

Title: Detecting change in conjunctival hyperemia using a pixel densitometry index

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Abstract

Purpose: To investigate a pixel densitometry index (PDI) for measuring ocular surface inflammation (OSI).

Methods: Efron's grading was performed by two independent observers. Color photographs and indocyanine green angiography (ICGA) were undertaken before and after instillation of phenylephrine hydrochloride 2.5% (PE).

Results: Fifteen patients with and 10 without OSI were included. The reduction in the PDI before and after PE was 73.29 ± 30.71 and 50.87 ± 17.46 ($p = 0.036$) in patients with inflammation and 52.86 ± 16.90 and 39.63 ± 12.04 ($p = 0.0024$) in those without OSI. The reduction in Efron grades following PE was 25% (mean 0.46 ± 0.50 , median 0.50; $p < 0.01$). The coefficient of variation was higher using the Efron grades (131%) than the PDI (65%).

Conclusion: The PDI allows the objective detection of change in conjunctival hyperemia and may be directly applicable to non-invasive angiography such as optical coherence tomography based angiography.

Introduction

Hyperemia caused by vascular injection of the bulbar conjunctiva and subconjunctival vessels is a key feature of ocular surface inflammation (OSI). OSI in clinical routine is usually graded subjectively based on biomicroscopic findings.^{1,2} Clinical grading of OSI is crucial for disease monitoring and the evaluation of treatment efficacy. Objective and repeatable assessment is of particular importance in clinical settings where consecutive patients need to be closely followed by more than one clinician and for monitoring systemic immunosuppressive medication, for example, in chronic allergic eye disease, graft-versus-host disease, and cicatrizing conjunctivitis. Different qualitative and quantitative grading scales including semi- or fully automated methods

have been proposed to measure conjunctival vascular injection. The grading scales proposed by Efron and McMonnies, which provide a score depending on the number, density, and tortuosity of vessels, are among the scales most frequently used in clinical routine.^{1,2} As with all qualitative or semi-quantitative methods, however, these classifications exhibit considerable variability among different observers³ or by the same observer over time.⁴ Most of the available semi-automated techniques are based on a combination of color quantification,^{5AD} edge detection,⁶ and fractal analysis.⁹ These methods, however, have not been adapted widely in clinical practice due to several disadvantages, including limited availability and setup costs of dedicated instruments, the need of special training for graders, and the amount of time needed to analyse images. The advent of optical coherence tomography based anterior segment angiography (OCTA) now allows the non-invasive three-dimensional depiction of ocular surface vasculature, although still limited by artefacts.^{10,11} In order to provide proof of concept, in this pilot study we investigated and compared angiographic pixel densitometry with the Efron grading scale to measure ocular surface vascular injection. In order to further evaluate and compare these methods to detect change, vasoconstriction was induced with topical phenylephrine.

Methods

Patients with OSI resulting from multiorigin conjunctivitis and or keratitis (study group) and patients with corneal neovascularisation (CoNV) in the fellow eye were included. Written informed consent was obtained from all subjects, and the study was performed according to the tenets of the Declaration of Helsinki with institutional review board approval. Standardized patient demographic and clinical data sheets were used to record age, race, sex, diagnosis, visual acuity (best-corrected visual acuity), previous ocular medical or surgical treatment, and the presenting clinical features of the ocular surface disease including the cause and duration of the current

episode of ocular inflammation. Patients with any contraindications to undergo ICGA (known allergy to iodides and shellfish, inability to fixate on a target, or continuous eye movements such as nystagmus) were excluded. The occurrence of adverse events and reactions was documented. Color photographs and indocyanine green angiography (ICGA) of the ocular surface were undertaken on each patient.

