

Reliability of a New Symptom Score in a Titrated Quantitative Conjunctival Provocation Test Supported by an Objective Photodocumentation

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Keywords

Allergic rhinoconjunctivitis · Clinical score · Conjunctival provocation test · Photodocumentation · Titrated quantitative conjunctival provocation test

Abstract

Background: Allergen provocation tests are useful methods for proving the clinical relevance of an allergen-specific sensitization. Among these methods, the conjunctival provocation test (CPT) represents an easy-to-use tool. However, its readout parameters have not yet been internationally standardized or validated. Photodocumentation has been shown as a good option for objectifying a CPT reaction, supporting the local investigator assessment. Based on test-retest reliability of the score and an objective digital photoanalysis of the conjunctival redness, this study aimed to prove the reproducibility of a new CPT scoring system for use in clinical trials (ClinicalTrials.gov identifier: NCT02690740). **Methods:** A titrated quantitative CPT was conducted outside of the pollen season in a final cohort of 23 adult patients with birch or grass pollen-induced allergic rhinoconjunctivitis. Conjunctival symptoms were analyzed using a standardized symptom score. Conjunctival redness was also evaluated by

an external observer and correlated with a digital photoanalysis using MATLAB software. **Results:** A test-retest correlation of 0.6 ($p < 0.01$) was found for the symptom score results. Likewise, a correlation of 0.65 ($p < 0.01$) was observed in the digital photoanalysis. The total symptom score showed a decrease in the mean value of 0.48 score points in the retest. **Conclusions:** This study reveals both a valuable test-retest correlation of the proposed score as well as a good correlation of eye redness with the (objective) photodocumentation. Based on our results, we can recommend the use of this scoring system as a valuable clinical protocol for future clinical trials.

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Introduction

Allergic rhinoconjunctivitis (ARC) is a common disease for adults and children in many countries [1, 2]. Besides an allergic history, skin prick tests are among the standard methods used to diagnose IgE-mediated

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allergic illnesses [2]. However, the results of these diagnostic procedures are inconclusive with respect to their correlation with clinical symptoms associated with observed sensitizations [3]. In other words, the clinical relevance of the sensitization is unclear. Thus, allergen provocation is an essential diagnostic technique to prove the clinical relevance of an allergic sensitization in the target organs [3–5] and is recommended by the European Medicines Agency for dose-range finding trials in allergen immunotherapy (AIT) [6]. Most often a nasal provocation test (NPT), recently standardized in a position paper of the European Academy of Allergy and Clinical Immunology (EAACI), is conducted in Europe [7].

Another possible diagnostic method is the conjunctival provocation test (CPT), developed decades ago [8, 9]. The respective test results often show strong correlations with NPT results [10–13]. In general, the CPT is an easy-to-use, inexpensive, and fast method that only rarely produces intolerable systemic side effects [14–19].

In many studies it has been confirmed that CPTs are reproducible and well suited for therapeutic studies [14, 17, 20–22]. Recently, a task force initiative of the EAACI outlined recommendations for conducting the conjunctival allergen provocation test in daily practice and has proposed a test protocol as well as positivity criteria [18]. However, no harmonized and clinically validated evaluation method exists on the national or international level [18, 23, 24]. The various protocols are highly heterogeneous with regard to the documentation and interpretation of evoked conjunctival symptoms [14, 17, 19, 21, 24, 25]. Global harmonization followed by a thorough validation of a symptom score in CPT testing has been emphasized as an unmet need in a current position paper on clinical outcomes in AIT trials by the EAACI [4]. Nevertheless, CPTs have been employed for monitoring clinical effects of pharmacotherapy [23] or AIT [26–29].

Besides determining the reaction of a CPT with a subjective composite score evaluation, photodocumentation has been shown as a good objective option for confirming a CPT reaction, supporting the local investigator assessment [30]. According to the new position paper [18], it is essential for assessing the reproducibility of the CPT to use subjective and objective criteria as well.

The objective of the present study was to determine the reproducibility of a new symptom score for evaluating CPT results. Furthermore, the reproducibility of an objective photodocumentation was conducted. Both were based on test-retest reliability using a CPT involving 23 patients with pollen-induced ARC. Another study aim

was to correlate conjunctival redness as evaluated by the investigator with that assessed via (objective) photodocumentation.

