

Quantification of conjunctival vascular reaction by digital imaging

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Background: No objective evaluation of the conjunctival provocation test (CPT) was possible until now.

Objective: The aim of this study was to develop a tool that would enable us to monitor conjunctival allergic inflammation by objective measurements.

Methods: Twenty-four patients allergic to grass pollen were challenged by a CPT with grass pollen and genuine grass pollen in the "Vienna Challenge Chamber." Patients were randomized to treatment with placebo or topical application of an H1-receptor antagonist (azelastine). Vascular reaction of the conjunctiva was repeatedly monitored by special video equipment. The signal was sent to a digital frame grabber, and digital image analysis was done with the aid of the WCUE-3 program (Olympus Optical Co., Hamburg, Germany).

Results: The CPT led to an immediate increase of the red density. This extension was linear within the first 15 minutes. During the long-term challenge, the increasing vascular effect lasted 2 hours. During active treatment a consistent reaction pattern was obvious; however, the corresponding levels were statistically lower ($p = 0.022$).

Conclusion: The calculation of red density of the conjunctiva by digital analysis proved to be a sensitive tool for measuring the conjunctival allergic reaction. It is possible to overcome the insufficient subjective evaluation of the CPT by objective measurements of the vascular reaction. (*J Allergy Clin Immunol* 1996;98:495-500.)

Key words: Conjunctival provocation test, conjunctival allergic inflammation, vascular reaction, video equipment, red density

The conjunctival provocation test (CPT) is very common in practical allergology and scientific work.¹ It is used to monitor allergen-specific reactions not only during the early phase but also during the late phase.² Taking cost-effectiveness into account, CPT is easy to perform and inexpensive, and it is the most sensitive test for allergic conjunctivitis, allergic rhinitis, and allergic asthma.^{3,4} Until now, clinical evaluations have been carried out by scoring the subjective symptoms, such as itching and lacrimation, and by physician's subjective assessment of hyperemia and eyelid swelling. However, no qualified objective evaluation of conjunctival hyperemia was possible until

Abbreviations used

CPT: Conjunctival provocation test
VCC: Vienna Challenge Chamber

now, although objective evaluations are desirable, especially in clinical trials.

The aim of the study was to develop a tool that would enable us to monitor conjunctival allergic inflammation by objective measurements of the vascular reaction during a CPT.

METHODS

Patients

Volunteers, aged 20 to 30 years, with a documented history of allergic conjunctivitis caused by *Dactylis* grass pollen (positive case history and positive skin prick test, RAST, and CPT results) were involved in the study. They were recruited out of the pollen season. An agreement from the Ethical Committee of the University of Vienna, Austria, was obtained, and written consent was given by all volunteers.

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Challenge procedure

We used two different modes of conjunctival challenge. First, we used the conventional method with a standardized grass pollen allergen solution for conjunctival challenge tests. A single drop of the grass pollen allergen solution (ALK, Copenhagen, Denmark) was applied to the conjunctiva. A dilution of 7.000 SQ allergen units was used (SQ units are biologically standardized allergen units, based on skin prick test according to the position paper on allergen standardization and skin tests).⁵

Second, a challenge was performed under physiologic conditions in the Vienna Challenge Chamber (VCC).^{6,7} The VCC is a closed, air-conditioned system in which a continuous, exactly defined aeroallergen concentration of genuine pollen grains is dispensed in the air and kept stable for several hours. It is possible to challenge up to 14 patients under physiologic conditions at the same time. The VCC satisfies the recommendation for specific challenge rooms of the Subcommittee on Bronchoprovocation for Occupational Asthma.⁸ A concentration of 2.000 genuine grass pollen grains (*Dactylis glomerata*) per cubic meter of air was used continuously for 4 hours. This concentration is consistent with the one found in the country on a hot summer day during the hay fever season.

