Cluster of Mycobacterium Chelonae Keratitis Cases Following Laser In-situ Keratomileusis

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- **PURPOSE:** To describe a cluster of Mycobacterium chelonae keratitis cases involving patients who underwent laser in-situ keratomileusis (LASIK) at a single refractive surgery center.
- **DESIGN:** Descriptive case series of four patients and cohort study to identify disease associations.
- **METHODS:** Examination schedules, diagnostic tests, and therapy were based on best medical judgment. Isolates from three patients were compared by pulsed-field gel electrophoresis. Epidemiologic studies were performed to identify the source of infection.
- **RESULTS:** Seven of eight eyes developed *M. chelonae* keratitis following bilateral simultaneous LASIK. Each patient was thought to have diffuse lamellar keratitis initially, but all seven eyes were noted to have opacities suggestive of infectious keratitis by 13 to 21 days after surgery. All eyes had undergone hyperopic LASIK over four days in April 2001 by one surgeon in a community-based refractive surgery center. A cohort study of all patients undergoing LASIK at the same center in April 2001 revealed that *M. chelonae* keratitis occurred only in persons undergoing correction of hyperopia (seven of 14 eyes vs. none of 217 eyes undergoing myopic LASIK, \( P < .001 \)). The only difference identified between procedures was use of masks created from a soft contact lens in hyperopic LASIK. Three isolates (three patients) were indistinguishable by pulsed-field gel electrophoresis. Eyes were treated with a combination of antimicrobial agents, including topical azithromycin in three patients, with resolution of infection in all eyes over 6 to 14 weeks. The source of infection was not identified on environmental cultures.
- **CONCLUSION:** Postoperative nontuberculous mycobacterial keratitis can occur in an epidemic fashion following LASIK. Topical amikacin, azithromycin, clarithromycin, ciprofloxacin, or a combination of these agents, appears to be effective treatment for these infections. (Am J Ophthalmol 2001;132:819–830. © 2001 by Elsevier Science Inc. All rights reserved.)

Infectious keratitis after refractive surgery is uncommon. Nevertheless, there have been several reports of bacterial, fungal, and mycobacterial keratitis after laser in-situ keratomileusis (LASIK).1–18 Rapidly growing mycobacteria (one group of the nontuberculous or “atypical” mycobacteria) account for a substantial proportion of these cases, with *Mycobacterium chelonae* being the most frequently reported species.1,4,5,9,16 To date, all reports of nontuberculous mycobacterial keratitis after LASIK in the medical literature have described single isolated cases.1,4,5,9,16

In May 2001, on behalf of the Centers for Disease Control and Prevention (CDC), the American Academy of Ophthalmology issued an alert to its members regarding nontuberculous mycobacterial infections after LASIK, in response to a reported cluster of cases from a single community-based refractive surgery center in Southern California (published in the American Academy of Ophthalmology’s EyeNet magazine, July 2001, pages 60 to 61). The cluster involved four patients (seven eyes) who developed *M. chelonae* keratitis after undergoing bilateral,
simultaneous hyperopic LASIK by one surgeon during a four-day period.

To provide the ophthalmic community with additional information about these cases, we present a description of their clinical findings, course of disease, and response to treatment (Figures 1–3), as well as information regarding attempts to identify the source of infection. We also describe the use of topical azithromycin as a possibly effective agent for treatment of nontuberculous mycobacterial keratitis.

**SUBJECTS AND METHODS:** Disease assessment was based on slit-lamp biomicroscopic examination at frequencies dictated by best medical judgment. Medical and surgical treatments, including changes in therapy, were based on best medical judgment. Indications for surgical excision of lamellar keratectomy flaps were nonadherence of the flap or lack of clinical improvement, as judged by the treating ophthalmologist.

Initial specimens for culture were obtained by scraping the surface of the cornea with a Kimura spatula in areas of epithelial defects. Subsequent specimens for culture were obtained by scraping the corneal stroma with a Kimura spatula after elevation of the lamellar keratectomy flap. Specimens were placed directly on the following culture media: blood agar, chocolate agar, thioglycolate broth, Sabouraud agar, and Middlebrook 7H11 agar. Specimens were also placed on glass slides for microscopic examination using the following stains: Gram, Giemsa, Ziehl–Nielson, and fluorochrome.

