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Corresponding Author: Ms. Natasha Spiteri, M.D., FRCOphth

Corresponding Author's Institution:

First Author: Natasha Spiteri, M.D., FRCOphth

Order of Authors: Natasha Spiteri, M.D., FRCOphth; Vito Romano, MD; Yalin Zheng, PhD; Sohraab Yadav, MBChB; Rahul Dwivedi, BSc(Hons), MBChB; Jern Chen, MD; Sajjad Ahmad, PhD, FRCOphth; Colin Willoughby, MD, FRCOphth; Stephen Kaye, MD, FRCOphth

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Natasha Spiteri MD1, FRCOphth, Vito Romano1 MD, Yalin Zheng PhD 2, Sohraab Yadav MBChB 1, Rahul Dwivedi BSc(Hons), MBChB1, Jern Chen1 MD, Sajjad Ahmad PhD, FRCOphth 1,2, Colin Willoughby MD, FRCOphth1, Stephen B Kaye MD, FRCOphth1,2

1. St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
2. Department of Eye and Vision Science, University of Liverpool, UK.

Tel: +44 1517062000

Corresponding Author:
Natasha Spiteri
St Paul's Eye Unit, 8Z link, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
nax024@gmail.com

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Running head: Targeted diathermy for corneal new vessels

Address for reprints:
St Paul's Eye Unit, 8Z link, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK

This article contains one video as additional online-only material.

Abstract

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Subjects: Patients with CoNV unresponsive to topical steroid therapy.

Methods: Visual acuity, color images, and fluorescein angiography (FA) and indocyanine green angiography (ICGA) were measured before and following FND with a minimum of three months follow-up. The number of afferent vessels crossing the limbus, time to fluorescein leakage, area and geometric properties of the CoNV were determined using an in-house automated program written in numerical computing language (MatLab R14; The MathWorks Inc., Natick, MA). The location of the afferent vessel was identified from the angiographic images and marked at the slit lamp using a needle to make a cut to the depth of the vessel. FND was then applied using an electrolysis needle.

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Conclusions: The differentiation of afferent and efferent vessels using corneal angiography enables treatment to be selectively applied to the afferent vessels of which, there are usually 1 to 2 for each CoNV complex.

POINT-BY-POINT RESPONSE FORM

Please list the editor's, reviewer(s)', and editorial office's comments in the left-hand column, spacing them so that you can insert the relevant response in the center column and the respective point(s) in the text (and tables or legends, if appropriate) in the right-hand column. Adding line numbers to the manuscript file and referring to specific line numbers will be useful in determining which parts of the manuscript changed.

Manuscript #: 2014-2033R1
Manuscript title: **Corneal angiography for guiding and evaluating fine needle diathermy treatment of corneal neovascularization**

Suggestion, Question, or Comment from the Editor	Author's Response	Change in the Manuscript
Please clarify the length of follow-up in the abstract, precis, and table(s), e.g. in the precis: "...appears effective with 4 months of follow-up	Minimum 3 months follow-up specified	Lines 39-40, 106
Please review, discuss and cite the paper by Trikha et al. as suggested by Reviewer #2. This paper had a mean follow-up of almost 19 months	This paper which had a range of follow-up of 1 -56months, has been cited and discussed in both introduction and discussion	Lines 78, 80-2, 194-8
Please add details about the length of steroid treatment prior to considering diathermy, and whether steroid treatment was continued beyond the first 4 weeks after treatment in any cases	Details regarding steroid treatment have now been included. Steroids post-treatment limited to 4 weeks in all. Pre-treatment steroid ranged between 6 and 10 weeks	Lines 101-2, 153

Suggestion, Question, or Comment from Reviewer #1	Author's Response	Change in the Manuscript
The authors need to acknowledge clearly that the afferent vs. efferent nature of the neovessels in the cornea can often be identified easily at the slit lamp and does	Whilst we respect the reviewer's comment "the afferent vs. efferent nature of the neovessels in the cornea can often be identified easily	Lines 89 - 90 Although in some cases it may be

not require imaging in all cases.	at the slit lamp", we would also offer evidence to the contrary (References 11-14). We do however, agree that Identifying and distinguishing afferent and efferent vessels may be possible in some cases but this is not the general situation. We have shown that not all the vessels can be seen or easily distinguished on slit lamp biomicroscopy even aided by the patients pulse. We hope the reviewer will accept our comment that 'once the afferent vessels or vessels have been identified on angiography, it then makes their identification on slit lamp biomicroscopy more evident and reliable' and that this is a fair reflection of the literature.	possible to differentiate afferent and efferent vessels on slit lamp biomicroscopy, aided for example by the patient's pulse, this can be difficult. Once the afferent vessels or vessels have been identified on angiography, however, it then makes the identification on slit lamp biomicroscopy more evident and reliable.
The authors make the erroneous assertion in their discussion (in citing reference 17) that the central epithelium of the cornea is entirely devoid of Langerhans cells. This misconception was laid to rest many years ago; the central epithelium of the cornea DOES contain a few LCs most are not functional. Thus, the authors need to modify their assertion and simply state that FND can increase the numbers of these cells.	Thank you. Statement regarding absence of LCs in central cornea deleted	Line 218

Suggestion, Question,	**Author's Response**	**Change in the**

or Comment from Reviewer #2		Manuscript
The technique described in the text lines (137-149) appear to show a bipolar approach to fine needle diathermy whereas the original description by Pillai et al (ref 7) used a unipolar approach. In the methods it is not clear whether the approach is bipolar or unipolar. A unipolar approach uses one electrode (suture needle) allowing a current to pass through the vascular complex (the patient is 'earthed'), allowing tissue to be coagulated. In a bipolar diathermy approach the current passes at the point of the electrode and superficial heating of tissue (vaporization effect) is created. This difference may not be that clinically significant but the authors could consider adding this to the discussion and indicating in the methods which type of diathermy was used.	Thank you. It was unipolar and this has been corrected.	Line 149
The table data include BCVA pre- and post-op but there is no statistical analysis on whether the improvement in BCVA is significant with this sample number.	Thank you. Statistical test result has been added. There was a significant difference between pre and post operative BCVA	Lines 156-7 176-7
The discussion mentions that long term results are poorly understood (lines 218-19), however, Trikha et al BJO 'Long-term outcomes of Fine Needle Diathermy for established corneal neovascularization" have already shown a positive effect in the long term with this method.	Thank you. We have acknowledged and added this to the manuscript. Trikha et al have shown a improvement in the reduction in CoNV using FND.	Lines 78, 80-2, 194-8

Suggestion, Question, or Comment from the Editorial Office	Author's Response	Change in the Manuscript
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***Precis / Highlights**

Fine needle diathermy is an easy and effective treatment for corneal neovascularization, however since its cellular effects are poorly understood, diathermy should be used sparingly. This can be achieved by angiographically-guided targeting of afferent vessels.

Corneal angiography for guiding and evaluating fine needle diathermy treatment of corneal neovascularization

Natasha Spiteri MD[1], FRCOphth, Vito Romano[1] MD, Yalin Zheng PhD[2], Sohraab Yadav MBChB[1], Rahul Dwivedi BSc(Hons), MBChB[1], Jern Chen[1] MD, Sajjad Ahmad PhD, FRCOphth[1,2], Colin Willoughby MD, FRCOphth[1], Stephen B Kaye MD, FRCOphth[1,2]

1. St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
2. Department of Eye and Vision Science, University of Liverpool, UK.