Methods

Study Cohort

An open, investigator-initiated trial was conducted from December 2012 through March 2013 (ClinicalTrials.gov identifier: NCT02690740). Twenty-three patients aged 18–67 years with a birch or grass pollen allergy were included in the analysis. The study was in compliance with the Declaration of Helsinki and was approved by the competent ethics committee (FF 118/2012, December 4, 2012; Landesärztekammer Hessen, Frankfurt, Germany). All participating patients were informed of the nature and objectives of the study and signed an informed consent document prior to any study procedures being performed.

Among the main inclusion criteria were moderate to severe ARC symptoms, which were certified by a positive score (≥ 5 out of 12) in a retrospective global assessment (adapted from Bousquet et al. [31]) and necessitating concomitant antiallergic medication in the last 2 years, as well as an allergen-specific positive reaction in the skin prick test (wheal size at least 3 mm larger than the negative control). Another inclusion criterion was a CPT score difference ≥ 6 between the provoked and the reference eye in the first (V1) and second visit (V2) of the study, which is described explicitly in the following.

The main exclusion criteria comprised, among others, acute diseases of the eye or of the nose/nasal sinuses, the presence of current allergic symptoms, or the intake of medicine which might compromise the CPT results (adapted from the German position paper on NPT [32]). A further exclusion criterion was a CPT score > 3 during the examination of the reference eye (see next section).

Titrated Quantitative CPT Procedure

The CPT was conducted using standardized and registered allergen extracts (ALK-Abelló, Hørsholm, Denmark). All CPTs were conducted by the same investigator. At V1, a titrated quantitative CPT (tqCPT) was performed according to the following protocol: 1 drop of the allergen solution was applied with a pipette to the conjunctival sac of the left eye, while 1 drop of the reference solution (allergen-free ALK diluent) was applied to the conjunctival sac of the right eye. A new evaluation protocol (based on similar assessments, e.g. by Abelson et al. [17], Radcliffe et al. [21], and others) was used, whereby the 4 symptoms of redness, tearing, itching, and irritations were examined. Each symptom was evaluated and categorized on a scale from 0 to 3, whereby the numerals denoted the following: 0 = no reaction, 1 = mild reaction, 2 = moderate reaction, and 3 = strong reaction with symptom-related specification (Table 1). Thus, a total score value ranging from 0 to 12 could be attained for all 4 symptoms. Also, a high-resolution macrophotograph of the left provoked eye was recorded and documented (see “Photodocumentation” section below).

Ten minutes after the application of the provocation solution to the patient's left eye, this provoked eye as well as the right eye, tested at the same time with the reference solution, were both evaluated according to the protocol. The reference eye was assessed

Table 1. Evaluation of conjunctival symptoms after CPT (total score value ranging from 0 to 12)

Itching
☐ 0 = Not itchy
☐ 1 = Slightly itchy but not bothersome
☐ 2 = Itchy, bothersome and/or urge to rub eye at times
☐ 3 = Very itchy, very bothersome and/or urge to rub eye often
Irritation
☐ 0 = Not irritated
☐ 1 = Dry eye or other unusual feeling in eye, but not bothersome
☐ 2 = Gritty or foreign object feeling in eye, bothersome and/or urge to rub eye
☐ 3 = Burning or painful eye, very bothersome and/or difficulty keeping eye open and/or urge to rub eye often
Tearing
☐ 0 = Not watery
☐ 1 = Watery eye, but no tears and not bothersome
☐ 2 = Watery eye with a few tears, bothersome and/or urge to wipe eyes at times
☐ 3 = Watery eye with several tears, very bothersome and/or urge to wipe eye often
Redness
☐ 0 = No redness
☐ 1 = Redness in conjunctiva bulbi
☐ 2 = Redness in conjunctiva bulbi and tarsi
☐ 3 = Redness in conjunctiva bulbi and tarsi and edema

only once (as in the following visits). If the total score in the reference eye exceeded 3, the test was regarded as invalid, and the patient was excluded from the trial. The tqCPT was evaluated as positive when a score difference ≥ 6 between both eyes resulted. In this case, no further provocation testing took place.

If no score difference ≥ 6 ensued after application of the initial drop of allergen solution, the test solution concentrations were raised stepwise from 100 to 1,000, 10,000, 20,000, 50,000, and 100,000 standardized quality units/mL after the 10-min waiting period followed by assessment of the previous concentration (“titrated quantitative CPT”). The test solution was applied to the left eye until a positive result was obtained; if no positive reaction resulted in V1, the patient was excluded from the study.

