Original Article

Delhi Infectious Keratitis Study: Update on Clinico-Microbiological Profile and Outcomes of Infectious Keratitis

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Abstract

Purpose: To study the clinico-microbiological profile and outcomes of infectious keratitis (IK) at a tertiary eye care center in North India.

Methods: This is a retrospective, hospital-based, cross-sectional study. One thousand seven hundred and eighty-six corneal microbiological reports were identified from January 2017 to December 2018, out of which 625 patients of IK fulfilled the inclusion criteria. They underwent microbiological examination which included corneal scrapings, culture, and antibiotic sensitivity. Demographic features, signs and symptoms, risk factors such as associated trauma, previous ocular surgery, and use of corticosteroids were also recorded.

Results: Of the 625 patients, 68.2% were male and 31.8% were female. The age group affected most was the sixth decade; 21.9% (137 cases). Trauma was the most common associated risk factor in 151 cases (24.2%) followed by previous ocular surgery in 111 (17.8%). Out of the 625 corneal scrapings, 393 (62.9%) were culture-positive. Bacterial culture accounted for 60.6% (238/393) and fungal cultures were 143 (36.4%). More than 50% of the bacterial keratitis cases and more than 60% of the fungal cases had a favorable outcome. *Staphylococcus* sp. and *Fusarium* sp. were the most common bacteria and fungus isolated, respectively. Only one-third of the cases required surgical intervention, and the remaining two-thirds were managed medically.

Conclusions: In the current study, cultures were positive in 63% of cases, and the majority of cases had bacterial growth. Surgical intervention was needed in one-third of the cases. Management of corneal infections is incomplete without a good microbiological workup. Ophthalmologists should be encouraged to learn and practice basic staining procedures, and this should start early in the training years.

Keywords: Delhi, Infectious, Keratitis, Microbiology, Outcomes

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Introduction

Corneal blindness constitutes 5% of all cases of blindness globally and is second only to cataract and glaucoma.[1] Of the various causes of corneal blindness, infectious keratitis (IK) is a major concern and the cause for long-term visual impairment. Report from South India has termed IK as a "blinding disease of epidemic proportions,"[2] and its incidence ranges from 11/100,000/year in the West[3] to 113 and 799/100,000/year in India[4] and Nepal, respectively.[4] By some estimates, IK blinds at least 1.5 million eyes every year in the world,[5] and it is projected that India alone will have 0.6 million people blind due to IK by 2020.[6]

Although clinical signs help distinguish the various causes of IK, some atypical organisms pose both diagnostic and therapeutic challenges. Thus, the need for meticulous

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microbiological workup and analysis is essential in managing such cases appropriately.

The antimicrobial susceptibility profile is also important in view of reports on the emergence of methicillin or fluoroquinolone resistance in North America and Asia in the past decade.[7-9]

Significant work has been done in South India with regard to IK,[2,6,7,10,11] and some literature is also available from central[12] and eastern[13] India. However, to the best of our knowledge (PubMed search of microbial keratitis, infectious, India, and north), there is no major epidemiological study describing the correlation between microbiological profile, risk factors, antibiotic susceptibility, and clinical outcome from North India in the last decade. This study was taken up as an update on the microbiological profile along with sensitivity pattern and treatment outcome in IK at a tertiary eye care center in North India.

Methods

Study design, patients, and approval

This retrospective audit included records of all patients of IK presenting to the cornea clinic of a tertiary eye hospital in Delhi, North India, over a period of 2 years (January 2017–December 2018). IK was defined as patients having corneal ulceration with loss of the corneal epithelium and underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.[2] As per institute protocol, all patients with IK underwent microbiological investigation for the identification of causative organisms. Patients who had received treatment before presenting to us were also included. Keratitis documented as sterile neuropathic, autoimmune, or of viral etiology without secondary infection were excluded, as were ulcers presenting in the setting of concurrent endophthalmitis. Patients with nil microbiological workup and follow-up <1 month were also excluded. Institutional review board approval was obtained, and the study adhered to the tenets of the Declaration of Helsinki.

Clinical and microbiological evaluation

For every IK patient, a standardized form was filled with ocular history, corneal findings with color coding, and other associated ocular conditions. Other relevant findings were also noted which included sociodemographic features, predisposing factors, history of corneal trauma, contact lens wear, use of corticosteroids, associated ocular conditions, and other systemic diseases.