Tel: +44 1517062000

Corresponding Author:
Natasha Spiteri
St Paul's Eye Unit, 8Z link, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
nax024@gmail.com

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Design: Retrospective interventional case series

Subjects: Patients with CoNV unresponsive to topical steroid therapy**.**

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Conclusions: The differentiation of afferent and efferent vessels using corneal angiography enables treatment to be selectively applied to the afferent vessels of which, there are usually 1 to 2 for each CoNV complex.

Introduction

Avascularity of the cornea is important for its homeostasis and function. Corneal neovascularization (CoNV), however, may develop in response to hypoxia or inflammations, as infectious, allergic, toxic and traumatic injuries.[1] The presence of CoNV reflects an imbalance between anti-angiogenic factors, such as pigment-epithelium-derived factor (PEDF), and angiogenic factors, such as fibroblast and vascular endothelial growth factors (VEGF).[2, 3] CoNV is however, part of the wound healing response and may be useful in the acute phase for the transport of, humoral and cellular elements involved in immune response, materials required for repair and regeneration, removal of toxic substances and drugs to the site of inflammation. Chronic up-regulation of the angiogenic response, however, results in the persistence of pathological new vessels, which have increased vascular permeability resulting in corneal oedema, lipid exudation, chronic or recurrent inflammation and scarring.[4] There is also the potential establishment of lymphatics, normally absent from the cornea, which may further disrupt the "immune privilege" status of the cornea.[4]

Various techniques have been employed to treat CoNV, including topical steroids, anti-VEGF, metalloproteinase inhibitors, photodynamic therapy, Argon laser, yellow dye laser, radiation, cryotherapy and conjunctival resection.[4-6] Fine-needle diathermy (FND) has been described by several groups for the treatment of CoNV.[5, 7-9] It involves the application of a coagulating current through a unipolar diathermy unit or thermal cautery, usually delivered through a needle such as a cutting or an electrolysis needle. ~~Although~~ FND has shown promise with the largest retrospective study reporting a series of 56 eyes, showing regression of CoNV in 89.3% of patients following two or less treatments.[9] It is, however not known what ~~long-term~~ effects diathermy has on the cornea at a cellular level, particularly if applied to the multitude of vessels in the CoNV complexes. Reducing the number of vessels that need to be closed may reduce the potential risks associated with FND and improve the efficacy of the procedure. It is questionable whether both the afferent (presumed arteriole) and efferent (presumed venule) systems of CoNV require treatment and selective treatment to the afferent system may be sufficient. It is of note, that Cursiefen et al found that on histology, that arterioles tend to comprise less than 1% of CoNV.[10] One option therefore, would be to only treat the afferent vessel(s) of the CoNV complex.[11] While it is sometimes possible to distinguish afferent from efferent CoNV using slit lamp biomicroscopy~~at the slit lamp~~, it has been shown that the full extent and origin of the CoNV complex is not apparent ~~often difficult to identify~~ on color images.[12-14] We have, however, recently shown that corneal angiography (fluorescein angiography, FA and indocyanine green angiography, ICGA) are particularly useful in identifying

vessels not seen on color images[12-14], especially in the presence of corneal scars or inflammation and facilitate identification and differentiation of afferent and efferent vessels and vessel leakage.[13] In order therefore, to minimise the amount of diathermy applied to the cornea, we describe the use of angiography to identify and specifically treat the afferent vessels using FND.

Methods

Patients who were undergoing FND for CoNV associated with previous corneal disease such as microbial keratitis and that had not responded to topical steroid therapy were included. Topical steroids had been usedgiven for between 66 and 12 weeks duration. Inclusion criteria were CoNV extending more than 3mm into cornea with varying degrees of lipid keratopathy and no active keratitis or corneal ulceration. Patients were followed up for a period of at least four months. Patients were examined with slit lamp biomicroscopy, and color and red free images, and FA and ICGA were obtained pre-treatment and three months post-treatment. IRB approval and informed consent were obtained and the Tenets of the Declaration of Helsinki were adhered to.

Color, FA and ICGA images.

Color images were captured using a slit lamp mounted digital system (Topcon SL-D Digital Slit Lamp, Tokyo, Japan) with 10 to 25 times magnification. An HRA2 Scanning Laser Ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany) with a 20 degree imaging lens set at 34 diopter (D), was used for ICGA and FA as previously described.[12, 13] After injection of 5ml of indocyanine green dye (5mg/ml) (Pulsion Medical Systems, Germany) videography was recorded for 25 seconds. Single frame ICGA images of the whole cornea capturing corneal blood vessel fluorescence were taken every 3-5 seconds for 3 minutes followed by late images at 5 and 10 minutes. An intravenous injection of 3mL of 20% Sodium fluorescein (Martindale Pharmaceuticals, Essex, UK) was then given and the videography repeated. During the acquisition of single frame ICGA and FA images hi-resolution mode with eye tracking automatic real-time (ART) software was used.

Image analysis

The region of interest (ROI) was defined pre-operatively as the area of the cornea containing the CoNV and was used to compare pre and post-operative images. Images of pre- and final post-operative angiograms of grade 3 or 4 were selected for analysis as previously described by two independent observers (SY and RD).[12] The number of afferent vessels crossing the limbus and time to leakage of fluorescein were recorded. The area and geometric properties of the vessels were determined on the selected images using an in-house automated program developed in Matlab R14

(The MathWorks Inc., Natick, MA).[13] In brief, the major steps of the program are as follows. For each image the pixel resolution (mm/pixel) was first defined as the ratio between the diameter of the cornea (mm) and the number of pixels measured manually from the image. A ROI containing all the corneal vessels was then defined by hand. The CoNV in the ROI was detected by applying Gaussian enhancement, selective vessel enhancement and thresholding techniques to the ROI in a sequential order. In the resulting binary image (1 indicating vessel pixels while 0 indicating background pixels) the area of CoNV (mm^2) was computed by multiplying the number of CoNV pixels and the area (mm^2) occupied by a pixel. The centrelines of the vessels were identified by a 'thinning' operation so that the branch points and terminal points can be identified. The branching and terminal points were then used to divide the vascular tree into individual vessel segments. The mean diameter (mm) of each segment can be computed so as to characterise the CoNV complex in the image.

FND technique (Video)

Afferent and efferent vessels were identified on videography (Figure 1) and the former labelled. The annotated image was used as a reference to mark the afferent vessel(s) with an inked needle. Using slit lamp biomicroscopy, under topical anaesthesia, a partial thickness incision was made using a 25 gauge needle on a 1 ml syringe into the posterior stroma over the identified afferent vessel(s) either at the limbus or at the level of marginal corneal arcades.[15] If the afferent vessel was transected, an interruption of flow was evident in the visible vessels carrying red blood cells (Video). Under the operating microscope, an Ellman MH-EL-A2D fine wire electrolysis needle was then applied into the incision. Energy was delivered using a unipolar approach by a Surgitron® Dual RF™ machine (Ellman International Inc, Oceanside, NY, USA) using the lowest setting (1 joule per second) until a visible blanching of the cornea around the afferent vessel was seen and segmentation of the blood flow in the larger efferent vessels if not already present. Patients received guttae prednisolone 1% qds and chloramphenicol 0.5% qds post-operatively for 4 weeks only.

Statistical analysis

A Mann Whitney test was used to compare pre- and post-operative area of CoNV and a paired samples t-test for change in visual acuity (SPSS Statistics 21).

