tissue hyperreactivity is usually associated with allergic inflammation. Indeed, patients with allergic rhinitis who display a late-phase response show a higher sensitivity to nonspecific agents such as histamine.¹⁴ Nevertheless, airway inhalation of a variety of agents can result in tissue hyperreactivity even in the absence of underlying allergic inflammation.¹⁵⁻¹⁷ Lung and nose hyperreactivity are identified by challenge with pharmacologic or physical stimuli such as histamine, methacholine, or hyperosmolar solutions, and their severity is measured by the change in functional parameters.¹⁸⁻²¹

In the eye, conjunctival hyperreactivity to nonspecific stimuli has been described previously in patients with allergy by conjunctival challenge with histamine.^{1,22,23} Similarly, nonspecific conjunctival hyperosmolar challenge with a glucose solution produced conjunctival redness in patients with allergic conjunctivitis at lower

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Abbreviations used

CPT: Conjunctival provocation test
 LPR: Late-phase reaction
 PD: Provoking dose
 ROC: Receiver operating characteristic

glucose concentrations than in healthy subjects.²⁴⁻²⁶ These studies demonstrate that the conjunctiva of patients with ocular allergy promptly reacts to a variety of nonspecific stimuli. However, these studies did not define (1) whether conjunctival hyperreactivity to nonspecific stimuli is related to underlying allergic inflammation or whether it was an independent pathophysiological feature also detectable in subjects without allergy, and (2) whether conjunctival hyperreactivity determined by nonspecific provocation is clinically relevant and related to a history of ocular discomfort.

The aim of this study was to investigate in subjects with and without allergy the conjunctival response to a hyperosmolar conjunctival provocation test (CPT) and to evaluate the diagnostic accuracy of this test in predicting a history of nonspecific ocular discomfort.

METHODS

Subjects

Fifty healthy subjects who were not using any ocular medication and nineteen asymptomatic subjects with allergic conjunctivitis were enrolled in this study after obtaining written informed consent. Subjects with allergy were sensitized to grass (8%), *Parietaria* spp (17%), *Dermatophagoides* spp (33%), grass and *Parietaria* (17%), or all 3 allergens (25%). The demographic and clinical characteristics of these subjects are described in Table I. A clinical history was taken from all subjects with special reference to their history of allergic diseases. Proof of sensitizations was obtained by skin prick test and confirmed by a specific conjunctival provocation test. No subject had any sign of active allergy, was using any form of systemic anti-inflammatory or topical ophthalmic therapy or contact lenses, or had undergone eye surgery in the past 3 months. This study was performed out of pollen season.

All subjects received a thorough eye examination. A complete history of symptoms of ocular discomfort such as itching, tearing, photophobia, and/or redness after exposure to nonspecific environmental agents was recorded. Six environmental agents (wind, sun/light, dust, smoke, cold/warm air and water) to which patients may be frequently exposed in their daily life were considered. An ocular discomfort score was arbitrarily calculated by adding the number of nonspecific agents inducing ocular discomfort, for a total maximum score of 6 (Table I).

Hyperosmolar conjunctival provocation test

Conjunctival hyperosmolar challenge was performed according to a previously published report.²⁴ Briefly, increasing concentrations of a glucose solution (10%, 20%, 30%, 40%, and 50%) in 0.03% saline albumin diluent (Bayopharm-DHS, Milan, Italy) were prepared at the time of conjunctival challenge. Fifty microliters of glucose solution was instilled into the lower conjunctival sac in the subject's right eye, starting with the 10% dose and increasing the concentration every 10 minutes until a provoking dose (PD) was achieved. This dose was arbitrarily defined as the minimal glucose solution that

TABLE I. Demographic and clinical characteristics of subjects enrolled in the study

Subjects	Total	Healthy	Allergic conjunctivitis
Number	69	50	19
Age (y)			
Range	7-37	20-37	7-31
Mean	24.8 ± 4.5	25.8 ± 2.7	22.2 ± 6.6
Sex (n)			
Male	59	43	16
Female	10	7	3
History of nonspecific ocular discomfort (n)	18	6	12
Ocular discomfort score,* mean	0.6 ± 1.1	0.3 ± 0.8	1.6 ± 1.4

*The ocular discomfort score was calculated by summing the number of environmental agents (wind, sun/light, dust, smoke, cold/warm air and water) that trigger discomfort symptoms.

elicited a clinical response, identified as a conjunctival hyperemia intensity equal to 2+ (according to the Abelson-Udell-Weston scale).²⁷ If no conjunctival reaction occurred with the highest dose (50% glucose solution), the subject was considered to be a nonresponder. The left eye of each subject was used as a control and challenged with the same amount of saline albumin diluent.