Corneal scrapings from both the leading edge and the base of each ulcer were collected under aseptic conditions by an ophthalmologist under the magnification of a slit-lamp beam after instillation of 0.5% proparacaine, using a no. 15 Bard Parker blade. Glass slides were used for 10% potassium hydroxide (KOH) wet mount, Gram-stain, and Giemsa-stain procedures.[14,15] Ziehl–Neelsen (ZN) 1% and 20% staining was done when required. The material was also inoculated on chocolate agar, sheep's blood agar, and Sabouraud's dextrose agar (SDA) using C-shaped streaks.[14,15] All procedures were performed under standard institutional protocols, which have already been described in detail in other studies.[2,14,15]

Interpretation of bacterial and fungal cultures

Bacterial culture plates were incubated at 37°C for 7 days. After overnight incubation, bacterial culture was confirmed by growth on blood agar and chocolate agar followed by standard biochemical tests according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] Bacterial culture plates were observed for growth at 24 h, 48 h, and till the 7th day. The growth on culture media was considered significant if the following criteria were met.

1. If the same organism was observed on more than one solid media
2. If there was confluent growth at the site of inoculation on one solid media
3. If growth of one media was consistent with direct microscopic findings after Gram's Stain and 1% and 20% ZN staining
4. Growth on one solid and one liquid media.

Inoculated SDA slants were incubated at 30°C for up to 14 days and inspected daily for growth. It was declared as fungal negative thereafter. Fungal growth was grossly identified by its colony morphology and pigment production.

Diagnosis of fungus was made when any of the following criteria was met:

1. Growth on two slants or
2. Growth on one medium with the presence of hyphae in 10% KOH preparations
3. Similar growth on more than one media.

In vitro susceptibility testing was performed by Kirby–Bauer disc diffusion method (Astra Zeneca Pvt. Ltd., India). The interpretation was done using CLSI's serum standards.[16] The antibacterial agents used were consistently tested for their efficacy against standard American Type Culture Collection (ATCC)[17] bacteria (*Staphylococcus aureus* ATCC, *Streptococcus pneumoniae* ATCC, *Haemophilus* influenzae ATCC, *Pseudomonas aeruginosa* ATCC, and *Escherichia coli* ATCC) as a general quality control laboratory procedure. The findings of the microbiological investigations and sensitivity pattern were noted in detail.

Clinical course and management

Treatment given to the patient was noted, which was initiated based on smears, without waiting for the results of culture and sensitivity. Initial empirical therapy for bacterial keratitis involves frequent instillation of broad-spectrum antibiotic drops. The combination mode of therapy was preferred, wherein a cephalosporin was combined with an aminoglycoside. The rationale was that the cephalosporin covers the Gram-positive cocci and some Gram-negative rods, and the aminoglycoside, the Gram-negative ones. This treatment was also used for all the smear-negative cases. Generally, 5% cefazolin or ceftazidime combined with amikacin was used. However, monotherapy using only one fluoroquinolone six times a day was reserved for keratitis, which was not severe or did not involve the visual axis.

Whatever the chosen strategy, monotherapy or combination therapy, drugs were started intensively. Initially, the loading dose was preferred followed by hourly administration of topical antibiotic for the first 48 h.

Natamycin 5% ophthalmic suspension was the initial drug of choice for most cases of fungal keratitis. As in bacterial infections, dosing was started on an hourly basis. Corneal epithelial debridement, every 24–48 h, was performed in cases of fungal keratitis. It was done to debulk the cornea of necrotic debris and also to enhance the penetration of topical antifungals. Oral antifungals were indicated in large or deep ulcers or if it involved the sclera.

For acanthameba keratitis, chlorhexidine (0.02%), polyhexanide biguanide (0.02%), propamidine isethionate (0.1%), or hexamidine (0.1%) was used.

Therapeutic penetrating keratoplasty (TPK) was done in cases of progressive ulceration, non-responsive to therapy or large areas of perforation requiring a corneal graft. Application of tissue adhesives with bandage contact lens was done to manage perforations <3 mm in size, impending perforations, and descemetoceles. These two surgical interventions were considered markers for poor outcomes. Tarsorrhaphy was another surgical procedure which was done to enhance the healing of resolving keratitis. Perforation or progression of keratitis to endophthalmitis or panophthalmitis was categorized as complications.