Color images were recorded using a slit-lamp mounted digital system (Topcon SL-D Digital Slit Lamp) using a 6 times magnification in four sections: Nasal, temporal, superior and inferior conjunctival quadrant. Illumination was from a 45 degree angled beam on slit lamp biomicroscopy with a diffuser filter and a variable flash intensity. Color photographs were saved in tiff format at an image dimension of 4,256 x 2,832 and 300 pixels per inch. Following color photography, angiographic images were recorded with red-free, infrared, and fluorescent filters (ICG: 825 nm) using the Heidelberg system with 20 and 30 degree angle lenses and between 32D and 53D focus. Preceding dye injection for angiography, images were taken with an ICG filter to exclude other causes of hyperfluorescence. After administration of 2.5 mL of 5mg/ml indocyanine green (ICG, Pulsion Medical Systems, Munich, Germany), a video was taken for 60 seconds (early ICG phase) of the superior bulbar conjunctiva, commencing 10 seconds after the injection.¹² Representative color photographic and ICG angiographic images are shown in figure 1. We have previously shown that the inferior (I) quadrant of the cornea fills first followed by the superior (S), nasal (N), and temporal (T) quadrants (ISNT)¹². Single images were taken for up to 3 minutes. 2.5% phenylephrine (PE) eye drops were then instilled and after 5 minutes, color photography was repeated, and a further injection of 2.5 ml of ICG and ICGA images were again recorded for 3 to 5 minutes.

Analysis

Video and still pictures taken immediately after the injection of ICG were analysed independently by two observers (VR and BS). Color images were analysed for quality and clarity of conjunctival vasculature. The best color photographs and ICGA images obtained between 60 and 120 sec after the first and second injection of ICG were independently selected by same two observers based on the following previously published qualitative subjective parameters:¹² Specifically, the best ICGA images were selected based on the following qualitative subjective parameters. Quality grading for ICGA was categorized as 0 to 4 (0, no vessel discernible; 1, poor vessel delineation; 2, good vessel delineation; 3, very good vessel delineation; 4, excellent vessel delineation).¹² The images with good vessel delineation would have at least 50% of vessels clearly evident with distinct boundaries and hyperfluorescence. A square-shaped region of interest (ROI) of 3x3 mm was chosen in the superior bulbar conjunctiva, and the same ROI was marked on both color photographs and ICG angiograms before and after PE. The superior conjunctival quadrant was chosen in order to analyse an area with the lowest likelihood of vascular anomalies secondary to degenerative conjunctival changes as e.g. pinguecula. Additionally, the degree of conjunctival vascular injection in other quadrants is expected to be more affected by environmental factors or lid margin pathology as potential bias to PDI results when monitoring ocular surface inflammation (OSI).

For analysis of color photographs, blood vessels within the ROI were extracted by an established segmentation method.¹³ The ratio between the number of vessel pixels and total pixel number within the ROI was computed. For Efron grading, a selected color photograph before and after PE from each patient was analysed and graded independently by VR and BS. For analysis of ICG angiograms, the alignment of pre- and post-PE images was attempted using i2k Retina software (DualAlign LLC, Clifton Park, NY), but this could not be achieved with this software. The mean intensity (\pm standard deviation) of the pixels in the ROI was defined as the pixel densitometry index

(PDI).

Statistical analysis was performed using the Statistical Package for the Social Sciences Software (IBM SPSS Version 22.0 for MAC; SPSS Inc., Chicago, IL). P values of less than 0.05 were considered statistically significant. **Data are presented as mean values \pm standard deviations.** Non-parametric tests (Kruskal-Wallis and Wilcoxon signed rank test) were used to compare clinical grading scores. Levels of agreement were tested using Fleiss' Kappa Statistic (k). Interpretation of levels of agreement were based on that described for two (binary) categories for each patient. A value of <0.2 , k was considered poor, $0.2 < k < 0.4$ fair; $0.4 < k < 0.6$ moderate, $0.6 < k < 0.8$ substantial and $k > 0.8$ as almost perfect.

Results

Fifteen patients with bulbar conjunctival redness of variable origin and duration and 10 patients with CoNV in the fellow eye but with no clinical evidence of OSI or history of inflammation in the studied eye were included. The demographic features and clinical details are summarized in Table 1. The mean patient age at presentation was 42.3 ± 17.1 years (min: 21 years and max: 75 years). In patients with inflammation the mean duration of conjunctival hyperemia was 4 days (min: 2 –7 days). The mean visual acuity was 0.30 ± 0.33 LogMAR (min: -0.10 and max: 1) and 0.1 ± 0.18 in patients with and without ocular surface disease. The most common aetiology of bulbar conjunctival hyperemia were microbial keratitis (9/15), atopic keratoconjunctivitis (3/15), and episcleritis and scleritis (3/15). Intravenous application of ICG was well tolerated and no any adverse event to angiography occurred.