V2 took place about 1 week after the first examination. Again, 1 drop of the allergen-free reference solution was applied to the conjunctival sac of the right eye. In order to eliminate any compounding effects from up dosing in CPT to reach the threshold dosage achieved at V1, only the last drop which elicited the positive test was administered. If this concentration again triggered a positive result, the patient was then scheduled for final testing at visit 3 (V3). Otherwise, as on the first test day, the dosage was increased stepwise at 10-min intervals until a positive symptom score resulted (≥ 6 compared to that of the reference eye). These patients were scheduled for visit 2A (V2A) 1 week later at which the respective threshold concentration determined during V2 was administered.

Patients who attained a positive CPT result at V2A were then scheduled for V3. Those patients who again failed to achieve a positive CPT result at the threshold concentration were excluded from the study evaluation.

The final testing took place at V3, which was 3–4 weeks after V2 (or V2A if applicable). At this last visit, the final CPT was performed using the concentration which had elicited a positive test result at V2 or V2A (Fig. 1).

Photodocumentation

To enable an objective evaluation of the clinical reactions after the CPT, the eye reaction was documented in a high-resolution ($4,032 \times 3,042$ pixels) macroimage that was analyzed using a MATLAB software program to calculate the degree of redness in percent developed by Astvatsatourov and Mösges [33] (alternative redness reconstruction was applied by Sárándi et al. [34]). For this purpose, a photograph was taken before testing and 10 min after each provocation, respectively. The images were taken under the same lighting conditions and using the same eye position for all patients. The “region of interest” was cut out manually by the image processor operator at the Institute of Medical Statistics, Informatics, and Epidemiology in Cologne, Germany. Besides the digital photoanalysis, an external, trained observer also evaluated the images with regard to conjunctival redness increase on a scale from 0 to 3 (none, mild, moderate, severe).

Statistical Analysis

The main objective of this trial, the reproducibility of the CPT symptom score between V2/V2A and V3, was presented descriptively. The acquired data were represented by means and root mean square deviation (RMS). The Pearson correlation coefficient was calculated for the current analysis. Furthermore, the results of the photodocumentation were evaluated and compared with the investigators’ evaluations of the conjunctival redness.