Treatment outcomes were categorized as favorable or poor. The patient was classified to have a favorable outcome when all the following criteria were present: elimination of infection, no associated complication of keratitis, and no surgical intervention required except tarsorrhaphy. Patients were classified to have a poor outcome if the disease progressed with persistence of infection, there were associated complications, or if any surgical intervention (TPK or tissue adhesive) was needed.

Results

Demographic profile and predisposing factors

A total of 625 patients with IK were included in the study. Of the total patients, 68.2% were male and 31.8% were female. The median age of the patients was 50 ± 19 years (range, 1–100 years), and most patients (137, 21.9%) were in the sixth decade of life. All patients had unilateral involvement. The majority of patients were from Delhi and surrounding suburbs (439 patients, 70.2%), and 186 (29.8%) patients were from outside Delhi. The average presenting visual acuity was 2.25 ± 1.34 logMAR (range, 0–5 logMAR). Predisposing risk factors and their microbiological association analyzed in the study are summarized in Table 1. Almost one-fourth of the patients presented with trauma, and different types of trauma and their microbiological association are described in Table 2. Among previous surgeries, keratoplasty alone accounted for 85.9% (95/111). Six cases had previously undergone vitrectomy, three were post-LASIK surgery, three were postcorneal laceration repair, one postmucous membrane graft, one postamniotic membrane graft each, and two were posttrabeculectomy.

Microbiological profile

One hundred and fifty (24%) smears were positive for fungus in the 10% KOH mount and 303 (48.5%) showed Gram-stain positivity. Only 172 (27.5%) were smear negative on examination on the 1st day of scraping. Two cases were positive on smear for acanthameba and two cases were positive for atypical mycobacteria. The sensitivity and specificity of Gram staining was 89% and 78%, respectively, when compared to those of culture results. Similarly, sensitivity and specificity for 10% KOH mount were calculated to be 87% and 94%, respectively. Out of the 625 corneal scrapings, 393 (62.9%) were culture-positive. On further subdivision of culture-positive samples, the bacterial culture accounted for 238 (60.6%), of which 179 (75.2%) were Gram-positive organisms and 59 (24.8%) were Gram-negative organisms. Further subgrouping is shown in Table 3. Patients with more than one bacteria growth on the culture were classified under mixed bacterial growth. A total of 21 patients had mixed bacterial growth, of which *Staphylococcus* and *Streptococcus* accounted for the majority (13/21) of cases. For the purpose of analysis, those mixed bacterial groups, which had both Gram-positive organisms, were taken in the Gram-positive

Table 1: Distribution of predisposing risk factors and microbiological profile associated with infectious keratitis

Predisposing factor	*n*	Percentage risk factor compared to total cases (625 cases)	Bacterial isolate detected	Fungal isolate detected	No organism detected	Mixed bacterial and fungal	*Acanthameba*
Trauma	151	24.2	37	49	61	4	-
Previous ocular surgery	111	17.8	74	12	25	-	-
History of prior steroid use	97	15.5	59	16	22	-	-
Associated diabetes mellitus	52	8.3	23	10	19	-	-
No associated risk factor	214	34.2	45	56	105	-	3
Total	625	100	238	143	232	9	3

Table 2: Type of trauma and microbiological profile associated with infectious keratitis

Nature of trauma	Male	Female	Total	Percentage	Bacterial isolate detected	Fungal isolate detected	No organism detected	Mixed bacterial and fungal isolates detected
Vegetative matter	48	16	64	42.4	15	22	26	1
Cement particle and brick powder	15	3	18	11.9	6	3	8	1
Wooden stick	6	9	15	9.9	4	3	8	-
Foreign body	14	0	14	9.3	2	5	6	1
Insect in eye	13	0	13	8.6	2	7	4	-
Dust particles	8	1	9	5.9	4	2	2	1
Animal tail	4	2	6	3.9	1	3	2	-
Chemical injury	2	2	4	2.6	1	1	2	-
Finger nail	3	1	4	2.6	-	2	2	-
Plastic	0	1	1	0.7	-	1	-	-
Pencil tip	1	0	1	0.7	1	-	-	-
Welding	1	0	1	0.7	-	-	1	-
Tyre burst	1	0	1	0.7	-	-	1	-
Total	116	35	151		36	49	62	4