Technical equipment

To obtain clear images, the patient's head was stabilized by a modified slit-lamp base during the conjunctival investigation. Instead of the original light source, we used a cold light source manufactured by Olympus Optical Co. (Europa) GmbH, Hamburg, Germany) (Highlight 3000). A prompt spectral analysis of the light source was done by WCUE-3 software (Olympus Optical Co. [Europa]).⁹ The microscope of the slit lamp was replaced by video equipment. We used a high-resolution RGB camera (CF15/2 MC RGB Kappa; KAPPA Messtechnik, Gleichen, Germany) and a microzoom objective with nine different moving grid positions. One individual area of interest (2.5×3.3 mm) to the right of the iris in each eye was documented repeatedly. The signal was saved on a Beta-cam SP videotape recorder (PVW-2800P; Sony, Tokyo, Japan). For immediate documentation it was possible to get prints from a color video printer (Mavigraph UP-3000P, Sony) with a resolution of 750×582 pixels. Simultaneously, the image was sent to a digital frame grabber (Matrox Magic modified by Galai, Olympus Optical Co. [Europa]). The frame grabber, which allows up to 1024×1024 pixel resolution in RGB-Real-colour 24-bit (16,7 million colors). The images were stored on an Intel 50 MHz personal computer (Fritsch & Jirak, Vienna, Austria). Digital image analysis, in terms of morphometry and densitometry, was done with the aid of the WCUE-3 Version 1.0 program.⁹ Before the evaluation, linear filters were used to increase the optical resolution.

Parameters used

We used the following objective parameter to calculate the clinical response to the CPT: the change of

the optical density of the red fraction of the total image. In addition and as a reference, we used the subjective parameter of itching eye, calculated by the visual analog scale on a 100 mm line. The parameters were obtained repeatedly, just before the challenge, during the challenge in strict intervals, and after the challenge.

Pilot study

A single patient allergic to grass pollen (as described previously) was challenged by conventional CPT with *Dactylis glomerata* allergen test extract with a concentration of 7.000 SQ units. The test solution was supplied by ALK. A single drop of this allergen test solution was applied to the conjunctiva after the initial parameters were obtained. Parameters were obtained repeatedly every 3 minutes for a period of 15 minutes.

Clinical study

Twenty-four patients allergic to grass pollen (as described previously) were recruited for this double-blind, randomized, placebo-controlled, crossover study. Patients were treated with 0.05% azelastine-HCl eye-drops¹⁰ by using a single dose of 0.02 mg azelastine versus a single dose of placebo eyedrops just 1 hour before the challenge. The washout period between the two crossover sessions was 2 weeks. Allergen challenge was performed in the VCC under physiologic conditions. In each of the two challenge sessions, patients were challenged by genuine allergens dispensed in the air. We used a concentration of 2.000 genuine grass pollen grains (*Dactylis glomerata*) per cubic meter of air continuously for 4 hours (240 minutes). After 225 minutes, an additional conjunctival challenge with an aqueous grass pollen extract was done in a mode previously described for the pilot study. This additional challenge was done to increase the mild and physiologic challenge procedure in the VCC to an artificial hard conjunctival challenge test (CPT). Parameters were obtained every 30 minutes for a period of 240 minutes.

Statistics

Testing the hypothesis of differences of the degree of vascularization (red density of the conjunctiva) between the verum test preparation and placebo was done by means of dependent *t* tests. If normal distribution and homogeneity of variances could not be accepted, a nonparametric test method (Wilcoxon matched-pairs signed-rank test) was used. The Bonferroni-Holm procedure was used to maintain a global alpha of 0.05. The vascularization raw data were transformed to differences in relation to the value at the start of the session (time, 0 minutes). By using these baseline-corrected values, the area between the beginning and end of the challenge period was calculated (area under the curve). All statistical tests were carried out at the 5% level of significance.