To evaluate the reproducibility of results, the test was repeated after 2 weeks in 13 randomly selected subjects following the same procedure.

Statistical analysis

The Mann-Whitney U-test was used to compare PD values between groups of subjects (subjects with allergy vs subjects without allergy and subjects with a history of nonspecific discomfort symptoms vs those with a negative history). The positive/negative response of subjects at or below each glucose concentration was related to the presence/absence of a nonspecific discomfort history by χ^2 analysis to evaluate whether hyperosmolar provocation was able to discriminate between patients with or without ocular discomfort.

The sensitivity and specificity of the hyperosmolar provocation test in identifying conjunctival hyperreactivity were calculated for each glucose concentration. A receiver operating characteristic (ROC) curve (MedCalc 7.30, MedCalc Software, Mariakerke, Belgium) was generated to identify the best cutoff value to differentiate between subjects with ocular discomfort from those without.²⁸ The Spearman ρ test was used to relate the ocular discomfort score to the provoking dose (SPSS Ver 8, Chicago, Ill). One-way ANOVA test was used to compare the results of the rechallenge to the results of the previous provocation test. A *P* value of <.05 was considered statistically significant.

RESULTS

Six healthy (6/50; 12%) and 12 allergic (12/19; 63%) subjects complained of symptoms of ocular discomfort after exposure to environmental stimuli (Table I). The conjunctival provocation test with a hyperosmolar glucose solution induced a prompt conjunctival hyperemia and chemosis with mild itching and/or burning in 46 of 69 (66.67%) subjects. The reaction occurred 2 to 3 minutes after challenge and gradually disappeared within 10 minutes without treatment (Figs 1 and 2, A).

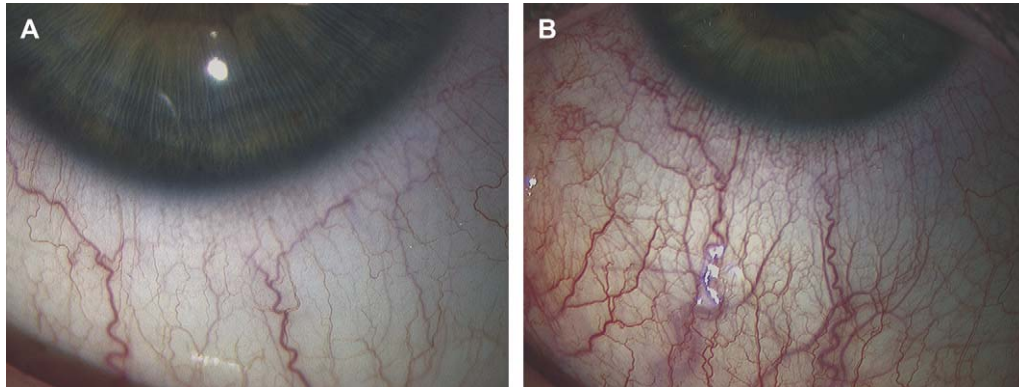


FIG 1. Significant (+2) conjunctival reaction. **A**, Subject was challenged using hyperosmolar provocation with increasing glucose concentrations until a positive conjunctival response was achieved. **B**, Conjunctival reaction 3 minutes after instillation of 40% glucose.

As shown in Fig 2, the majority (85%) of subjects with allergy reacted to a glucose concentration lower than 50%, whereas most of the healthy subjects (86%) needed the highest glucose concentration (50%) to develop a reaction or did not react at all. Subjects with allergy developed a conjunctival reaction at significantly lower glucose concentrations (mean PD, $39.4\% \pm 5\%$; range, 30% to 50% glucose) than subjects without allergy (mean PD, $47\% \pm 4\%$; range, 30% to 50% glucose concentration; $P < .001$). In subjects with allergy, PD was not correlated to a specific allergen sensitization.

Interestingly, 7 out of 50 (14%) subjects without allergy showed a significant conjunctival reaction at concentrations lower than 50% glucose, suggesting the presence of a conjunctival hyperreactivity even in the absence of allergic disease (Fig 2, C).