Table 3: Distribution of bacterial agents causing infectious keratitis

Bacterial isolate	Total number of isolates	Percentage	95% CI
Staphylococcus sp.	104	43.7	37.55-50.05
Streptococcus sp.	39	16.4	12.22-21.62
Pseudomonas	32	13.4	9.69-18.36
Mixed bacterial growth	21	8.8	5.84-13.11
Diphtheroids	8	3.4	1.71-6.49
Klebsiella	5	2.1	0.9-4.82
Neisseria sp.	4	1.7	0.66-4.24
Bacillus	4	1.7	0.66-4.24
Sphingomonas	4	1.7	0.66-4.24
Nocardia	4	1.7	0.66-4.24
Corynebacterium diphtheriae	4	1.7	0.66-4.24
Moraxella	2	0.8	0.23-3.01
Atypical mycobacteria	2	0.8	0.23-3.01
Enterobacter	2	0.8	0.23-3.01
Escherichia coli	1	0.4	0.07-2.34
Proteus	1	0.4	0.07-2.34
Actinomyces	1	0.4	0.07-2.34
Total	238		

CI: Confidence interval

group and those which had one-Gram-positive organism and one-Gram-negative organism were taken in the Gram-negative group.

A total of 143 patients showed fungal growth, of which *Fusarium* sp. was the most commonly isolated fungus (37.1%) followed by *Aspergillus* sp. (30%) of fungal keratitis. *Microsporidia* accounted for 11 cases in this study. The details are shown in Table 4.

Antibacterial sensitivity was performed by Kirby–Bauer method with drugs such as gatifloxacin, cefazolin chloramphenicol, ceftazidime, vancomycin, ciprofloxacin, moxifloxacin, and amikacin. Sixty-seven and four-tenth percent of *Streptococcus* sp., 52% of *Pseudomonas*, 100% of *Nocardia*, and 95.9% of *Staphylococcus* sp. were sensitive to amikacin [Table 5]. Ninety-three and a half percent of *Streptococcus* sp., 8% of *Pseudomonas*, 50% of *Nocardia*, and 96.6% of *Staphylococcus* sp. were sensitive to vancomycin. Eighty-seven and one-fifth percent of *Streptococcus* sp., 53.6% of *Pseudomonas*, 100% of *Nocardia*, and 93.2% of *Staphylococcus* sp. were sensitive to moxifloxacin. *Nocardia* was 100% sensitive to amikacin and ciprofloxacin. *Staphylococcus* sp. and *Streptococcus* sp. had maximum sensitivity to vancomycin eye drops. *Pseudomonas* showed only 52% sensitivity to amikacin eye drops but showed 90.5% sensitivity to imipenem. However, not all isolates were tested to all the antibiotics mentioned (such as *Streptococcus* sensitivity to imipenem).

Clinical outcomes

Keratitis caused by bacterial agents had a favorable outcome in 59.2%. In the bacterial group, Gram-positive organisms had a favorable outcome in 60.3% and Gram-negative organism had a favorable outcome in 55.9%. The keratitis caused by fungal agents had a favorable outcome in 60.8% [Table 6].

The keratitis caused by bacterial agents had a poor outcome in 40.8%. In the bacterial group, Gram-positive organisms had a poor outcome in 39.7% and Gram-negative organism had a poor outcome in 44.1%. The keratitis caused by fungal agents had a poor outcome in 39.2%. Culture-proven Gram-negative bacteria had the highest percentage of poor outcomes [Table 6]. The average final visual acuity was 1.87 ± 1.48 logMAR (range, 0–5 logMAR).

Tarsorrhaphy was done in 96 of the 625 IK cases. It was not included in the surgical intervention as it was done to enhance healing. Tarsorrhaphy was done in fifty patients as an additional procedure combined with therapeutic keratoplasty, whereas in 46 patients, it was done as a primary procedure to enhance healing.