Results

30 patients (mean age 56 years, range: 23 to 95 years, male:female::1:1) undergoing FND for CoNV were included. Demographic details as well as diagnoses and previous treatments are provided in

Table 1. The causes of the CoNV were previous herpes simplex keratitis (HSK) (13), clinically suspected bacterial keratitis (13), vascularization associated with intrastromal corneal ring segments (2), ectodermal dysplasia (1) and corneal choristoma (1). The duration of CoNV at time of FND was over six months in the 23 patients (77%), between three and six months in 3 patients (10%), and less than three months in 5 patients (13%). The number of FND treatments performed was single treatment for 20 patients (66.6%), two for 8 patients (26.6%) and three for 2 patients (6.6%) (Table 1).

The number of afferent vessels per CoNV complex ranged from 1 to 2 with a mean diameter of 40μm (SD: 10μm) (Table 2). The mean time to leakage from the apical vessels on fluorescein angiography was 44.22 seconds (min: 27.43s, max: 63.59s). The area of CoNV reduced from 2.42mm^2 (SD: 1.59) to 0.62mm^2 (SD: 0.73) post-operatively ($p<0.01$), a mean reduction of 1.80mm^2 (SD: 1.40). The percentage reduction in area of CoNV for each patient is shown in Table 2. Although the area of CoNV reduced post-treatment (Figure 2), FND did not lead to a complete closure of the entire CoNV complexes in all patients with some residual vessels in the periphery of the cornea.

There was ~~statistically~~ significant improvement in BCVA from 0.59 (SD: 0.71) to 0.40 (SD: 0.42) post-treatment ~~, t(29) = 2.32, two tail~~ (p =0.027) with~~, and~~ a mean change of 0.17 LogMAR (Table 1).

Adverse events and reactions included peripheral corneal thinning (Patient 6), intrastromal haemorrhage, which cleared spontaneously (Patient 11), recurrence of herpes simplex keratitis (Patients 9 and 11) and a change in refractive error (Patient 1).

Discussion

The presence of CoNV threatens the functional integrity of the cornea with the risk of lipid exudation, loss of transparency, loss of visual acuity, increased inflammatory response and an increased risk of graft failure. To date, there is no universally accepted treatment strategy to successfully treat CoNV although topical steroids are perhaps the most commonly employed treatment.[4] Topical and injected anti-VEGF has been utilised in the treatment of CoNV, however, it has been suggested that once vessels mature and acquire a pericyte-covered wall, they are no longer dependent on VEGF for growth and are thus unresponsive to anti-VEGF.[16,17] Argon laser has been shown to have limited efficacy and with a risk of complications such as iris atrophy.[4, 7] The speed of red blood cell movement in afferent vessels and their usual deeper location in the cornea makes them relatively insensitive to Argon laser.

FND has shown promising ~~short-term~~ results for treatment of CoNV. Trikha et al reported FND to be ~~a~~ safe and effective in the medium to long term, with mean follow-up of 18.9 months (range 1 - 56

months). They reported only one complication, that of corneal and subconjunctival haemorrhage ~~in their large series of patients,~~ and also demonstrated a significant improvement in LogMAR VA from 0.82 pre-treatment to 0.72 post-treatment.[9] FND, ~~has~~ however, has been applied without distinction to afferent or efferent vessels, usually to the larger vessels identified on color images.[18] The larger vessels and more numerous vessels that are identified on color images, slit lamp biomicroscopy are efferent vessels so that FND applied to these vessels may not be as effective as if applied to the afferent vessels, which are narrower and slightly less tortuous. In particular, the differentiation of afferent and efferent vessels aided by corneal angiography enables such treatment to be applied to the afferent vessels of which, there are usually only 1 to 2 for each CoNV complex. Although in some cases it may be possible to differentiate afferent and efferent vessels on slit lamp biomicroscopy, aided for example by the patient's pulse, this can be difficult. Once the afferent vessel or vessels have been identified on angiography, however, it then makes the identification on slit lamp biomicroscopy more apparent and reliable.~~In particular, the differentiation of afferent and efferent vessels using corneal angiography enables such treatment to be applied to the afferent vessels of which there are usually only 1 to 2 for each CoNV complex.~~

FND is a relatively easy procedure to perform with only a few minor complications reported, including transient whitening of the cornea and intrastromal haemorrhage, which usually resolves without sequelae,[5, 7] recurrent HSK and localised thinning and ectasia.[7] The cellular changes that occur following FND, however, are less well understood. In rats, a significant increase in the number of B7+ MHC II^{+} Langerhans cells in the limbal surface epithelium occurs within hours of cautery, and later throughout the entire corneal epithelium, suggesting an inflammatory reaction~~as well as of these cells the central cornea, where they are normally absent~~.[19] ~~Langerhans cells are the professional antigen-presenting cells of the corneal epithelium, and their absence from the central cornea plays an important role in maintaining the immune privilege of the cornea.~~ In addition, Feldman et al, ~~in an experimental study on rabbits~~ showed that radial thermokeratoplasty caused significant damage to the corneal endothelium beneath and surrounding the coagulation site.[20] Furthermore corneal heating reduces corneal curvature, with therapeutic potential for correction of myopia and as a treatment for corneal ectasia.[21,22] A~~However a~~t a molecular level, however, it results in shrinkage of corneal stromal collagen.[22] It is also unclear what the long-term effects of diathermy to the cornea may be. The process of corneal diathermy itself may be a stimulus for further CoNV. It would therefore seem reasonable, to try and minimise the application of FND to the cornea. ICGA or FA offers the ability to identify the afferent or feeder vessels for FND, and application of anti-angiogenic factors such as anti-VEGF may be better applied to less mature or

Comment [NS1]: Have changed back to American spelling for Ophthalmology

immature vessels,[17] which can be identified using time to leakage on FA.[12, 13] In particular topically applied pazopanib, a selective multi-targeted receptor tyrosine kinase inhibitor of VEGF receptor and platelet derived growth factor (PDGF) receptor has shown promise in the treatment of CoNV.[15]

Our results suggest that angiographically-guided FND, targeting the afferent vessels may be effective in reducing the area of CoNV. In our series, although around a third of patients required retreatment, this may reflect identification of vessels on angiography that are not apparent on colour images.[11-14] Similar to Trikha et al[9], adverse events were uncommon and mostly transient, and there was an ~~small~~ improvement in visual acuity. ~~In those patients undergoing corneal transplantation, the reduction in CoNV may be of benefit.~~ Since the long-term effects of FND are poorly understood, it is the authors' opinion that diathermy should be used sparingly. The differentiation of afferent and efferent vessels aided by~~using~~ corneal angiography, enables such treatment to be applied to the afferent vessels, of which there are usually only 1 to 2 for each CoNV complex.

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References

1. Chang JH, Gabison EE, Kato T, Azar DT. Corneal neovascularization. Curr Opin Ophthalmol 2001;12:242-9.

2. Manzano RP, Peyman GA, Khan P, et al. Inhibition of experimental corneal neovascularisation by bevacizumab (Avastin). Br J Ophthalmol 2007;91:804-7.

3. Clements JL, Dana R. Inflammatory corneal neovascularization: etiopathogenesis. Semin Ophthalmol 2011;26:235-45.

4. Gupta D, Illingworth C. Treatments for corneal neovascularization: a review. Cornea 2011;30:927-38.

5. Thatte S. Fine needle diathermy - a choice for managing corneal vascularization. Nepal J Ophthalmol 2011;3:23-6.

6. Bucher F, Parthasarathy A, Bergua A, et al. Topical Ranibizumab inhibits inflammatory corneal hem- and lymphangiogenesis. Acta Ophthalmol 2014;92:143-8.