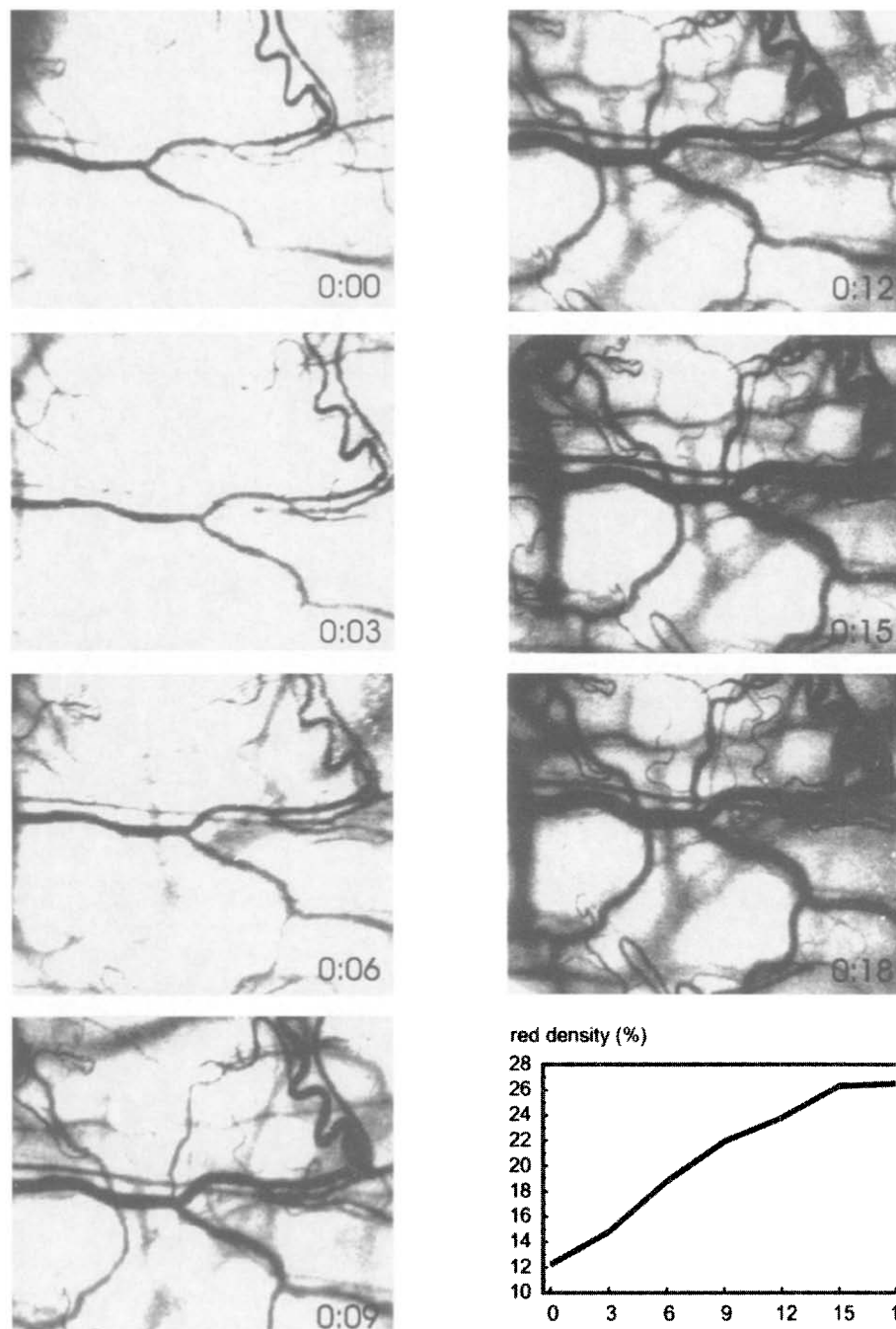


FIG. 1. Vascular reaction of the conjunctiva (area of 2.5×3.3 mm) after a CPT. Consecutive digital imaging of the vascular reaction every 3 minutes. Linear increase of red density in conjunctiva until 15 minutes after challenge is demonstrated.