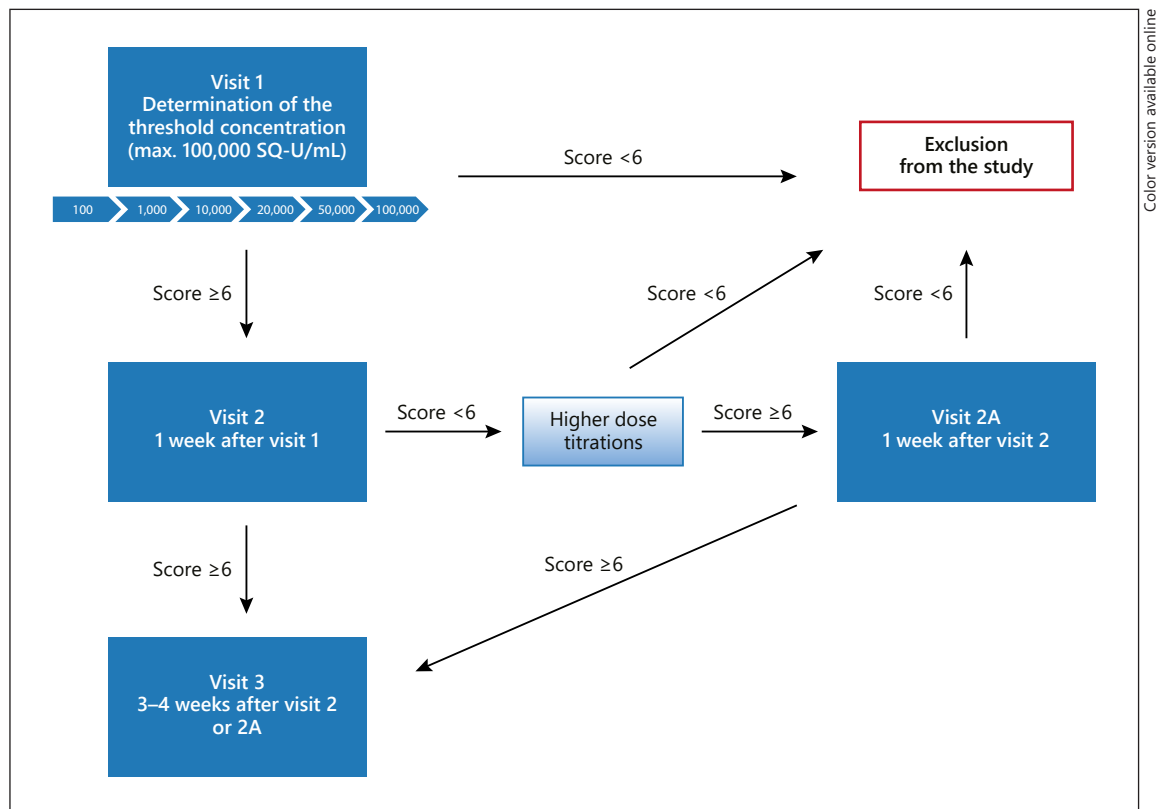


Fig. 1. Flow diagram showing the study procedure. To get access to the final visit 3, a CPT score difference ≥ 6 between the provoked and the reference eye was necessary in visit 2 or visit 2A. SQ-U, standardized quality units.

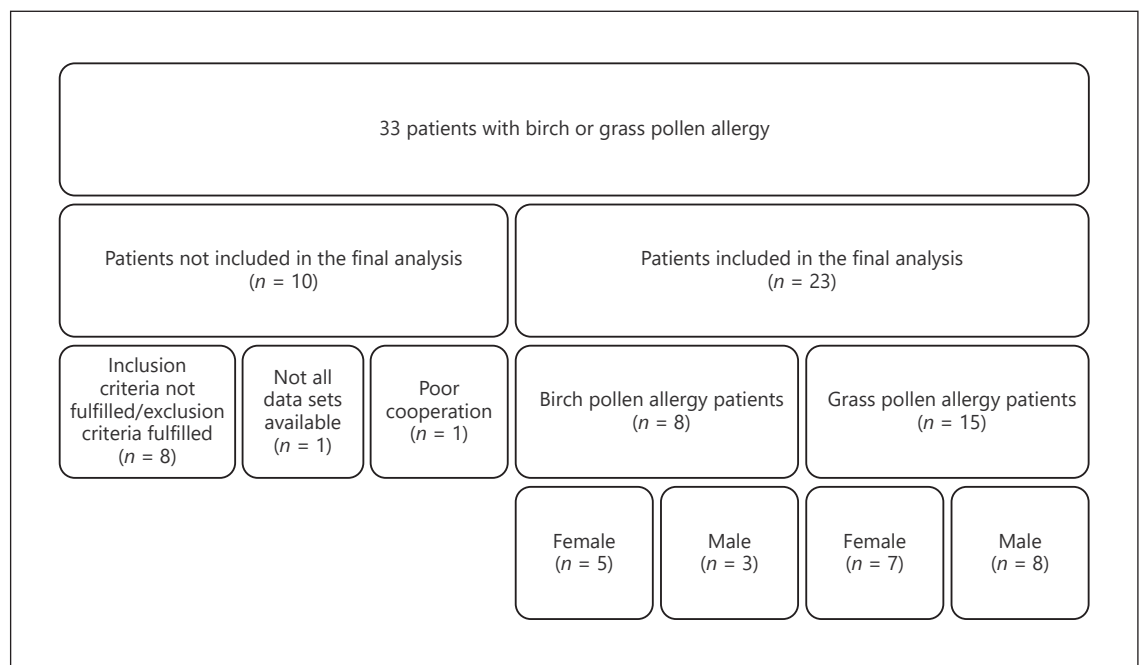


Fig. 2. Flowchart of patient distribution.

Fig. 3. Frequency distribution. **a** Total score values. **b** Differences between the results of visit 2/2A and visit 3 according to the investigator's evaluation. μ , mean value; RMS, root mean square deviation; Δ score, score difference.