In both healthy and allergic groups, subjects who had a history of nonspecific ocular discomfort had lower provoking glucose doses (mean PD, $39.5\% \pm 5\%$; range, 30% to 50% glucose) than those with no history (mean PD, $47.5\% \pm 5\%$; range, 30% to 50% glucose; $P < .001$; Fig 3). Sensitivity and specificity of the hyperosmolar provocation test in detecting conjunctival hyperreactivity at each glucose concentration are shown in Table II. ROC curve analysis demonstrated a good accuracy of the test, with an area under the ROC curve of 0.904 (Fig IV). By evaluating test sensitivity and specificity through ROC curve analysis, a 40% glucose PD was shown to be the cutoff value with the highest accuracy. Statistical analysis showed that the 40% glucose provocation accurately identified subjects with a history of ocular discomfort in the entire population of 69 subjects ($P < .001$), as well as in the 19 subjects with allergy ($P = .0013$) and 50 subjects without allergy ($P < .001$).

The ocular discomfort score showed a statistically significant inverse correlation to the PD values in both the entire population ($P < .001$; $\rho = -0.688$), and the healthy ($P = .007$; $\rho = -0.496$) and allergic ($P = .008$; $\rho = -0.616$) subgroups.

To investigate the reproducibility of hyperosmolar conjunctival provocation, 13 randomly selected subjects

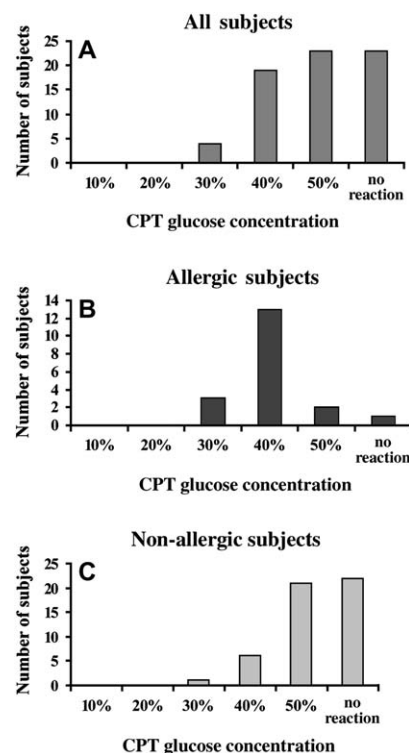


FIG 2. Conjunctival provocation with increasing glucose concentrations used to achieve a 2+ hyperemic reaction. **A**, The nonspecific response of the entire population. **B**, Most subjects with allergy responded with a dose less than or equal to 40% glucose. **C**, Seven healthy subjects without allergy responded with a 2+ conjunctival reaction to 40% glucose or lower.

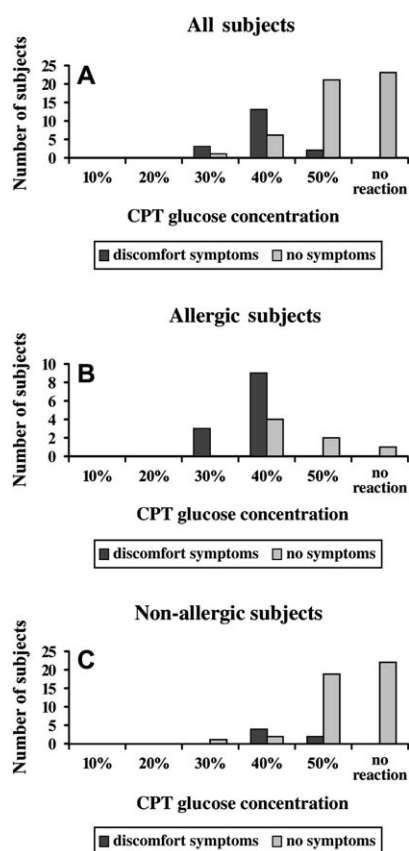
(10 healthy subjects and 3 subjects with allergy) were challenged again after 2 weeks. Ten subjects (77%) responded at the same PD, and 3 subjects (23%) who had previously responded at 40% PD showed a PD of 30% after 2 weeks. In these patients, a positive response was maintained within the 40% glucose concentration (established as our cutoff value), and therefore the diagnosis of conjunctival hyperreactivity was nevertheless confirmed.

TABLE II. Sensitivity and specificity of each glucose dose used for hyperosmolar conjunctival provocation to detect the presence of nonspecific ocular discomfort in subjects with and without allergy

Glucose concentration	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio	Negative likelihood ratio
<30% Glucose	0% (0-18.7)	100% (93-100)	—	1.00
30% Glucose	17% (3.8-41.4)	98% (89.5-99.7)	8.5	0.85
40% Glucose*	89% (65.2-98.3)	86% (73.7-94.3)	6.48	0.13
50% Glucose	100% (81.3-100)	45% (31.1-59.7)	1.82	0.00
Nonresponders	100% (81.3-100)	0% (0-7)	1.00	—

CI, Confidence interval.