The level of inter-observer agreement using Efron's grading was poor before PE ($k=0.21$) but moderate after PE ($k=0.45$). There was, however, substantial inter-observer agreement on the difference before and after PE ($k=0.65$). There were significant differences between patients with

and without inflammation using Efron's grading 2.93 ± 0.89 [min: 1.50, max: 4.00] versus 0.90 ± 0.39 [min: 0, max: 1.5 $p < 0.001$) and the PDI 73.29 ± 30.71 (min: 36.48, max: 130.19) versus 52.87 ± 16.90 (min: 34.62, max: 77.08 $p = 0.04$). The PDI using the color images (ratio between the number of vessel pixels and total pixel number) gave a very low ratio (mean 0.114 ± 0.04).

There was a significant reduction in vascular injection following the instillation of PE in all subjects.

The overall PDI of all cases before and after instillation of PE was 65.11 ± 27.60 and 44.87 ± 22.48 .

($p = 0.002$) The PDI before and after PE in patients with inflammation was 73.29 ± 30.71 and 48.49

± 27.60 ($p = 0.004$), and 52.87 ± 16.90 and 39.44 ± 10.22 ($p = 0.0024$) in those without. The mean

overall Efron grading before PE was 2.12 ± 1.24 and after PE was 1.66 ± 1.19 ($p = 0.07$) (table 2).

The coefficient of variation (SD/mean $\times 100$) both pre and post phenylephrine was lower using the PDI (42% and 50%) than with the Efron grades (59% and 72%).

The mean reduction in Efron grades following PE was 25% (mean 0.46 ± 0.50 , median 0.50; $p < 0.01$), and of PDI units was 29% (mean 20.25 ± 20.85 , median 15.84; $p < 0.01$). There were significant linear associations between the grade of inflammation pre and post PE for both Efron ($R^2 = 0.95$, $b = 0.81$, $p < 0.01$) and the PDI ($R^2 = 0.89$, $b = 0.67$, $p < 0.01$), reflecting a 33% change following PE for the PDI and 19% using the Efron grades (figure 2).

Discussion

We report a method to objectively quantify ocular hyperemia using densitometric analysis of early ICG angiographic images. The PDI is calculated from the number of white and black pixels in analysed images, where vessels with dye are seen as white pixels. For image comparison either longitudinally or following PE as in this study, and different from previous reports,^{5,14,15} the presented system is based on digital vessel alignment and subtraction analysis providing a less operator dependent method. This avoids the need for white balance standardization of a

photograph, which introduces a level of subjectivity when the observer selects a white spot on the images for the purpose of color balance adjustment. ICGA densitometry also enables quantitative analysis. Furthermore the use of a continuous scale of measurement lends simplicity to the clinical interpretation of the results and allows for more analysis of the data using continuous rather than ordinate or nominal scales.

In order to assess the feasibility and sensitivity of this method to detect and measure intra-individual change, we used topical phenylephrine to constrict the superficial ocular surface vasculature. Topical PE led to a smaller reduction and with greater variability using the Efron grades than with the PDI for both patients with and without OSI. The coefficient of variation using the Efron grades was higher than that with the PDI. This may be due to the difference in scales but may also indicate that the PDI may be subject to less variability. The PDI does not carry the limitation of inter-observer variability, reducing the need for a clinically trained grader. This is a weakness of many commonly used grading scales such as McMonnies and Efron,^{1,2} who suggested the use of photographic reference scales to grade conjunctival hyperemia when recording the level of severity of ocular complications of contact lens wear. Observer experience significantly improved grading reliability in these widely used grading scales^{16,17} which are also limited by a high inter- and intra-observer variability.^{3,4}