Susceptibility testing was performed in the UCLA Clinical Laboratories according to National Committee for Clinical Laboratory Standards guidelines. Isolates were susceptible to amikacin (mean inhibitory concentration [MIC]: 8 μg/ml) and clarithromycin (MIC: ≤ 0.5 μg/ml) in vitro. There are no standards for susceptibility testing to azithromycin in vitro. Clarithromycin is used as the class agent for the newer macrolides when performing suscepti-

**FIGURE 1.** (Left) The right eye of case 1, 26 days after LASIK, showing focal and diffuse infiltrates at the interface below the lamellar keratectomy flap. (Right) The same eye, 1 month later. There is complete necrosis and nonadherence of the lamellar keratectomy flap, which was subsequently excised. A portion of the central white material is precipitated clarithromycin that is adherent to necrotic tissue.

**FIGURE 2.** (Left) The left eye of case 2, 23 days after LASIK, showing diffuse infiltrates at the interface below the lamellar keratectomy flap. (Right) The same eye, 2 months later, after resolution of infection. There is a central, nonvascularized scar at the level of the interface that also involves the lamellar keratectomy flap. The surrounding cornea is clear.
bility testing; organisms susceptible to clarithromycin are considered to be susceptible to azithromycin.20

All positive culture isolates from patients were sent to the CDC for molecular analysis. Isolates were compared using pulse-field gel electrophoresis.

Topical fortified amikacin (50 mg/ml) was prepared by local pharmacies from drug for intravenous injection, according to routine procedures. A commercially available preparation was used for topical ciprofloxacin (Ciloxan, Alcon Laboratories, Inc., Fort Worth, TX). Topical clarithromycin (10 mg/ml) was prepared as a suspension from clarithromycin granules for oral suspension (Abbott Laboratories, Inc., North Chicago, IL), as described by Ford and associates.21 Topical azithromycin was prepared from lyophilized drug for intravenous injection (Pfizer Inc., New York, NY) according to the following procedures. One vial of drug was reconstituted with 4.8 ml of sterile water per the manufacturer's instructions. The resulting solution was diluted further with normal saline to achieve a concentration of 2 mg/ml. This solution was then filtered through a 0.22 µm filter into a sterile ophthalmic dropper vial. Patients were instructed to refrigerate the solution between applications. According to the manufacturer's package insert, azithromycin solution containing 2 mg/ml of drug will be stable for 7 days when refrigerated.

When initial culture results from the patients suggested a common source of infection related to surgery, the local health department (the Department of Health and Human Services, Long Beach, CA) was notified. That agency then contacted the California Department of Health Services in Berkeley, CA and the Epidemic Intelligence Service, Centers for Disease Control and Prevention (CDC) for assistance in epidemiologic studies to identify the source of infection.

A case was defined as any patient who developed keratitis after LASIK by the identified surgeon. The surgeon agreed to alert the CDC regarding any new cases of M. chelonae keratitis that were discovered during post-operative care of all surgical patients. To help identify other possible cases, an e-mail alert was sent to all

FIGURE 3. The right eye (top left) and left eye (top right) of case 4, shown 20 days after bilateral, simultaneous LASIK. The right eye shows focal round corneal opacities at the interface below the lamellar keratectomy flap. The left eye shows a similar focal infiltrate in front of the inferior pupillary margin, as well as early diffuse cellularity at the interface. The right eye (bottom left) and left eye (bottom right) of case 4, 1 month after photographs shown in top left and top right. A hypopyon has developed in the right eye, and there is substantial irritation (corneal epitheliopathy, conjunctival redness and chemosis, eyelid swelling and redness) attributed to topical clarithromycin. Increased diffuse opacity at the level of the interface can be seen in the left eye. Precipitated clarithromycin that is adherent to mucus strands can be seen in both eyes.
members of the American Academy of Ophthalmology, asking for information about any recent cases of nontuberculous mycobacterial corneal infection throughout the country. Similar alerts were posted on the CDC e-mail list-server “Epi-X” and on a national e-mail list-server for clinical microbiology laboratories, “ClinMicroNet.”