Two hundred and twelve patients needed surgical intervention either in the form of TPK (157) or application of tissue adhesives (55) in the course of treatment. The percentage of surgical intervention was highest in the Gram-negative keratitis group [Table 7]. Ninety-two patients were culture-positive for bacteria (67 Gram-positive and 25 Gram-negative) and 54 were culture-positive for fungi, whereas 66 were culture-negative. The mean duration between the date of presentation and surgical intervention in bacterial keratitis was 8.9 days, whereas it was 8.5 days in fungal keratitis ($P = 0.82$). However, within bacterial growth, the Gram-negative group required surgical intervention much earlier (mean = 4.8 days) as compared to Gram-positive type (mean = 10.2 days) ($P = 0.014$).

A total of 55 of the 625 IK patients needed tissue adhesive and glue as surgical intervention in this study. Of these, 26 (10.9%) were culture-positive for bacteria (19 Gram-positive and 7 Gram-negative) and 7 (4.9%) were culture-positive for fungi, whereas 22 (9.5%) were culture-negative. Forty-four (80%) of these cases healed with scar and with elimination of infection, whereas 11 cases did not resolve after glue application. A total of 157 IK patients needed therapeutic keratoplasty. Sixty-six (27.7%) of these cases were culture-positive for bacteria, 47 (32.9%) were culture-positive for fungi, and 44 (19%) were culture-negative. Out of 66 bacterial culture-positive cases, 48 (26.8%) had grown Gram-positive organisms and 18 (30.5%) had grown Gram-negative organisms. At the end of the study period, 36 eyes became blind (negative to perception of light) and could not be salvaged.

Table 4: Distribution of fungal agents causing infectious keratitis

Fungal isolate	Total number of isolates	Percentage	95% CI
Fusarium sp.	53	37.1	29.58-45.22
Aspergillus sp.	43	30	23.16-38.03
Unidentified dematiaceous fungi	25	17.5	12.13-24.54
Microsporidia	11	7.7	4.35-13.25
Curvularia	6	4.2	1.94-8.85
Cladosporium	3	2.1	0.72-5.99
Candida	1	0.7	0.12-3.85
Trichothecium	1	0.7	0.12-3.85
Total	143		

CI: Confidence interval

Discussion

IK continues to be a major cause of visual loss in developing countries.[18] An important aspect of management of this disease is to understand its epidemiology, risk factors, and etiological agents.[18] The trends may vary demographically, and hence, regular regional updates become important for ophthalmologists in framing treatment protocols. Through this article, we have tried to review the current trends of IK presenting to a tertiary eye care center in Delhi. Almost 30% of our patients traveled from outside Delhi to our center for treatment. With lack of health-care facilities in smaller towns and villages, it is no surprise that one-third of our patients are from outside Delhi. Interestingly, according to government estimates, of all the overnight trips made by people from rural India, 47.9% of them are for health-related reasons.[19] This reflects the workforce shortage that India is facing in the medical sector, and urgent policy changes are needed at the grassroots level to increase doctor-to-patient ratio, which currently stands at <1/1000 patients,[20] and is even less for ophthalmologists (one/100,000).[21] As expected, almost 69% of our patients were male, and ocular trauma was the most important predisposing factor. Vegetative matter was the most common cause of trauma, and North India being an agricultural hub, this has been an expected trend. Limited facilities for farmers and poor infrastructure call for a major overhaul of our system with focus on eye-care safety. Most of the studies done in this field have similar trends, and as has been done in previous studies,[22] we also want to highlight the importance of protective eyewear in the workplace. Local doctors should be given basic training in tackling eye emergencies, and reports from Burma, India, and Bhutan have shown that prophylactic use of 1% chloramphenicol in trauma cases has helped heal corneal abrasions without infection.[22] In addition, among predisposing factors, of all the previous ocular surgeries

Table 5: Antibacterial susceptibility pattern of common bacterial isolates to common antibiotics