7. Pillai CT, Dua HS, Hossain P. Fine needle diathermy occlusion of corneal vessels. Invest Ophthalmol Vis Sci 2000;41:2148-53.

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8. Koenig Y, Bock F, Kruse FE, et al. Angioregressive pretreatment of mature corneal blood vessels before keratoplasty: fine-needle vessel coagulation combined with anti-VEGFs. Cornea 2012;31:887-92.

9. Trikha S, Parikh S, Osmond C, et al. Long-term outcomes of Fine Needle Diathermy for established corneal neovascularisation. Br J Ophthalmol 2014;98:454-8.

10. Cursiefen C, Hofmann-Rummelt C, Kuchle M, Schlotzer-Schrehardt U. Pericyte recruitment in human corneal angiogenesis: an ultrastructural study with clinicopathological correlation. Br J Ophthalmol 2003;87:101-6.

11. Romano V, Spiteri N, Kaye SB. Angiographic guided treatment of corneal neovascularization. JAMA Ophthamol. In press.

12. Kirwan RP, Zheng Y, Tey A, et al. Quantifying changes in corneal neovascularization using fluorescein and indocyanine green angiography. Am J Ophthalmol 2012;154:850-8.

13. Anijeet DR, Zheng Y, Tey A, et al. Imaging and evaluation of corneal vascularization using fluorescein and indocyanine green angiography. Invest Ophthalmol Vis Sci 2012;53:650-8.

14. Zheng Y, Kaye AE, Boker A, Stewart RK, Tey A, Ahmad S, Willoughby CE, Bron AJ, Kaye SB. Marginal corneal vascular arcades. Invest Ophthalmol Vis Sci 2013;54:7470-7.

15. Amparo F, Sadrai Z, Jin Y, et al. Safety and efficacy of the multitargeted receptor kinase inhibitor pazopanib in the treatment of corneal neovascularization. Invest Ophthalmol Vis Sci 2013;54:537-44.

16. Chaoran Z, Zhirong L, Gezhi X. Combination of vascular endothelial growth factor receptor/platelet-derived growth factor receptor inhibition markedly improves the antiangiogenic efficacy for advanced stage mouse corneal neovascularization. Graefes Arch Clin Exp Ophthalmol 2011;249:1493-501.

17. Asena L, Akova YA, Cetinkaya A, Kucukerdonmez C. The effect of topical bevacizumab as an adjunctive therapy for corneal neovascularization. Acta Ophthalmol 2013;91:246-8.

18. Faraj LA, Elalfy MS, Said DG, Dua HS. Fine needle diathermy occlusion of corneal vessels. Br J Ophthalmol 2014;98:1287-90.

19. Chen W, Lin H, Dong N, et al. Cauterization of central cornea induces recruitment of major histocompatibility complex class II+ Langerhans cells from limbal basal epithelium. Cornea 2010;29:73-9.

20. Feldman ST, Ellis W, Frucht-Pery J, et al. Experimental radial thermokeratoplasty in rabbits. Arch Ophthalmol 1990;108:997-1000.

21. Ehrlich JS, Manche EE. Regression of effect over long-term follow-up of conductive keratoplasty to correct mild to moderate hyperopia. J Cataract Refract Surg 2009;35:1591-6.

22. Barsam A, Patmore A, Muller D, Marshall J. Keratorefractive effect of microwave keratoplasty on human corneas. J Cataract Refract Surg 2010;36:472-6.

Figure Captions

Figure 1. Identification of afferent and efferent vessels.
The 3 afferent vessels (two superiorly and one inferiorly are identified as the first to fluoresce in the angiogram (arrows in A). As the angiogram is continued (B and C) the efferent vessels fill and become prominent (arrows). Note the larger efferent vessels partially overlying and obscuring the narrower afferent vessels (D).

Figure 2. Treatment of CoNV effect following FND.
Pre (A) and post-treatment closure of CoNV (B) following occlusion of the afferent vessel with FND. Note the narrower and less tortuous afferent vessel compared to the more obvious efferent vessels draining the complex.

Video
Transection of afferent vessel using a 25 gauge needle. Note the stagnation of flow in the larger efferent vessels.

Corneal angiography for guiding and evaluating fine needle diathermy treatment of corneal neovascularization

Natasha Spiteri MD[1], FRCOphth, Vito Romano[1] MD, Yalin Zheng PhD[2], Sohraab Yadav MBChB[1], Rahul Dwivedi BSc(Hons), MBChB[1], Jern Chen[1] MD, Sajjad Ahmad PhD, FRCOphth[1,2], Colin Willoughby MD, FRCOphth[1], Stephen B Kaye MD, FRCOphth[1,2]

1. St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
2. Department of Eye and Vision Science, University of Liverpool, UK.

Tel: +44 1517062000

Corresponding Author:
Natasha Spiteri
St Paul's Eye Unit, 8Z link, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK
nax024@gmail.com

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Address for reprints:
St Paul's Eye Unit, 8Z link, Royal Liverpool University Hospital, Liverpool, L7 8XP, UK

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1. St Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, UK
2. Department of Eye and Vision Science, University of Liverpool, UK.

Abstract

Purpose: To investigate the outcome of selective occlusion of the afferent vessel of corneal neovascular complexes (CoNV), using angiographically guided fine needle diathermy (FND).

Design: Retrospective interventional case series

Subjects: Patients with CoNV unresponsive to topical steroid therapy**.**

Methods: Visual acuity, color images, and fluorescein angiography (FA) and indocyanine green angiography (ICGA) were measured before and following FND with a minimum of three months follow-up. The number of afferent vessels crossing the limbus, time to fluorescein leakage, area and geometric properties of the CoNV were determined using an in-house automated program written in numerical computing language (MatLab R14; The MathWorks Inc., Natick, MA). The location of the afferent vessel was identified from the angiographic images and marked at the slit lamp using a needle to make a cut to the depth of the vessel. FND was then applied using an electrolysis needle.

Main Outcome Measure: Area of CoNV

Results: 30 patients underwent FND for CoNV that had not responded to treatment with topical steroids. The CoNV was associated with previous microbial keratitis (26), intrastromal corneal ring segments (2), ectodermal dysplasia (1) and corneal choristoma (1). Duration of CoNV was over six months in the 23 patients (77%), between three and six months in 3 patients (10%), and less than three months in 5 patients (13%). The number of afferent vessels per CoNV complex ranged from 1 to 3 with a mean diameter of 40μm (SD 10μm) and mean time to leakage from apical vessels was 44.22 seconds (min: 27.43s max: 63.59s). The number of episodes of FND treatments that were required was one for 20 patients (66.6%), two for 8 patients (26.6%) and three for 2 patients (6.6%). Following FND, the area of CoNV reduced by 1.80mm^2 (SD: 1.40); from 2.42mm^2 (SD: 1.59) to 0.62mm^2 (SD: 0.73) up to 12 weeks post-operatively ($p<0.01$).

Conclusions: The differentiation of afferent and efferent vessels using corneal angiography enables treatment to be selectively applied to the afferent vessels of which, there are usually 1 to 2 for each CoNV complex.