On May 8, 2001 (24 days after the last procedure associated with a known M. chelonae infection), physicians from the CDC, as well as from state and local health agencies, visited the surgical practice in question. The surgeon and refractive surgery center staff were interviewed regarding the reported cases and the details of the LASIK procedures performed. Included were questions about the handling of surgical instruments during and between surgical procedures. Information was requested from the surgeon about all surgical procedures performed 2 weeks before and 2 weeks after the week during which case patients had undergone LASIK. For each procedure, the following information was obtained: patient demographic data, type of procedure (hyperopic LASIK, myopic LASIK), date of procedure, postsurgical examination findings, and staff present during procedures.

Investigators returned to the refractive surgery center on May 19, 2001 (the date of the next scheduled hyperopic LASIK) to observe in detail all presurgical, surgical, and postsurgical practices at the center. As part of this investigation, the sterilizer at the refractive surgery center was evaluated. Routine procedures employed by the staff in using the sterilizer were reviewed and compared with the manufacturer’s instructions. The sterilizer was tested three times with Bacillus subtilis spore strips (SGM Biotech, Bozeman, MT) to assess efficacy. The first test involved one instrument and three spore strips; the second test involved a full load of instruments and four spore strips, and the third test included trephines as well as instruments and five spore strips. Two spore strips, left unopened and not placed in the sterilizer, were used as controls. All spore strips were incubated and cultured according to manufacturer’s instructions.

Environmental cultures from potential contaminants in the surgical center were performed at the time of the initial visit on May 8, 2001. Because the case patients had been diagnosed with diffuse lamellar keratitis, the surgeon had replaced some materials related to the surgeries, including distilled water, dish soap used for cleaning surgical instruments before sterilization, and balanced salt solution used intraoperatively, before resuming LASIK procedures on April 18, 2001. The stocks of distilled water and dish soap used during the outbreak time-period were still stored at the surgeon’s office, however, and samples from these stocks were obtained for culture. One bottle of balanced salt solution used during the outbreak time period was also available and was obtained for culture. Four packages of gentian violet corneal ink used during the outbreak time period were also obtained for culture. Because tap water is a common habitat for nontuberculous mycobacteria, tap water samples from the sink outside the operating room were obtained for culture on May 21, 2001. The sink drain was also swabbed for culture at that time.

The contact lens fragments used as masks in each hyperopic LASIK case (see description in Results section below) had been discarded at the end of each procedure, and therefore could not be cultured. A copy of the surgeon’s lens ordering and shipment information was obtained, and the State of California Food and Drug Branch obtained 25 soft contact lenses of the same lot number as those used during the outbreak time period from the lens manufacturer.

Water and sink drain cultures were performed at the Long Beach (CA) Department of Health and Human Services Laboratory. All other environmental cultures were performed at the California Department of Health Sciences Microbial Diseases Laboratory (Berkeley, CA). Tap and distilled water samples were filtered through 45 μm filters. Filters were placed into Bactec media and on Lowenstein–Jensen slants. Sink drain swabs were inoculated directly into the same media. Samples of gentian violet corneal ink, dish soap, and balanced salt solution were inoculated directly into Bactec, Lowenstein–Jenson, and Middlebrook 7H10 media. Lens packaging fluid from the 25 contact lens containers and five ground contact lenses were plated onto Middlebrook 7H10 media and inoculated into MGIT broth media. All cultures were incubated at 35°C and held for 6 weeks.

Ground contact lenses and packaging fluid were also sent to CDC for polymerase chain reaction (PCR) analysis, using mycobacteria-specific PCR probes.

We performed a MEDLINE literature search to identify previously published cases of infectious keratitis following refractive surgery. A separate MEDLINE literature search revealed no previous publications describing the use of topical azithromycin for the treatment of nontuberculous mycobacterial keratitis in any setting.