We reported previously that the anterior segment angiography is useful tool in grading of ocular surface disease including corneal neovascularization atopic keratoconjunctivitis.¹⁸⁻²⁰ Our results suggest that densitometric analysis of conjunctival angiograms has the potential to be useful for monitoring OSI. ICGA was well tolerated by all study subjects, which is in line with previous data showing ICG to be much better tolerated and to carry a lesser risk of anaphylactic reactions compared to fluorescein dye (0.05 %vs 0.3 %).^{21,22}

We included the uninvolved fellow eyes of patients with CoNV as representative of patients

without inflammation. Although there was no clinically apparent inflammation (hence Efron grade of zero), there may have been circulating cytokines from the disease (CoNV) in the fellow eyes leading to increased vessel flow in the apparently un-inflamed eye and a higher PDI.

As a limitation at this stage, the creation of a fixed PDI grading scale is difficult because of inter-individual differences in vessel characteristics. Currently the PDI may not therefore be as amenable to inter-patient comparison compared to color image comparison. In contrast to angiography, we did not find the PDI to be informative using color images possibly due to reduced small vessel detection with color photographs compared to angiography. It would be feasible to further standardize ICGA for measurement of the PDI in addition to the standardized scanning protocol and with larger numbers to have clinical PDI standards. For example, in order to compensate variable laser power and scanning sensitivity, an internal reference could be used to calibrate the PDI.²³ This would then enable comparisons both between and within subjects at different time points. Also given the limitation of the i2kRetina programme and of its kind, it is desirable to have an effective approach to align images before and following PE injection so as to analyse the exact same areas. Both of these would then enable the determination of changes over time or between individuals. In the clinical setting, intra-individual comparability is probably the relevant feature for detecting longitudinal change in ocular surface inflammatory disease.

Our results show that ocular surface angiography based on white pixel densitometry allows the objective detection of change in conjunctival hyperemia. While ICGA may be considered invasive for routine assessment of conjunctival inflammation, this investigation serves as a proof of principle to establish a methodology which may be directly applicable to non-invasive angiography such as optical coherence tomography based angiography.¹⁰

References

1. McMonnies CW, Chapman-Davies A. Assessment of conjunctival hyperemia in contact lens wearers. Part I. *Am J Optom Physiol Opt*. 1987;64(4):246-250.
2. Efron N. Grading scales for contact lens complications. *Ophthalmic Physiol Opt J Br Coll Ophthalmic Opt Optom*. 1998;18(2):182-186.
3. Terry R, Wong R, Papas E. Variability of clinical investigators in contact lens research. *Optom Vis Sci*. 1995;72:16.
4. Chong E, Simpson T, Fonn D. The repeatability of discrete and continuous anterior segment grading scales. *Optom Vis Sci Off Publ Am Acad Optom*. 2000;77(5):244-251.
5. Amparo F, Wang H, Emami-Naeini P, Karimian P, Dana R. The Ocular Redness Index: a novel automated method for measuring ocular injection. *Invest Ophthalmol Vis Sci*. 2013;54(7):4821-4826. doi:10.1167/iops.13-12217.
6. Fieguth P, Simpson T. Automated measurement of bulbar redness. *Invest Ophthalmol Vis Sci*. 2002;43(2):340-347.
7. Papas EB. Key factors in the subjective and objective assessment of conjunctival erythema. *Invest Ophthalmol Vis Sci*. 2000;41(3):687-691.
8. Rodriguez JD, Johnston PR, Ousler GW, Smith LM, Abelson MB. Automated grading system for evaluation of ocular redness associated with dry eye. *Clin Ophthalmol Auckl NZ*. 2013;7:1197-1204. doi:10.2147/OPHTH.S39703.
9. Schulze MM, Hutchings N, Simpson TL. The use of fractal analysis and photometry to estimate the accuracy of bulbar redness grading scales. *Invest Ophthalmol Vis Sci*. 2008;49(4):1398-1406. doi:10.1167/iops.07-1306.
10. Brunner M, Romano V, Steger B, et al. Comparison of optical coherence tomography angiography and indocyanine green angiography for imaging of corneal neovascularization. *Invest Ophthalmol Vis Sci Press*. 2017.
11. Ang M, Cai Y, MacPhee B, et al. Optical coherence tomography angiography and indocyanine green angiography for corneal vascularisation. *Br J Ophthalmol*. January 2016. doi:10.1136/bjophthalmol-2015-307706.
12. Anijeet DR, Zheng Y, Tey A, Hodson M, Sueke H, Kaye SB. Imaging and evaluation of corneal vascularization using fluorescein and indocyanine green angiography. *Invest Ophthalmol Vis Sci*. 2012;53(2):650-658. doi:10.1167/iops.11-8014.
13. Zhao Y, Rada L, Chen K, Harding SP, Zheng Y. Automated Vessel Segmentation Using Infinite Perimeter Active Contour Model with Hybrid Region Information with Application to Retinal