Introduction

Avascularity of the cornea is important for its homeostasis and function. Corneal neovascularization (CoNV), however, may develop in response to hypoxia or inflammations, as infectious, allergic, toxic and traumatic injuries.[1] The presence of CoNV reflects an imbalance between anti-angiogenic factors, such as pigment-epithelium-derived factor (PEDF), and angiogenic factors, such as fibroblast and vascular endothelial growth factors (VEGF).[2, 3] CoNV is however, part of the wound healing response and may be useful in the acute phase for the transport of, humoral and cellular elements involved in immune response, materials required for repair and regeneration, removal of toxic substances and drugs to the site of inflammation. Chronic up-regulation of the angiogenic response, however, results in the persistence of pathological new vessels, which have increased vascular permeability resulting in corneal oedema, lipid exudation, chronic or recurrent inflammation and scarring.[4] There is also the potential establishment of lymphatics, normally absent from the cornea, which may further disrupt the "immune privilege" status of the cornea.[4]

Various techniques have been employed to treat CoNV, including topical steroids, anti-VEGF, metalloproteinase inhibitors, photodynamic therapy, Argon laser, yellow dye laser, radiation, cryotherapy and conjunctival resection.[4-6] Fine-needle diathermy (FND) has been described by several groups for the treatment of CoNV.[5, 7-9] It involves the application of a coagulating current through a unipolar diathermy unit or thermal cautery, usually delivered through a needle such as a cutting or an electrolysis needle. FND has shown promise with the largest retrospective study reporting a series of 56 eyes, showing regression of CoNV in 89.3% of patients following two or less treatments.[9] It is, however not known what effects diathermy has on the cornea at a cellular level, particularly if applied to the multitude of vessels in the CoNV complexes. Reducing the number of vessels that need to be closed may reduce the potential risks associated with FND and improve the efficacy of the procedure. It is questionable whether both the afferent (presumed arteriole) and efferent (presumed venule) systems of CoNV require treatment and selective treatment to the afferent system may be sufficient. It is of note, that Cursiefen et al found that on histology, that arterioles tend to comprise less than 1% of CoNV.[10] One option therefore, would be to only treat the afferent vessel(s) of the CoNV complex.[11] While it is sometimes possible to distinguish afferent from efferent CoNV using slit lamp biomicroscopy, it has been shown that the full extent and origin of the CoNV complex is not apparent on color images.[12-14] We have, however, recently shown that corneal angiography (fluorescein angiography, FA and indocyanine green angiography, ICGA) are particularly useful in identifying vessels not seen on color images[12-14], especially in the presence of corneal scars

or inflammation and facilitate identification and differentiation of afferent and efferent vessels and vessel leakage.[13] In order therefore, to minimise the amount of diathermy applied to the cornea, we describe the use of angiography to identify and specifically treat the afferent vessels using FND.

Methods

Patients who were undergoing FND for CoNV associated with previous corneal disease such as microbial keratitis and that had not responded to topical steroid therapy were included. Topical steroids had been used for between 6 and 12 weeks duration. Inclusion criteria were CoNV extending more than 3mm into cornea with varying degrees of lipid keratopathy and no active keratitis or corneal ulceration. Patients were followed up for a period of at least four months. Patients were examined with slit lamp biomicroscopy, and color and red free images, and FA and ICGA were obtained pre-treatment and three months post-treatment. IRB approval and informed consent were obtained and the Tenets of the Declaration of Helsinki were adhered to.

Color, FA and ICGA images.

Color images were captured using a slit lamp mounted digital system (Topcon SL-D Digital Slit Lamp, Tokyo, Japan) with 10 to 25 times magnification. An HRA2 Scanning Laser Ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany) with a 20 degree imaging lens set at 34 diopter (D), was used for ICGA and FA as previously described.[12, 13] After injection of 5ml of indocyanine green dye (5mg/ml) (Pulsion Medical Systems, Germany) videography was recorded for 25 seconds. Single frame ICGA images of the whole cornea capturing corneal blood vessel fluorescence were taken every 3-5 seconds for 3 minutes followed by late images at 5 and 10 minutes. An intravenous injection of 3mL of 20% Sodium fluorescein (Martindale Pharmaceuticals, Essex, UK) was then given and the videography repeated. During the acquisition of single frame ICGA and FA images hi-resolution mode with eye tracking automatic real-time (ART) software was used.

Image analysis

The region of interest (ROI) was defined pre-operatively as the area of the cornea containing the CoNV and was used to compare pre and post-operative images. Images of pre- and final post-operative angiograms of grade 3 or 4 were selected for analysis as previously described by two independent observers (SY and RD).[12] The number of afferent vessels crossing the limbus and time to leakage of fluorescein were recorded. The area and geometric properties of the vessels were determined on the selected images using an in-house automated program developed in Matlab R14 (The MathWorks Inc., Natick, MA).[13] In brief, the major steps of the program are as follows. For

each image the pixel resolution (mm/pixel) was first defined as the ratio between the diameter of the cornea (mm) and the number of pixels measured manually from the image. A ROI containing all the corneal vessels was then defined by hand. The CoNV in the ROI was detected by applying Gaussian enhancement, selective vessel enhancement and thresholding techniques to the ROI in a sequential order. In the resulting binary image (1 indicating vessel pixels while 0 indicating background pixels) the area of CoNV (mm^2) was computed by multiplying the number of CoNV pixels and the area (mm^2) occupied by a pixel. The centrelines of the vessels were identified by a 'thinning' operation so that the branch points and terminal points can be identified. The branching and terminal points were then used to divide the vascular tree into individual vessel segments. The mean diameter (mm) of each segment can be computed so as to characterise the CoNV complex in the image.

FND technique (Video)

Afferent and efferent vessels were identified on videography (Figure 1) and the former labelled. The annotated image was used as a reference to mark the afferent vessel(s) with an inked needle. Using slit lamp biomicroscopy, under topical anaesthesia, a partial thickness incision was made using a 25 gauge needle on a 1 ml syringe into the posterior stroma over the identified afferent vessel(s) either at the limbus or at the level of marginal corneal arcades.[15] If the afferent vessel was transected, an interruption of flow was evident in the visible vessels carrying red blood cells (Video). Under the operating microscope, an Ellman MH-EL-A2D fine wire electrolysis needle was then applied into the incision. Energy was delivered using a unipolar approach by a Surgitron® Dual RF™ machine (Ellman International Inc, Oceanside, NY, USA) using the lowest setting (1 joule per second) until a visible blanching of the cornea around the afferent vessel was seen and segmentation of the blood flow in the larger efferent vessels if not already present. Patients received guttae prednisolone 1% qds and chloramphenicol 0.5% qds post-operatively for 4 weeks only.

Statistical analysis

A Mann Whitney test was used to compare pre- and post-operative area of CoNV and a paired samples t-test for change in visual acuity (SPSS Statistics 21).

Results

30 patients (mean age 56 years, range: 23 to 95 years, male:female::1:1) undergoing FND for CoNV were included. Demographic details as well as diagnoses and previous treatments are provided in Table 1. The causes of the CoNV were previous herpes simplex keratitis (HSK) (13), clinically

suspected bacterial keratitis (13), vascularization associated with intrastromal corneal ring segments (2), ectodermal dysplasia (1) and corneal choristoma (1). The duration of CoNV at time of FND was over six months in the 23 patients (77%), between three and six months in 3 patients (10%), and less than three months in 5 patients (13%). The number of FND treatments performed was single treatment for 20 patients (66.6%), two for 8 patients (26.6%) and three for 2 patients (6.6%) (Table 1).