- **STATISTICAL ANALYSIS:** Relative risk (RR), 95% confidence intervals, and P values (Fisher exact test) were calculated using Epi Info 2000 software version 1.1 (CDC, Atlanta, GA). A per eye analysis was performed because one patient had a unilateral infection; such an analysis might allow identification of factors related to infection between first and second procedures on an individual patient.

- **REPRESENTATIVE CASE REPORT:** A 57-year-old woman (case 4) with no history of ocular problems underwent bilateral, simultaneous hyperopic LASIK. She was in good general health. On the second postoperative day, diffuse lamellar keratitis was diagnosed in both eyes, and therapy with prednisolone acetate 1% hourly while awake was initiated. Oral prednisone (60 mg/d) was added to her treatment regimen on the third postoperative day. On examination 2 weeks after surgery, the diffuse lamellar
keratitis had resolved in both eyes, and the topical corticosteroid therapy was tapered but not stopped. Examination during the third postoperative week revealed multiple white anterior stromal granular opacities (each less than 0.5 mm diameter) in the central cornea at the level of the interface below the lamellar keratectomy flap in the right eye (Figure 3 [top left]), and a single similar lesion in the left eye (Figure 3 [top right]). She had no ocular or visual symptoms. She was referred to the Jules Stein Eye Institute for evaluation and management. Visual acuity without correction was 20/20 in both eyes. The lamellar keratectomy flap was lifted in the right eye to obtain specimens by scraping for culture and microscopic examination. Acid-fast bacteria were seen on smears, and cultures on Middlebrook 7H11 agar grew. Fast bacteria were seen on smears, and cultures on Middlebrook 7H11 agar grew. M. chelonae keratitis was diagnosed as probable diffuse lamellar keratitis, in contrast to none of 171 eyes undergoing myopic LASIK (P < .001; RR is undefined statistically because no myopic LASIK cases were associated with infection). All myopes underwent bilateral simultaneous LASIK (seven patients), while 104 myopes underwent unilateral LASIK (nine patients). A patient analysis also revealed a significant association between hyperopic procedures and infection (P < .001).

With the exception of one case patient (case 3), who relocated to Michigan during the first postoperative week after surgery, all patients who underwent LASIK during the period April 4–28, 2001 were examined for at least 3 weeks postoperatively by the surgeon. Because of the infections that had occurred in the four case patients, particular attention was paid to the other three patients who underwent hyperopic LASIK; each was followed for at least 14 weeks with no signs of infection.