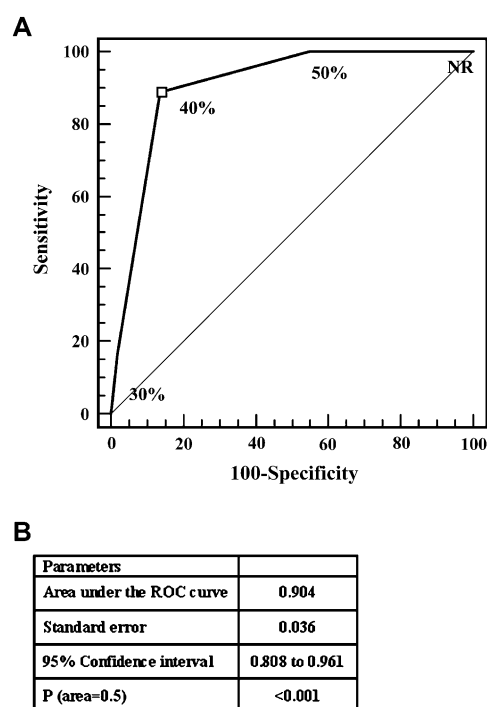
*Cutoff value with the highest accuracy.

**FIG 3.** All subjects with a history of nonspecific ocular discomfort responded to a dose of 50% glucose or lower in the hyperosmolar conjunctival provocation test: the entire population (A), subjects with allergy (B), and subjects without allergy (C).

The responses to the first challenge were not statistically different from the response to the rechallenge after 2 weeks (ANOVA $P = .445$; mean PD of the first challenge, 43 ± 6.7 ; mean PD of the rechallenge, 40 ± 9.4).

DISCUSSION

This study provides evidence of a conjunctival non-specific hyperreactivity detectable by hyperosmolar glucose provocation in subjects with and without allergy.

**FIG 4.** A, ROC curve analysis was used to define the accuracy of the hyperosmolar provocation test for identifying subjects with a history of nonspecific ocular discomfort. B, ROC analysis demonstrated good accuracy of the test. NR, Nonresponder.

The conjunctival reaction to hyperosmolar glucose—that is, mild hyperemia, itching, and burning—reproduces the signs and symptoms of ocular surface irritation after exposure to nonspecific environmental stimuli.

Conjunctival hyperreactivity is often associated with allergic conjunctivitis and other ocular surface diseases,¹⁻⁶ yet it is not considered as a distinct clinical feature, and no diagnostic tests are available for its identification and measurement. In this study, conjunctival hyperreactivity was identified in the majority of subjects with allergy (63%) but also in 12% of healthy subjects without allergy with a history of nonspecific ocular discomfort. Because all patients who used eye drops were excluded and subjects who self-medicated for alleviation of symptoms of non-specific irritation were also excluded, the percentage of subjects in both the allergic and nonallergic populations

who have conjunctival hyperreactivity might have been underestimated. Results of this study demonstrate that conjunctival hyperreactivity appears to be a distinct pathophysiological phenomenon independent from the presence of allergic inflammation.

The hyperosmolar conjunctival provocation test with glucose solution was used in other studies^{24-26,29} to detect conjunctival hyperreactivity in allergic patients. Ciprandi et al²⁹ hypothesized that subjects with mite allergy have developed a conjunctival hyperreactivity, reflecting a "minimal persistent inflammation" caused by the constant exposure to perennial allergens. However, in the current study, conjunctival hyperreactivity was also detected in patients with seasonal allergy and subjects without allergy; thus, it was not exclusively related to perennial allergy. Other mechanisms should therefore be advocated to justify the occurrence of a conjunctival hyperreactivity to nonspecific stimuli.

Because of its swift onset and resolution, conjunctival reaction to hyperosmolar CPT appears to be the result of mast cell degranulation and histamine release with the possible generation of a subsequent late-phase reaction (LPR).³⁰⁻³³ Nevertheless, a potential role of neuroinflammatory mediators released by inflammatory and epithelial cells and/or a stimulation of sensory nerves cannot be excluded, because it is known to occur in reactive airway dysfunction syndrome.^{11,24,31,32,34} Its uncertain pathophysiology and a lack of diagnostic tests render it difficult to propose and evaluate the effects of treatments on non-specific conjunctival hyperreactivity. In future studies, it will be interesting to evaluate the pathogenetic mechanisms of conjunctival hyperreactivity by studying the involvement of inflammatory cells and neuroinflammatory mediators in both the early and late-phase components of this reaction.