- Images. *IEEE Trans Med Imaging*. 2015;34(9):1797-1807. doi:10.1109/TMI.2015.2409024.
14. Ferrari G, Rabiolo A, Bignami F, et al. Quantifying Ocular Surface Inflammation and Correlating It With Inflammatory Cell Infiltration In Vivo: A Novel Method. *Invest Ophthalmol Vis Sci*. 2015;56(12):7067-7075. doi:10.1167/iovs.15-17105.
15. Rabiolo A, Bignami F, Rama P, Ferrari G. VesselJ: A New Tool for Semiautomatic Measurement of Corneal Neovascularization. *Invest Ophthalmol Vis Sci*. 2015;56(13):8199-8206. doi:10.1167/iovs.15-17098.
16. Efron N, Morgan PB, Farmer C, Furuborg J, Struk R, Carney LG. Experience and training as determinants of grading reliability when assessing the severity of contact lens complications. *Ophthalmic Physiol Opt J Br Coll Ophthalmic Opt Optom*. 2003;23(2):119-124.
17. Efron N, Pritchard N, Brandon K, et al. A survey of the use of grading scales for contact lens complications in optometric practice. *Clin Exp Optom*. 2011;94(2):193-199. doi:10.1111/j.1444-0938.2010.00549.x.
18. Romano V, Steger B, Brunner M, Ahmad S, Willoughby CE, Kaye SB. Method for Angiographically Guided Fine-Needle Diathermy in the Treatment of Corneal Neovascularization. *Cornea*. 2016;35(7):1029-1032. doi:10.1097/ICO.0000000000000865.
19. Romano V, Steger B, Zheng Y, Ahmad S, Willoughby CE, Kaye SB. Angiographic and In Vivo Confocal Microscopic Characterization of Human Corneal Blood and Presumed Lymphatic Neovascularization: A Pilot Study. *Cornea*. 2015;34(11):1459-1465. doi:10.1097/ICO.0000000000000609.
20. Steger B, Romano V, Kaye SB. Corneal Indocyanine Green Angiography to Guide Medical and Surgical Management of Corneal Neovascularization. *Cornea*. 2016;35(1):41-45. doi:10.1097/ICO.0000000000000683.
21. Olsen TW, Lim JI, Capone A, Myles RA, Gilman JP. Anaphylactic shock following indocyanine green angiography. *Arch Ophthalmol Chic Ill 1960*. 1996;114(1):97.
22. Bearely S, Rao S, Fekrat S. Anaphylaxis following intravenous fluorescein angiography in a vitreoretinal clinic: report of 4 cases. *Can J Ophthalmol J Can Ophtalmol*. 2009;44(4):444-445. doi:10.3129/i09-068.
23. Delori F, Greenberg JP, Woods RL, et al. Quantitative measurements of autofluorescence with the scanning laser ophthalmoscope. *Invest Ophthalmol Vis Sci*. 2011;52(13):9379-9390. doi:10.1167/iovs.11-8319.

Figure legends

Figure 1. Color photo (A) and angiographic images before (C) and after (D) topical phenylephrine 2.5%. The histogram plot (B) shows the frequency of pixels in the image (vertical axis) with a particular brightness value ranging from 0 to 255 (horizontal axis).

Figure 2. Measurement of ocular surface inflammation. Plot of the linear association before and following phenylephrine using Efron grading (A) and the PDI (B).