A representative case history is presented above, and specific details for each case patient are listed in Table 1. Initial clinical characteristics were similar in all four case patients. By the second postoperative day, each exhibited cellularity at the interface below the lamellar keratectomy flap, which was diagnosed as probable diffuse lamellar keratitis. All patients were treated with prednisolone acetate 1% (hourly while awake). Signs resolved in two patients (cases 1 and 2), and their topical corticosteroid treatments were stopped after 1 week. Oral prednisone (60 mg daily) was also prescribed for two patients (cases 3 and 4). The patient who relocated to Michigan (case 3) stopped oral prednisone after 6 days, but was still using topical prednisolone acetate 1% at the time a diagnosis of presumed M. chelonae keratitis was made, as described below. One patient (case 4) was still receiving both oral prednisone and topical prednisolone acetate 1% when...
<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Eye(s) Involved</th>
<th>Symptoms</th>
<th>Slit Lamp Biomicroscopic Findings</th>
<th>Basis of ( M. ) chelonae Keratitis Diagnosis</th>
<th>Clinical Features at Diagnosis</th>
<th>Drug Treatment</th>
<th>Visual Acuity</th>
<th>Excision of Flap (Interval After Diagnosis)</th>
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<tbody>
<tr>
<td>1</td>
<td>56/F</td>
<td>RE</td>
<td>Ache, irritation, burning, blurring</td>
<td>Multiple linear opacities at level of flap interface; AFB in flap biopsy specimen</td>
<td>Positive culture from flap interface</td>
<td>Initial:</td>
<td>Immediate, Ciprofloxacin, amikacin, clarithromycin, oral doxycycline</td>
<td>Subsequent:</td>
<td>6 weeks, Resolution of inflammatory signs after 6 weeks</td>
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<tr>
<td>2</td>
<td>56/F</td>
<td>OU</td>
<td>photophobia, burning, blurring</td>
<td>Multiple round opacities at level of flap interface (LE)</td>
<td>Positive culture from flap interface</td>
<td>Initial OU:</td>
<td>Immediate, Ciprofloxacin, amikacin, clarithromycin, oral doxycycline</td>
<td>Subsequent OU:</td>
<td>6 weeks, Resolution of stromal infiltration, (4 weeks)</td>
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<tr>
<td>3*</td>
<td>64/F</td>
<td>OU</td>
<td>Mild irritation</td>
<td>Diffuse keratitis at level of flap interface</td>
<td>Positive culture from flap interface</td>
<td>Initial OU:</td>
<td>Immediate, Ciprofloxacin, amikacin, clarithromycin, oral doxycycline</td>
<td>Subsequent OU:</td>
<td>6 weeks, Resolution of inflammatory signs after 6 weeks</td>
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**TABLE 1.** Characteristics of \( Mycobacterium \) chelonae Keratitis in Patients Following Bilateral Laser In-situ Keratomileusis
<table>
<thead>
<tr>
<th>Case*</th>
<th>Age (years)/Gender</th>
<th>Eye(s)</th>
<th>Symptoms</th>
<th>Basis of M. chelonae Keratitis Diagnosis</th>
<th>Initial OU: Drug Combination†</th>
<th>Interval From Diagnosis to Start of Drugs</th>
<th>Duration of Drugs</th>
<th>Response</th>
<th>Excision of Flap (Interval After Diagnosis)</th>
<th>Visual Acuity</th>
<th>Final‡</th>
<th>At Diagnosis</th>
<th>Final‡</th>
<th>Best Spectacle</th>
<th>Final Corneal Status³</th>
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<td>4</td>
<td>57/F</td>
<td>OU</td>
<td>Asymptomatic</td>
<td>Multiple round opacities at level of flap</td>
<td>Ciprofloxacin, amikacin, clarithromycin, oral</td>
<td>Immediate 6 weeks</td>
<td></td>
<td>No</td>
<td>RE: 20/20 NA 20/80-</td>
<td>RE: Diffuse stromal scars</td>
<td>LE: 20/20 NA 20/30 20/25-</td>
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<td>Positive culture from flap</td>
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AFB = acid fast bacteria; F = female; HM = hand motion; NA = not available.
*Cases are presented in the order of surgery: cases 1 and 2 underwent LASIK on April 11, 2001; case 3 underwent surgery on April 12, 2001; case 4 underwent surgery on April 14, 2001.
†All drugs listed were administered topically unless otherwise specified.
‡Article prepared at 19 weeks after LASIK procedures were performed.
§Cases 3 and 4 were being treated with topical prednisolone acetate 1% at diagnosis of M. chelonae keratitis; case 4 was being treated with oral prednisone at diagnosis. Corticosteroids had been prescribed for presumed diffuse lamellar keratitis.
¶Best corrected visual acuity with spectacles at onset of antimycobacterial drug treatment was RE: 20/40; LE: 20/40.
Signs of infectious keratitis developed. Signs of infectious keratitis (focal corneal stromal opacities at the level of the interface) were first noted 13 to 21 days after surgery (mean, 17 days) (Figure 3 [top left and top right]). The three patients still in California were referred to the Jules Stein Eye Institute for additional evaluation and management when evidence of infectious keratitis developed.

Diagnosis of \textit{M. chelonae} keratitis for the three patients in California (cases 1, 2, and 4) was based on culture results. Initial cultures of specimens obtained from the corneal surface in areas of epithelial defects overlying deeper infiltrates were negative in all three cases. Additional specimens were then obtained from all three patients by lifting the lamellar keratectomy flap of the worse eye and scraping the interface with a Kimura spatula. Acid-fast bacteria were identified on slides prepared with the same material in case 4 only. Growth was first detected on Middlebrook 7H11 agar between 3 and 7 days in all cases. All three \textit{M. chelonae} isolates were indistinguishable by pulsed-field gel electrophoresis (Figure 4). In one patient (case 1), biopsy of necrotic flap tissue from the same eye was performed after the flap was raised initially, but before results of the initial cultures were obtained; this specimen also contained acid-fast bacteria, and repeat culture of the interface taken at the same time grew \textit{M. chelonae}.