The number of afferent vessels per CoNV complex ranged from 1 to 2 with a mean diameter of 40μm (SD: 10μm) (Table 2). The mean time to leakage from the apical vessels on fluorescein angiography was 44.22 seconds (min: 27.43s, max: 63.59s). The area of CoNV reduced from 2.42mm^2 (SD: 1.59) to 0.62mm^2 (SD: 0.73) post-operatively ($p<0.01$), a mean reduction of 1.80mm^2 (SD: 1.40). The percentage reduction in area of CoNV for each patient is shown in Table 2. Although the area of CoNV reduced post-treatment (Figure 2), FND did not lead to a complete closure of the entire CoNV complexes in all patients with some residual vessels in the periphery of the cornea. There was significant improvement in BCVA from 0.59 (SD: 0.71) to 0.40 (SD: 0.42) post-treatment ($p=0.027$) with a mean change of 0.17 LogMAR (Table 1). Adverse events and reactions included peripheral corneal thinning (Patient 6), intrastromal haemorrhage, which cleared spontaneously (Patient 11), recurrence of herpes simplex keratitis (Patients 9 and 11) and a change in refractive error (Patient 1).

Discussion

The presence of CoNV threatens the functional integrity of the cornea with the risk of lipid exudation, loss of transparency, loss of visual acuity, increased inflammatory response and an increased risk of graft failure. To date, there is no universally accepted treatment strategy to successfully treat CoNV although topical steroids are perhaps the most commonly employed treatment.[4] Topical and injected anti-VEGF has been utilised in the treatment of CoNV, however, it has been suggested that once vessels mature and acquire a pericyte-covered wall, they are no longer dependent on VEGF for growth and are thus unresponsive to anti-VEGF.[16,17] Argon laser has been shown to have limited efficacy and with a risk of complications such as iris atrophy.[4, 7] The speed of red blood cell movement in afferent vessels and their usual deeper location in the cornea makes them relatively insensitive to Argon laser.

FND has shown promising results for treatment of CoNV. Trikha et al reported FND to be safe and effective in the medium to long term, with mean follow-up of 18.9 months (range 1 - 56 months). They reported only one complication, that of corneal and subconjunctival hemorrhage and also

196 demonstrated a significant improvement in LogMAR VA from 0.82 pre-treatment to 0.72 post-
197 treatment.[9] FND, however, has been applied without distinction to afferent or efferent vessels,
198 usually to the larger vessels identified on color images.[18] The larger vessels and more numerous
199 vessels that are identified on color images, slit lamp biomicroscopy are efferent vessels so that FND
200 applied to these vessels may not be as effective as if applied to the afferent vessels, which are
201 narrower and slightly less tortuous. In particular, the differentiation of afferent and efferent vessels
202 aided by corneal angiography enables such treatment to be applied to the afferent vessels of which,
203 there are usually only 1 to 2 for each CoNV complex. Although in some cases it may be possible to
204 differentiate afferent and efferent vessels on slit lamp biomicroscopy, aided for example by the
205 patient's pulse, this can be difficult. Once the afferent vessel or vessels have been identified on
206 angiography, however, it then makes the identification on slit lamp biomicroscopy more apparent
207 and reliable.
208
209 FND is a relatively easy procedure to perform with only a few minor complications reported,
210 including transient whitening of the cornea and intrastromal haemorrhage, which usually resolves
211 without sequelae,[5, 7] recurrent HSK and localised thinning and ectasia.[7] The cellular changes that
212 occur following FND, however, are less well understood. In rats, a significant increase in the number
213 of B7+ MHC II^+ Langerhans cells in the limbal surface epithelium occurs within hours of cautery, and
214 later throughout the entire corneal epithelium, suggesting an inflammatory reaction.[19] In addition,
215 Feldman et al, showed that radial thermokeratoplasty caused significant damage to the corneal
216 endothelium beneath and surrounding the coagulation site.[20] Furthermore corneal heating reduces
217 corneal curvature, with therapeutic potential for correction of myopia and as a treatment for
218 corneal ectasia.[21,22] At a molecular level, however, it results in shrinkage of corneal stromal
219 collagen.[22] It is also unclear what the long-term effects of diathermy to the cornea may be. The
220 process of corneal diathermy itself may be a stimulus for further CoNV. It would therefore seem
221 reasonable, to try and minimise the application of FND to the cornea. ICGA or FA offers the ability to
222 identify the afferent or feeder vessels for FND, and application of anti-angiogenic factors such as
223 anti-VEGF may be better applied to less mature or immature vessels,[17] which can be identified using
224 time to leakage on FA.[12, 13] In particular topically applied pazopanib, a selective multi-targeted
225 receptor tyrosine kinase inhibitor of VEGF receptor and platelet derived growth factor (PDGF)
226 receptor has shown promise in the treatment of CoNV.[15]
227
228 Our results suggest that angiographically-guided FND, targeting the afferent vessels may be effective
229 in reducing the area of CoNV. In our series, although around a third of patients required

retreatment, this may reflect identification of vessels on angiography that are not apparent on colour images.[11-14] Similar to Trikha et al[9], adverse events were uncommon and mostly transient, and there was an improvement in visual acuity. Since the long-term effects of FND are poorly understood, it is the authors' opinion that diathermy should be used sparingly. The differentiation of afferent and efferent vessels aided by corneal angiography, enables such treatment to be applied to the afferent vessels, of which there are usually only 1 to 2 for each CoNV complex.

References

1. Chang JH, Gabison EE, Kato T, Azar DT. Corneal neovascularization. Curr Opin Ophthalmol 2001;12:242-9.

2. Manzano RP, Peyman GA, Khan P, et al. Inhibition of experimental corneal neovascularisation by bevacizumab (Avastin). Br J Ophthalmol 2007;91:804-7.

3. Clements JL, Dana R. Inflammatory corneal neovascularization: etiopathogenesis. Semin Ophthalmol 2011;26:235-45.

4. Gupta D, Illingworth C. Treatments for corneal neovascularization: a review. Cornea 2011;30:927-38.

5. Thatte S. Fine needle diathermy - a choice for managing corneal vascularization. Nepal J Ophthalmol 2011;3:23-6.

6. Bucher F, Parthasarathy A, Bergua A, et al. Topical Ranibizumab inhibits inflammatory corneal hem- and lymphangiogenesis. Acta Ophthalmol 2014;92:143-8.

7. Pillai CT, Dua HS, Hossain P. Fine needle diathermy occlusion of corneal vessels. Invest Ophthalmol Vis Sci 2000;41:2148-53.

8. Koenig Y, Bock F, Kruse FE, et al. Angioregressive pretreatment of mature corneal blood vessels before keratoplasty: fine-needle vessel coagulation combined with anti-VEGFs. Cornea 2012;31:887-92.

9. Trikha S, Parikh S, Osmond C, et al. Long-term outcomes of Fine Needle Diathermy for established corneal neovascularisation. Br J Ophthalmol 2014;98:454-8.

10. Cursiefen C, Hofmann-Rummelt C, Kuchle M, Schlotzer-Schrehardt U. Pericyte recruitment in human corneal angiogenesis: an ultrastructural study with clinicopathological correlation. Br J Ophthalmol 2003;87:101-6.

11. Romano V, Spiteri N, Kaye SB. Angiographic guided treatment of corneal neovascularization. JAMA Ophthamol. In press.

12. Kirwan RP, Zheng Y, Tey A, et al. Quantifying changes in corneal neovascularization using fluorescein and indocyanine green angiography. Am J Ophthalmol 2012;154:850-8.