Antibiotic	Organism (%)			
	Staphylococcus	***Streptococcus***	***Pseudomonas***	***Nocardia***
Amikacin	117/122 (95.9)	33/49 (67.4)	13/25 (52)	4/4 (100)
Cefazolin	109/119 (91.6)	45/49 (91.8)	2/28 (7.1)	3/4 (75)
Ceftazidime	80/118 (67.8)	39/47 (83.0)	16/27 (59)	2/4 (50)
Moxifloxacin	109/117 (93.2)	41/47 (87.23)	15/28 (53.6)	3/3 (100)
Vancomycin	113/117 (96.6)	43/46 (93.5)	2/25 (8)	2/4 (50)
Gatifloxacin	54/118 (45.8)	28/49 (57.1)	11/27 (40.7)	2/4 (50)
Ciprofloxacin	76/120 (63.3)	36/50 (72)	12/26 (46.2)	4/4 (100)
Chloramphenicol	105/122 (86.07)	46/50 (92)	6/27 (22.2)	2/4 (50)
Imepenam	3/3 (100)	-	19/21 (90.5)	2/2 (100)

Not all isolates were tested to all the antibiotics mentioned

Table 6: Outcome of keratitis with different culture results

Organisms	Favorable outcome (%)	Poor outcome (%)	Total
Bacteria	141 (59.2)	97 (40.8)	238
Gram-positive bacteria	108 (60.3)	71 (39.7)	179
Gram-negative bacteria	33 (55.9)	26 (44.1)	59
Fungal	87 (60.8)	56 (39.2)	143
Culture-negative	165 (71.1)	67 (28.9)	232
Mixed growth	9 (100)	0 (0)	9
Acanthameba	3 (100)	0 (0)	3
Total	405	220	

Table 7: Details of surgical intervention

Organisms	Need of TPK (%)	Need of TA BCL (%)	Total organisms in the group
Bacteria	66 (27.7)	26 (10.9)	238
Gram-positive bacteria	48 (26.8)	19 (10.6)	179
Gram-negative bacteria	18 (30.5)	7 (11.2)	59
Fungal	47 (32.9)	7 (4.9)	143
Culture-negative	44 (19)	22 (9.5)	232
Total	157 (25.1)	55 (8.8)	

TPK: Therapeutic penetrating keratoplasty, TA BCL: Tissue adhesive with bandage contact lens

performed, 85% of patients had a previous keratoplasty done, which subsequently developed an infiltrate. Again, this points back at limited resources available for postkeratoplasty patients in terms of follow-up.

Our culture-positive cases were 62.9%. Previous reports have shown the culture-positive rates to vary from 25.6% in Thailand to 78% in Australia.[23] Out of the culture-positive bacterial cases, 75.2% were Gram-positive bacilli and *Staphylococcus* spp. was the most common Gram-positive organism. Over the years, various reports from all over India have shown similar results. *Staphylococcus* spp. and *Streptococcus* spp. have been the most commonly isolated organisms.[24] In our study, both showed good sensitivity to vancomycin (>90%) and variable sensitivity to various fluoroquinolones. A recent update from South India showed similar sensitivity toward vancomycin; however, they had good sensitivity toward fluoroquinolones as well,[11] which was not seen in our patients. Furthermore, as expected, *Pseudomonas* spp. was the most common Gram-negative organism. However, its sensitivity was <60% for all the five antibiotics tested in our analysis and is thus alarming. It was most sensitive to imipenem. Other studies have showed better sensitivity patterns,[11] but at the same time, one should not miss the worrying trends of multidrug-resistant *Pseudomonas* spp. from around the world,[25,26] a reflection of which we are also beginning to see in our clinics. One hundred and forty-three specimens grew fungi, with *Fusarium* spp. being the most common. India, especially the southern part, is known for fungal keratits, mostly due to the hot and windy climate.[27] The majority of our patients had vegetative trauma and were from an agricultural background, as opposed to the West, where contact lens wear has been found to be the most common risk factor.[28]

In conclusion, one-third of our patients (25.1%) had to undergo a TPK. We would like to highlight that the remaining 75% of the eyes were managed because of targeted treatment toward the microorganisms isolated. Laboratory setup is still missing in a majority of corneal practices, and work should be done to integrate basic laboratory services to the ophthalmology departments across the country. Ophthalmologists should be encouraged to learn and practice basic staining procedures, and this should start early in the training years. In addition, every ophthalmology resident should be trained in performing tarsorrhaphy and tissue adhesives. These are eye-saving procedures, and we could salvage 151 eyes (55 tissue adhesives and 96 tarsorrhaphy) using these procedures. One of the recommendations from this article would be to have training sessions for ophthalmologists in performing these procedures.

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Conflicts of interest
There are no conflicts of interest.

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