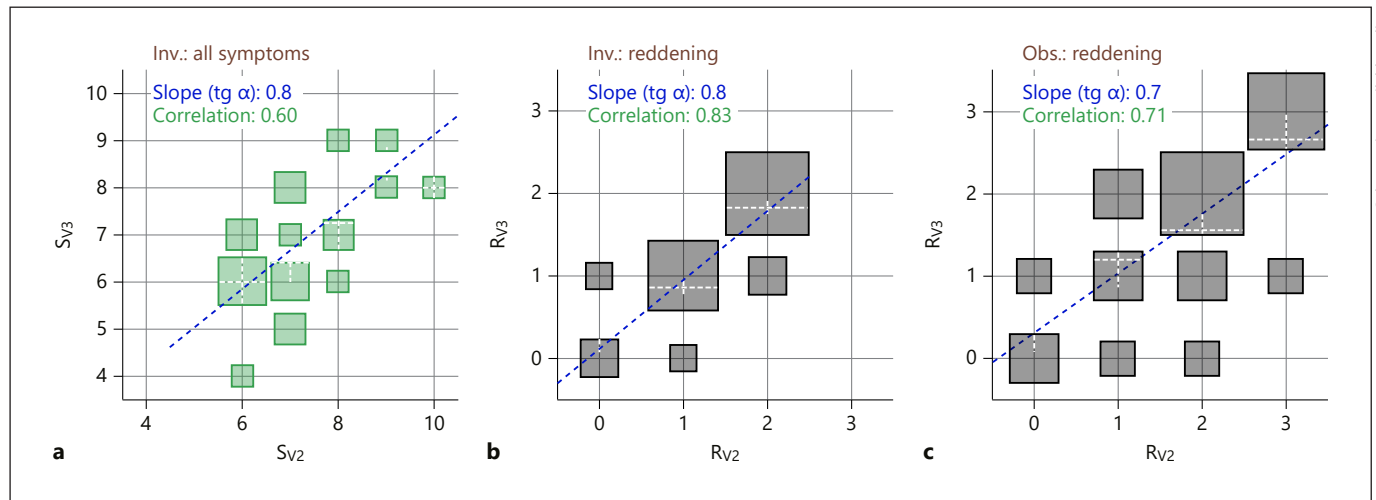
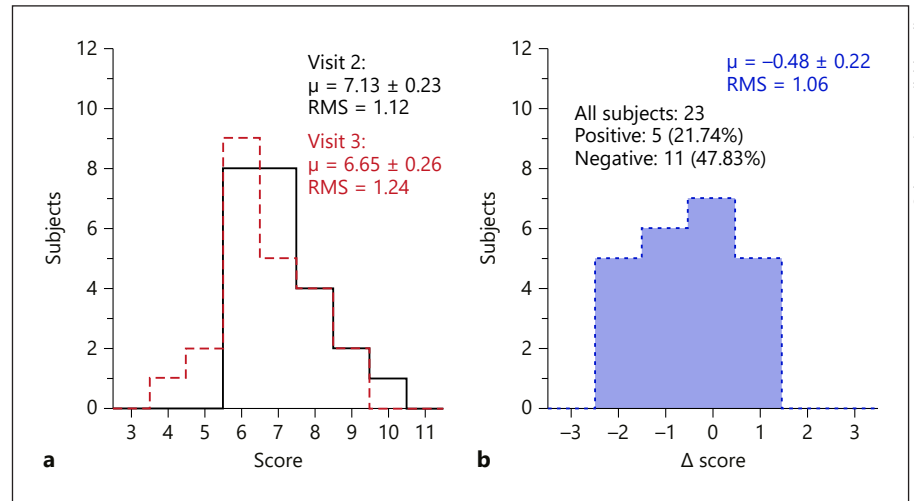


Fig. 4. Correlations of the scores. **a** Correlations of the respective total score values at visit 2/2A (S_{V2}) and visit 3 (S_{V3}). **b, c** Correlations of the redness score values between visit 2/2A (R_{V2}) and visit 3 (R_{V3}) according to the respective evaluations of the investigator (Inv.) and the external observer (Obs.). tg α , weighted slope of the linear fit ($y = \text{tg } \alpha \times x + c$).

Results

Of the 33 birch or grass pollen allergy patients who had been initially screened in the study, 10 patients were excluded from the full analysis (Fig. 2). The analysis included 23 patients, 15 of whom were tested with grass pollen whereas 8 individuals were tested with birch pollen (Fig. 2). After determining the individual subject's threshold value at V1 by applying incremental concentrations of the allergen solution, this threshold concentration was confirmed at V2, respectively V2A. At V3, the threshold concentration was applied again, and the total score of the

provoked eye was compared to the one of V2/V2A in order to confirm the reproducibility of this scoring system in CPT (Fig. 1).

Figure 3a shows the frequency distribution of the total score (Table 1) at V2/V2A and V3. Here, one sees that the mean value for the total scores dropped by 0.48 points from 7.13 at V2/V2A to 6.65 at V3. Although the score distributions between V2/V2A and V3 show no meaningful difference, the deviations of the test-retest range from -2 to $+1$ points. The deviations between the total scores of V2/V2A and V3 are shown in Figure 3b. To show the range of the symptom severity distribution, we