13. Anijeet DR, Zheng Y, Tey A, et al. Imaging and evaluation of corneal vascularization using fluorescein and indocyanine green angiography. Invest Ophthalmol Vis Sci 2012;53:650-8.

14. Zheng Y, Kaye AE, Boker A, Stewart RK, Tey A, Ahmad S, Willoughby CE, Bron AJ, Kaye SB. Marginal corneal vascular arcades. Invest Ophthalmol Vis Sci 2013;54:7470-7.

15. Amparo F, Sadrai Z, Jin Y, et al. Safety and efficacy of the multitargeted receptor kinase inhibitor pazopanib in the treatment of corneal neovascularization. Invest Ophthalmol Vis Sci 2013;54:537-44.

16. Chaoran Z, Zhirong L, Gezhi X. Combination of vascular endothelial growth factor receptor/platelet-derived growth factor receptor inhibition markedly improves the antiangiogenic efficacy for advanced stage mouse corneal neovascularization. Graefes Arch Clin Exp Ophthalmol 2011;249:1493-501.

17. Asena L, Akova YA, Cetinkaya A, Kucukerdonmez C. The effect of topical bevacizumab as an adjunctive therapy for corneal neovascularization. Acta Ophthalmol 2013;91:246-8.

18. Faraj LA, Elalfy MS, Said DG, Dua HS. Fine needle diathermy occlusion of corneal vessels. Br J Ophthalmol 2014;98:1287-90.

19. Chen W, Lin H, Dong N, et al. Cauterization of central cornea induces recruitment of major histocompatibility complex class II+ Langerhans cells from limbal basal epithelium. Cornea 2010;29:73-9.

20. Feldman ST, Ellis W, Frucht-Pery J, et al. Experimental radial thermokeratoplasty in rabbits. Arch Ophthalmol 1990;108:997-1000.

21. Ehrlich JS, Manche EE. Regression of effect over long-term follow-up of conductive keratoplasty to correct mild to moderate hyperopia. J Cataract Refract Surg 2009;35:1591-6.

22. Barsam A, Patmore A, Muller D, Marshall J. Keratorefractive effect of microwave keratoplasty on human corneas. J Cataract Refract Surg 2010;36:472-6.

Figure Captions

Figure 1. Identification of afferent and efferent vessels.

The 3 afferent vessels (two superiorly and one inferiorly are identified as the first to fluoresce in the angiogram (arrows in A). As the angiogram is continued (B and C) the efferent vessels fill and become prominent (arrows). Note the larger efferent vessels partially overlying and obscuring the narrower afferent vessels (D).

Figure 2. Treatment of CoNV effect following FND.

Pre (A) and post-treatment closure of CoNV (B) following occlusion of the afferent vessel with FND. Note the narrower and less tortuous afferent vessel compared to the more obvious efferent vessels draining the complex.

Video

Transection of afferent vessel using a 25 gauge needle. Note the stagnation of flow in the larger efferent vessels.

Table 1

Table 1 Patient demographics, diagnosis, treatment and outcomes.

Patient	Age	Diagnosis**	Sex	Duration (months) of CoNV	Treatment prior to FND *	N°. of FND	Adverse reactions	Pre-op BCVA (logMAR)	Post-op BCVA (logMAR)
1	74	HSK	F	>6	Aciclovir	1	None	0.18	0
2	56	BK (*S.aureus)*	F	3-6	Antimicrobial	1	None	0	0.2
3	23	Corneal choristoma	F	>6	Bevacizumab	1	None	0	0
4	54	HSK	M	<3	Aciclovir	1	None	0.6	0.6
5	70	BK (*S.aureus)*	M	>6	Aciclovir, Antimicrobial	1	None	0.18	0.18
6	70	HSK	M	>6	Aciclovir	1	Peripheral thinning	0.6	0.48
7	79	BK (*S. aureus*)	M	>6	Antimicrobial	3	None	0.3	0.3
8	78	BK (*S. aureus*)	F	>6	Antimicrobial	1	None	0.3	0.5
9	70	HSK	F	>6	Aciclovir	2	Recurrence of HSK	0.18	0.18
10	54	BK	M	>6	Antimicrobial	1	None	1	0.78
11	47	HSK	M	>6	Aciclovir	2	Recurrence of HSK , intrastromal hemorrhage	0.18	0.18
12	70	BK (*S.aureus*)	M	>6	Antimicrobial	1	None	0.3	0.3
13	22	BK (*P. aeruginosa*)	M	>6	Antimicrobial	1	None	-0.2	-0.2
14	84	BK (*S.aureus*)	M	3-6	Antimicrobial	1	None	0.18	0.18
15	52	HSK	M	>6	Aciclovir, Antimicrobial	1	Refractive error	0.18	0.18
16	39	BK (*S.aureus)*	F	3-6	Antimicrobial	2	None	0.3	0.18
17	34	KC (Intacs)	M	<3		1	None	3	2
18	34	HSK	M	>6	Aciclovir, Foscarnet	1	None	0.18	0
19	59	BK (S.aureus)	M	>6	Antimicrobial, Argon laser	1	None	0.5	0.6
20	67	HSK	F	>6	Aciclovir	2	None	1.3	0.67
21	47	Ectodermal dysplasia	F	>6	Retinoic acid	3	None	0.48	0.48
22	69	HSK	F	<3	Aciclovir	1	None	0.18	0.18
23	44	BK (*S.aureus)*	M	<3	Antimicrobial	2	None	1	0.3
24	71	BK (*P. aeruginosa*)	M	>6	Antimicrobial	2	None	0.6	1
25	95	HSK	F	>6	Argon laser	2	None	1	0.8
26	25	HSK	F	<3	Aciclovir	1	None	2	0.48
27	35	KC (Intacs)	F	>6		1	None	0.18	0.18
28	62	HSK	F	>6	Aciclovir	1	None	2	0.6
29	24	HSK	F	>6	Aciclovir	2	None	0	0
30	62	BK (*S. aureus)*	F	>6	Antimicrobial	1	None	1	0.78
Mean	56							0.59	0.40
SD	19.72							0.71	0.42

Footnotes: *All the patient had received topical stereoids before and after treatment.; HSK: Herpes Simplex Keratits, KC: keratoconus; BK: bacterial keratitis, LSCD: Limbal stem cell deficiency, ACV: aciclovir, AM: antimicrobials. Diagnosis** type of bacteria if isolated