To evaluate conjunctival hyperreactivity, we used increasing concentrations of a hyperosmolar glucose solution. We cannot exclude that the positive response observed at a given concentration was not influenced by the priming effect of previous ineffective provocations or by the overimposing effects of a LPR induced by previous challenges. However, this is not likely to be the case. In fact, we originally described an ocular LPR in the human conjunctiva after specific (allergen) challenge.^{35,36} The occurrence of a LPR after nonspecific challenge is more controversial, and contrasting data have been reported by our group and by Ciprandi et al.²⁴

Moreover, LPR appears to be unique in the eye compared with other allergic target tissues. In fact, (1) the ocular response is a continuous event, starting 10 to 15 minutes after allergen challenge (no LPR without an early-phase or dual reaction); (2) the more intense is the early phase, the more are the chances to have a clinical and cellular reaction in the late-phase period; and (3) the ocular LPR is dose-dependent.³⁵⁻³⁷

Hyperosmolar CPT with a glucose solution was shown to be a safe and easily performed test to assess conjunctival hyperreactivity with good sensitivity and specificity values. Its significant correlation with the presence of

ocular surface discomfort symptoms in subjects with and without allergy suggests that an abnormal nonspecific conjunctival hyperreactivity may be involved in the pathogenesis of this frequent syndrome, and that hyperosmolar CPT may be a useful test to diagnose and monitor symptoms. In particular, the 40% glucose concentration illustrated good diagnostic accuracy in identifying conjunctival hyperreactivity in subjects with and without allergy.

In conclusion, this study demonstrated the presence of conjunctival hyperreactivity in subjects with and without allergy, indicating a mechanism not only related to allergic inflammation. Moreover, the hyperosmolar CPT was shown to be a useful test for the objective diagnosis and study of conjunctival hyperreactivity in subjects with a history of ocular sensitivity to nonspecific environmental stimuli.

REFERENCES

- Bonini S, Bonini S. IgE and non-IgE mechanisms in ocular allergy. *Ann Allergy* 1993;71:296-9.
- Bonini S, Bonini S, Lambiasi A, Marchi S, Pasqualetti P, Zuccaro O, et al. Vernal keratoconjunctivitis revisited: a case series of 195 subjects with long-term followup. *Ophthalmology* 2000;107:1157-63.
- Rolando M, Zierhut M. The ocular surface and tear film and their dysfunction in dry eye disease. *Survey Ophthalmol* 2001;45(suppl 2):S203-10.
- De Paiva CS, Pflugfelder SC. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. *Am J Ophthalmol* 2004;137:109-15.
- Gilbard JP. Dry eye, blepharitis and chronic eye irritation: divide and conquer. *J Ophthalmic Nurs Technol* 1999;18:109-15.
- Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol* 2000;118:615-21.
- Ono SJ, Abelson MB. Allergic conjunctivitis: update on pathophysiology and prospects for future treatment. *J Allergy Clin Immunol* 2005;115:118-22.
- Bielory L. Allergic and immunologic disorders of the eye, part II: ocular allergy. *J Allergy Clin Immunol* 2000;106:1019-32.
- de Vries K, Nadel JA, Pauwels R, Nashall PD, editors. Clinical significance of bronchial hyperresponsiveness: normal and abnormal control, assessment, and therapy. Oxford: Blackwell Scientific; 1987. p. 359-71.
- Magnussen H, Nowak D. Role of hyperresponsiveness and airway inflammation in bronchial asthma. *Respiration* 1989;55:65-74.
- Smith L, McFadden ER Jr. Bronchial hyperreactivity revisited. *Ann Allergy Asthma Immunol* 1995;74:454-69.
- Gerth van Wijk RG, de Graaf-in't Veld C, Garrelds IM. Nasal hyperreactivity. *Rhinology* 1999;37:50-5.
- Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651-7.
- de Graaf-in't Veld C, Garrelds IM, van Toorenbergen AW, Gerth van Wijk R. Nasal responsiveness to allergen and histamine in patients with perennial rhinitis with and without a late phase response. *Thorax* 1997;52:143-8.
- Anderson SD, Silverman M, Konig P, Godfrey S. Exercise-induced asthma. *Br J Dis Chest* 1975;69:1-39.
- Konno A, Terada N, Okamoto Y, Togawa K. The role of chemical mediators and mucosal hyperreactivity in nasal hypersecretion in nasal allergy. *J Allergy Clin Immunol* 1987;79:620-7.
- Nelson HS. Advances in upper airway diseases and allergen immunotherapy. *J Allergy Clin Immunol* 2005;115:676-84.
- Van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000;16:514-33.
- James A, Ryan G. Testing airway responsiveness using inhaled methacholine or histamine. *Respirology* 1997;2:97-105.