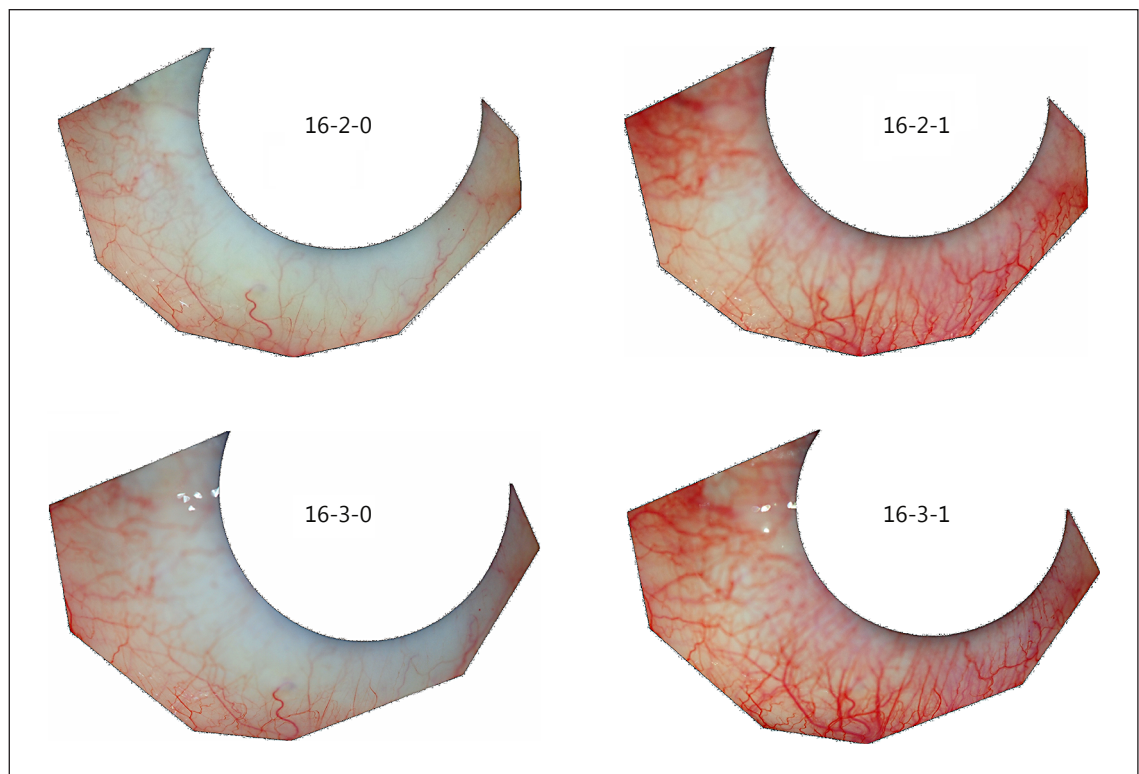


Fig. 5. Region of interest exemplified by patient 16. Before and after the provocation at visit 2, the MATLAB program evaluated subject 16 as having a redness degree of 8.24% (16-2-0) and 20.96% (16-2-1), respectively. According to the computer analysis, this patient exhibited a respective redness degree of 7.7% (16-3-0) and 30.34% (16-3-1) at visit 3.

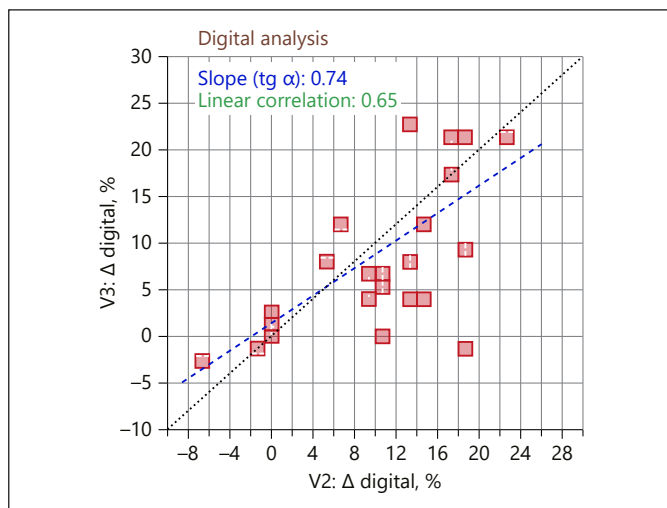


Fig. 6. Correlation of allergic redness as assessed by digital photoanalysis at visit 2/2A versus visit 3. Allergic redness for each subject as assessed by digital photoanalysis at V2/V2A (x axis) and V3 (y axis) is represented in percent; tg α, weighted slope of the linear fit ($y = \text{tg } \alpha \times x + c$).

used an RMS. If one then adds the difference of the total score mean value of 0.48 to the RMS of 1.12 (V2/V2A) and of 1.24 (V3), the result is a score value deviation of 2.84 points. The correlation of the total score values between V2 and V3 is 0.6 with a weighted slope of 0.8 (Fig. 4a). The Pearson correlation coefficient of 0.6 was found to be significant at the 0.01 two-tailed level.