Table 2

Table 2. Data on pre- and post-operative outcomes

Patient number	Area of CoNV (mm^2)			Percentage change	Afferent vessels		Time to leakage on pre-op FFA (s)
	Pre-op	Post-op	Change		Diameter (mm)	Number on pre-op angiogram	
1	0.89	0	-0.89	100	0.04	1	27.43
2	2.89	0	-2.89	100	0.04	2.5	28.28
3	0.5	0	-0.5	100	0.06	1	38.26
4	3.13	0	-3.13	100	0.03	1	30.83
5	0.25	0	-0.25	100	0.04	3	63.59
6	0.98	0	-0.98	100	0.07	2	48.9
7	2.42	0	-2.42	100	0.05	2	41.7
8	0.96	0	-0.96	100	0.04	1.5	33.91
9	4.04	0.12	-3.92	97	0.03	1	30
10	5.09	0.44	-4.65	91	0.05	2	41.82
11	3.81	0.36	-3.45	91	0.03	2	32.37
12	5.67	0.61	-5.06	89	0.05	2.5	41
13	2.85	0.41	-2.44	86	0.04	1	27.98
14	2.84	0.44	-2.4	85	0.05	2.5	34.79
15	0.95	0.19	-0.75	79	0.05	1	35.71
16	0.13	0.04	-0.1	77	0.03	1	43.18
17	0.5	0.12	-0.38	76	0.03	2	33.41
18	2.52	0.61	-1.9	75	0.04	2	42.31
19	2.11	0.61	-1.5	71	0.08	3	55.8
20	5.54	1.62	-3.91	71	0.05	2	32.01
21	4.6	1.48	-3.11	68	0.04	3	50.91
22	3.17	1.11	-2.07	65	0.04	1	38.19
23	3.02	1.16	-1.86	62	0.03	1	33.64
24	2.1	1.02	-1.08	51	0.04	2	28.15
25	2.49	1.46	-1.03	41	0.05	1	28.01
26	0.77	0.51	-0.25	32	0.03	1	30.5
27	0.6	0.41	-0.19	32	0.04	1	27.87
28	1.82	1.26	-0.56	31	0.05	3	40.1
29	1.52	1.13	-0.39	26	0.05	3	49.19
30	4.37	3.46	-0.91	21	0.05	2	36.85
Mean	2.42	0.62	-1.80	74	0.04	1.80	37,55
SD	1.59	0.73	1.40		0.01	0.75	9.06

Figure 1

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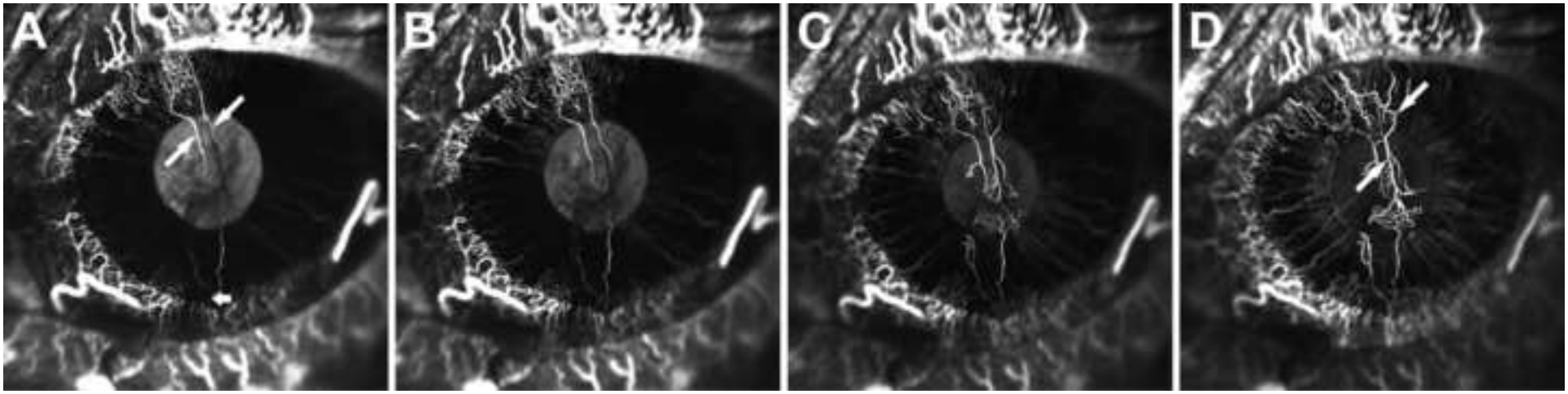

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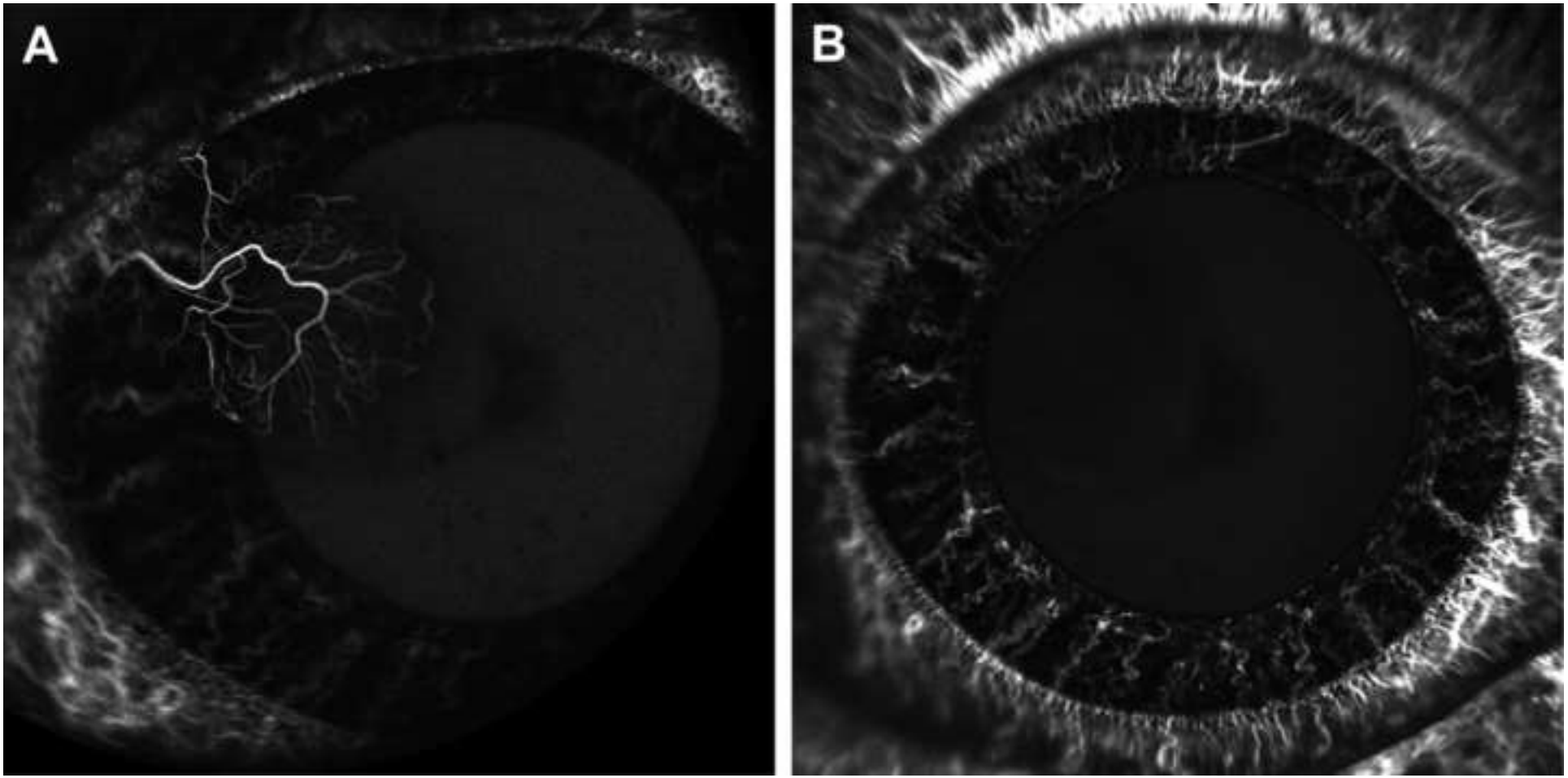

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