RESULTS

Pilot study

A conjunctival challenge with a single drop of grass pollen extract led to an early-phase allergic reaction in the conjunctiva. According to this reaction, the objective evaluation of the CPT demonstrated an immediate increase of the red

density in the conjunctival image (Fig. 1). This extension was linear within the first 15 minutes and resulted in a more than twofold value within this period. The calculation of the red density by digital analysis of the conjunctiva proved to be a sensitive tool for measuring the conjunctival allergic reaction.

red density (%) left eye

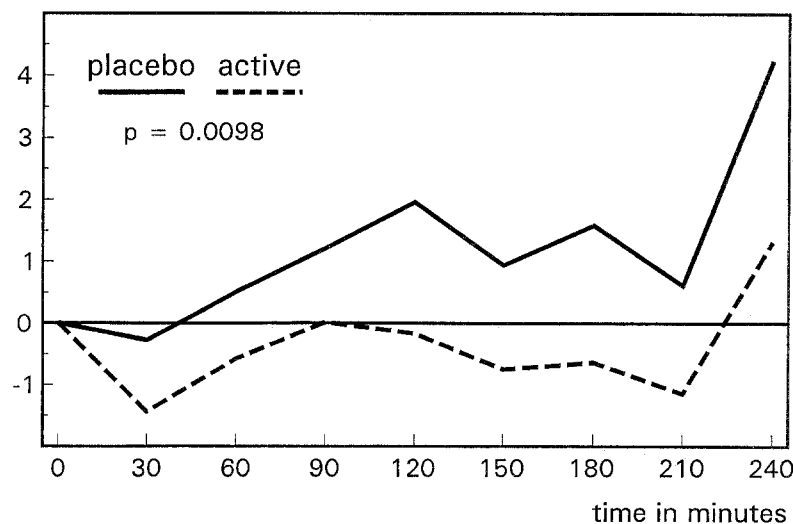


FIG. 2. Objective red density of conjunctiva (mean red density of digital image analysis of left eye) during a long-term challenge with grass pollen grains. Patients were given active (azelastine) or placebo treatment 1 hour before the challenge session. Statistical results are listed in Table I.

TABLE I. Testing the hypothesis of differences of red density in the conjunctiva

	Red density during challenge session of both eyes (AUC)		Red density at end point, left eye		Red density at end point, right eye	
	Placebo-treated	Active-treated	Placebo-treated	Active-treated	Placebo-treated	Active-treated
Mean	7.09	-3.26	4.24	1.32	3.06	-1.14
SD	22.42	15.23	5.56	5.15	6.10	2.30
Wilcoxon test	$p = 0.0221$		$p = 0.0098$		$p = 0.0010$	

AUC, Area under the curve; SD, standard deviation.

Clinical study

Two groups of patients were evaluated: patients treated with an active drug, suppressing the immediate conjunctival reaction, and patients treated with placebo. In both groups the objective parameter for the optical density of the red fraction of the conjunctiva's picture demonstrated an increasing vascular effect during the first 2 hours of the challenge period in the VCC (Fig. 2). This period of increasing redness of the conjunctiva was followed by a plateau reaction with more or less stable vascularization. Finally, the additional challenge with 1 drop of allergen extract results in a further increase of the red intensity (means, standard deviations, and statistical results are listed in Table I). During placebo treatment and active treatment, a consistent reaction pattern was obvious; however, the corresponding levels during active treatment were significantly lower. The statis-

tical analysis of the digital information of the red intensity indicated a significant difference between the active group and the placebo group during the whole challenge session ($p = 0.022$) and after the additional final challenge ($p = 0.009$).