At V2/V2A and V3, 87% of the subjects reacted with conjunctival redness. Only in 13% of the cases was no conjunctival redness detected. While 83% of the subjects had a constant redness score, 13% showed a weaker score value for redness at V3, and only 1 subject had a higher score than that found at V2/V2A.

Figure 4b and c depicts the correlations of the redness score values R_{V2} and R_{V3} between V2/V2A and V3 according to the evaluation of the investigator and of the external observer. These Pearson correlations were 0.83 for the investigator and 0.71 for the observer. Both of these correlation coefficients were found to be significant at the 0.01 two-tailed level.

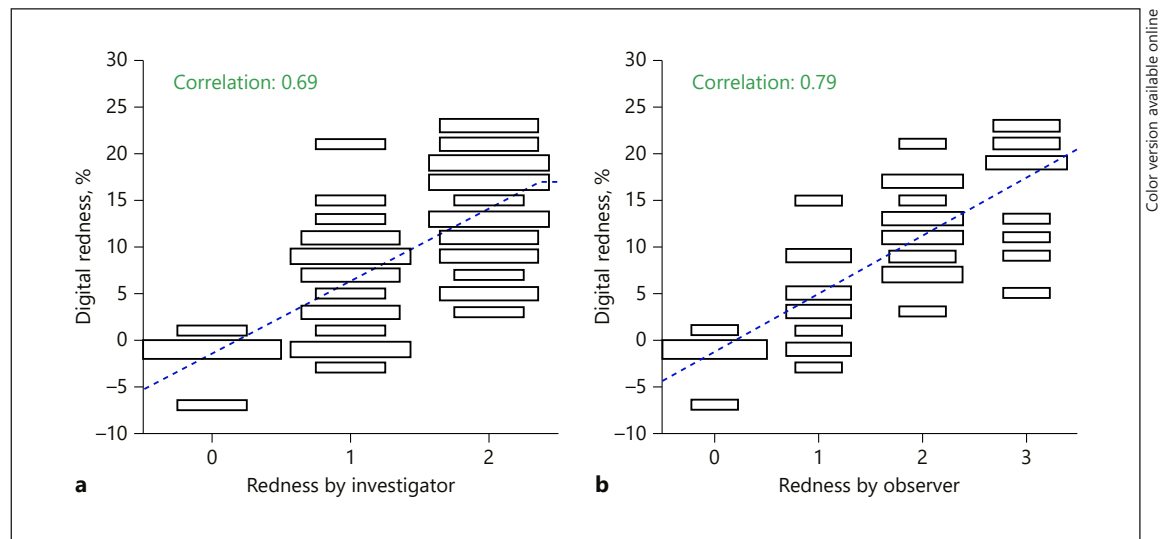


Fig. 7. Correlations between allergic conjunctival redness as assessed by digital photoanalysis versus redness as evaluated by the investigator (**a**) and by an observer (**b**). The data of visit 2/2A and visit 3 are combined in both images.

In addition to the subjective evaluation of the degree of conjunctival redness made by the investigator and the observer, we applied digital photoanalysis (Fig. 5) as an objective technique to ascertain the degrees of redness.

Figure 6 illustrates the difference in the degrees of redness in percent as determined by the MATLAB software program [33] for the measurements at V2/V2A and V3. The term “digital redness” represents the percent change in the redness area within the region of interest which was caused by the CPT. The correlation between the visits was 0.65, and the weighted slope of the linear fit was found to be 0.74. The average increase after allergen application in conjunctival redness was 10.3% for V2/V2A and 8.01% for V3.

The results of the digital photoanalysis were correlated with the results of the redness scores evaluated by the investigator and by the observer (Fig. 7). By comparison, the correlation values of 0.69 for the investigator’s evaluation and 0.79 for the observer’s evaluation with respect to the digitally determined change in redness were high.

Discussion

Our study aimed to investigate the reproducibility of a new symptom scoring method for evaluating CPT results including test-retest reliability and objective photoanalysis of the conjunctival redness.