20. Hakonarson H, Grunstein MM. Autocrine regulation of airway smooth muscle responsiveness. *Respir Physiol Neurobiol* 2003;137:263-76.
21. Wojnarowski C, Storm Van's Gravesande K, Riedler J, Eichler I, Gartner C, Frischer T. Comparison of bronchial challenge with ultrasonic nebulized distilled water and hypertonic saline in children with mild-to-moderate asthma. *Eur Respir J* 1996;9:1896-901.
22. Bonini S, Bonini S, Schiavone M, Centofanti M, Allansmith MR, Bucci MG. Conjunctival hyperresponsiveness to ocular histamine challenge in subjects with vernal conjunctivitis. *J Allergy Clin Immunol* 1992;89:103-7.
23. Ciprandi G, Buscaglia S, Pesce GP, Bagnasco M, Canonica GW. Ocular challenge and hyperresponsiveness to histamine in subjects with allergic conjunctivitis. *J Allergy Clin Immunol* 1993;91:1227-30.
24. Ciprandi G, Buscaglia S, Pesce G, Passalacqua G, Rihoux P, Bagnasco M, et al. Effects of conjunctival hyperosmolar challenge in allergic subjects and normal controls. *Int Arch Allergy Immunol* 1994;104:92-6.
25. Tosca M, Ciprandi G, Passalacqua G, Canonica GW. Cetirizine reduces conjunctival nonspecific hyperreactivity in children with mite allergy. *J Investig Allergol Clin Immunol* 1998;8:23-6.
26. Ciprandi G, Catrullo A, Tosca M, Cerqueti P, Mondino C, Passalacqua G, et al. Azelastine eye drops reduce conjunctival hyperresponsiveness to hyperosmolar glucose challenge in children with asymptomatic mite conjunctivitis. *J Investig Allergol Clin Immunol* 1999;9:35-8.
27. Abelson MB, Udell IJ, Weston JH. Standardization of ocular surface injection intensity. *Ann Ophthalmol* 1981;13:1225.
28. Shapiro DE. The interpretation of diagnostic tests. *Stat Methods Med Res* 1999;8:113-34.
29. Ciprandi G, Buscaglia S, Pesce G, Pronzato C, Ricca V, Permiani S, et al. Minimal persistent inflammation is present at mucosal level in subjects with asymptomatic rhinitis and mite allergy. *J Allergy Clin Immunol* 1995;96:971-9.
30. Krayenbuhl MC, Hudspeth BN, Brostoff J, Scadding GK, Guesdon JL, Latchman Y. Nasal histamine release following hyperosmolar and allergen challenge. *Allergy* 1989;44:25-9.
31. Anderson SD. Exercise-induced asthma and the use of hypertonic saline aerosol as a bronchial challenge. *Respirology* 1996;1:175-81.
32. Proud D, Bailey GS, Naclerio RM, Reynolds CJ, Cruz AA, Eggleston PA, et al. Tryptase and histamine as markers to evaluate mast cell activation during the responses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. *J Allergy Clin Immunol* 1992;89:1098-110.
33. Bonini S, Bonini S, Berruto A, Tomassini M, Carlesimo S, Bucci MG, et al. Conjunctival provocation test as a model for the study of allergy and inflammation in humans. *Int Arch Allergy Appl Immunol* 1989;88:144-8.
34. Nockher WA, Renz H. Neurotrophins and asthma: novel insight into neuroimmune interaction. *J Allergy Clin Immunol* 2006;117:67-71.
35. Bonini S, Bonini S, Todini V, Adriani E, Allansmith MR. Late-phase ocular reaction induced by conjunctival allergen challenge. A dose-response study in humans. *J Allergy Clin Immunol* 1988;81:173.
36. 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;86:869-76.
37. Bonini S, Centofanti M, Schiavone M, Lambiase A, Bonini S. The pattern of the ocular late phase reaction induced by allergen challenge in hay fever conjunctivitis. *Ocul Immunol Inflamm* 1994;2:191-7.