The evaluation of the subjective parameters led to consistent results although not as clear as the objective ones. During placebo treatment the subjective rating of a patient's itching eye according to the visual analog scale showed a slight increase after the onset of the long-term challenge and continued in a plateau reaction after 120 minutes. The final challenge induced an increase of the subjective symptoms (Fig. 3). During the first hours of the challenge session, the subjective scoring was lower during active treatment than during placebo treatment but without significant difference. The statistical exploration of the subjective scoring (itching eye according to a 100 mm visual

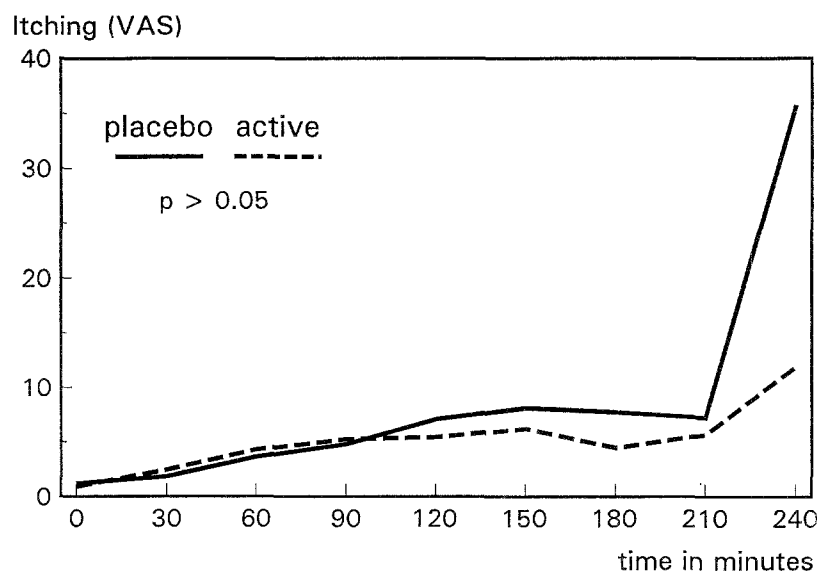


FIG. 3. Subjective scoring of itching eye (mean of a 100 mm visual analog score) during a long-term challenge with grass pollen grains. Patients were given active (azelastine) or placebo treatment 1 hour before the challenge session. Subjective parameters are consistent with the objective data (Fig. 2); however, differences are not so clear. VAS, Visual analog scale.

analog scale) displayed a significant difference between active and placebo treatment only at the end of the trial session ($p = 0.0007$).

DISCUSSION

The objective parameters chosen to estimate the CPT depend on vascularization only. As a result of the allergen challenge, the diameter of the small vessels increased and more vessels were visible, even in some of the deeper layers. This led to an increase of the red fraction of the conjunctiva. Comparable results have already been obtained by analyzing the surface of the nasal mucosa.¹¹ It is not possible to monitor the allergic inflammation itself (e.g., the increasing number of eosinophils). However, the shift in the vessels' parameters is a sufficient marker of allergic inflammation. The objective data led to a much more sensitive evaluation of the allergen challenge than subjective ratings of ocular itching. Furthermore, it was an advantage to obtain objective scaled data for statistical evaluation (Table I), especially in terms of clinical trials. Trials based on subjective conjunctival parameters only lack convincing arguments.^{12, 13} Attempts to improve the CPT procedure by evaluating the results by thermography were not sensitive enough and thus not successful in identifying positive CPT results.¹⁴ In contrast, digital imaging of the conjunctival vascular reaction leads to a sensitive evaluation.

The WCUE-3 software program is very potent and multifunctional, and it is easy to modify and extend. This special tool for the digital analysis of the conjunctiva suggests a variety of further investigations. With the aid of this unique tool, factual analysis and spectral intensity of the picture and digitalization of the vascular system would lead to more information about the vascular reaction in the conjunctiva during allergic inflammation. The new tool can be easily adopted by other investigators, not only for clinical trials but also for evaluating CPT results in routine work. The minimal equipment consists of the slit-lamp stand, the video camera with microzoom objective, a digital frame grabber, and the WCUE-3 software for image analysis. An assistant who is accustomed to handling the equipment, especially the slit-lamp stand and the WCUE-3 software, can obtain a complete report from a single patient within 1 minute. For the very first time, it is possible to overcome the insufficient subjective evaluation of the CPT by objective measurements of the vascular reaction, even in routine work.

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