As the primary objective, the total score values of the challenged eye between both visits V2 (or V2A if applicable) and V3 were found to be reproducible with a significant correlation coefficient of 0.6 ($p < 0.01$) (Fig. 4a). Nevertheless, the total score value for 11 of 23 patients (48%) was lower at V3 than at V2/V2A (Fig. 3), also indicated by a weighted slope of 0.8 (Fig. 4a). Recently, Gloistein et al. [35] have provided a hypothesis that repeated CPTs may cause a local desensitization of the conjunctiva but emphasized that more pilot studies are warranted to confirm this. Another possible explanation for this discrepancy could be that by V3, patients had become accustomed to the symptoms occurring after CPT differently and were less troubled by them as the total score (Table 1) largely consisted of purely subjective descriptions provided by the patient.

The maximum decrease (without any therapeutic influence) of a patient’s total score in the test-retest was 2 points (Fig. 3b). This observation would mean that any treatment effect on the patient’s reactivity would have to exceed this value to be detected. This difference in the total score is confirmed by the values of the frequency distribution (Fig. 3a). Upon considering the difference in the mean value of the total scores and adding the respective RMS error of both distributions, one obtains a total score value difference of 2.84 (Fig. 3), which could be considered in future clinical trials in ARC patients involving CPT.

For V2/V2A and V3, a high correlation coefficient of 0.83 can be recognized between the single conjunctival redness scores for the evaluation by the investigator (Fig. 4b). Conjunctival redness (as an objective parameter not influenced by subjective perception of the patient) can be regarded as the most reproducible parameter in the total of the individual conjunctival symptoms which are proposed in our classification (Table 1). The assessments of the conjunctival redness by an external observer (Fig. 4c), which is described as a good method of assessing CPT results [30], show also a good test-retest correlation coefficient of 0.71.

Even if the rating by the investigator of the single conjunctival redness score (Table 1) could be improved (no edema occurred, and therefore no score value of 3 was detected), the correlation of the digital analysis results with the investigators' evaluations of the conjunctival redness (0.69) shows that the degree of redness is comparable in terms of reproducibility (Fig. 7). It can be seen that the correlation between the digital analysis of conjunctival redness and the evaluation by the external observer is higher (0.79) than by the internal/local investigator (0.69) (Fig. 7). Kjaergaard et al. [36] previously conducted photo analyses in conjunctival provocation tests and also thereby ascertained the higher sensitivity of photographic evaluation compared to the rating of a local investigator following CPT. However, this difference was not that high in our study. In contrast to the analysis of the observer and the investigator, the digital photoanalysis as an objective measurement technique also allows an evaluation of detailed changes in the intensity of conjunctival redness.

In our study the digital analysis of conjunctival redness showed an average increase in 10.3% at V2/V2A and of 8.01% at V3. Similar to the total score there was also a decrease between the tests, whereby the frequency distribution was nevertheless relatively high due to the individual eye reactions. The correlation between the visits was significant ($p < 0.01$) with a coefficient of 0.65 in the digital analysis of conjunctival redness (Fig. 6). Noteworthy here is that in the test-retest (V2/V2A and V3), the ascertained total score correlation of 0.6 (Fig. 4a) is close to the correlation of 0.65 in the digital analysis of conjunctival redness. This highlights the validity of the total evaluation score applied in our study. The CPTs, performed at different time points, often showed various conjunctival reactions in the individual patients. Even so, the evaluation score appeared reproducible, with a deviation in individual patient score of a maximum of 2 points. On the basis of the maximum decrease in 2 points in the test-retest and

the calculated total score difference value of 2.84 points, a score difference of a minimum of 3 may need to be achieved to have the ability to detect a therapeutic effect. Although this *proof-of-concept* methodological trial was not planned and powered for any therapeutic intervention and the analysis is based on a limited number of patients, this assumption should be considered and investigated in future interventional trials with CPT as an end point measure.

In conclusion, our study reveals a valuable test-retest correlation of the proposed score in CPT and a good correlation of eye redness between local assessments and photodocumentation. Based on these results, we can recommend the use of tqCPT together with the tested scoring system as a valuable clinical outcome measure in future clinical trials involving ARC patients.

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Author Contributions

O. Pfaar was coordinating investigator of the trial. D.P. Claßen participated as investigator and wrote the first draft of the manuscript. O. Pfaar, D.P. Claßen, A. Astvatsatourov, L. Klimek, and R. Mösges were involved in the study design and conduct of the trial. A. Astvatsatourov analyzed the photodocumentation. R. Mösges and A. Astvatsatourov were responsible for the statistical analysis. O. Pfaar, D.P. Claßen, A. Astvatsatourov, L. Klimek, and R. Mösges contributed to the manuscript preparation from the draft stage by reviewing, commenting, critically revising the text where applicable, and approving journal submission.

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