Because of the experience with these three patients, the surgeon contacted the fourth patient (case 3) in Michigan. She was being followed by a local optometrist, still with a diagnosis of presumed diffuse lamellar keratitis. Her findings, as described by the optometrist, were similar to those in the three patients in California, and a presumptive diagnosis of \textit{M. chelonae} keratitis was made. The same antitycobacterial treatment being used for the other three patients was initiated empirically, and the patient was eventually referred to Michigan Cornea Consultants for additional evaluation and management.

Initial antibiotic therapy for all four patients consisted of topical ciprofloxacin 0.3%, fortified amikacin (50 mg/ml), and clarithromycin (10 mg/ml), as well as oral doxycycline (100 mg, twice daily). One patient (case 2) responded well to this treatment regimen, and no further modifications in medical therapy were made. The other patients had persistence of active infection on this treatment regimen and developed irritation and signs of ocular surface toxicity caused by topical clarithromycin (corneal epitheliopathy, increased conjunctival redness, chemosis, eyelid margin redness, and pain with instillation) (Figure 3 [bottom left and bottom right]). Oral clarithromycin was substituted for oral doxycycline, and topical azithromycin (2 mg/ml) was substituted for topical clarithromycin after 6 weeks of treatment for each patient. All three patients tolerated this revised treatment regimen well, with eventual resolution of inflammatory signs over the next 5 to 6 weeks. All medications were stopped for three patients (cases 1, 2, and 4) and there were no clinical signs of recurrent infection during 3 to 6 weeks of additional follow-up. One patient (case 3) had no sign of active disease, but was still being treated with topical azithromycin and ciprofloxacin at a reduced frequency at the time of this writing.

In one patient (case 1), the lamellar keratectomy flap was removed from the right cornea at week 4, when it became diffusely necrotic and nonadherent (Figure 1 [right]). In the other two patients being managed at the Jules Stein Eye Institute (cases 2 and 4), all four flaps remained adherent, although there were focal areas of necrosis and thinning. The flap was excised from the worse (left) cornea of the patient being managed at Michigan Cornea Consultants (case 3), because of dense flap infiltrates, lack of appreciable response to medical therapy, and published experience suggesting a therapeutic benefit of flap removal. Improvement was noted in the left cornea after flap removal; therefore, excision of the flap from the right cornea was recommended as well, as inflammatory signs had become more severe in that eye. On the same day, topical azithromycin was added to the patient's
treatment regimen. There was subsequent improvement in the status of both eyes.

Following resolution of infection, findings were remarkably similar in all seven involved corneas. All had anterior stromal scarring and superficial vascularization to varying degrees. In addition, there were variable amounts of necrosis and tissue loss from the lamellar keratectomy flap in all eyes. It is our clinical impression that among those patients whose flaps were not excised, stromal scarring and superficial vascularization was most severe in areas where the overlying flap was absent because of either necrosis or biopsy. Areas of remaining flap that had been necrotic, but healed, were diffusely opaque. Both corneas of one patient (case 3) had focal deep stromal loss, with up to 90% thinning (a 2 × 4 mm peripheral area in the right eye and a 1 mm diameter central area in the left eye).

Epidemiologic studies identified one surgical factor that was related to the cluster of infections, but failed to identify the source of the infecting organisms. Evaluation of the myopic and hyperopic LASIK procedures revealed only one difference between procedures; during hyperopic LASIK, the surgeon utilizes soft contact lens fragments to mask a portion of the cornea during laser ablation. Three masks of various sizes (3.5 mm, 4.5 mm, 5.5 mm) are created from a single contact lens, using nondisposable trephines that have been resterilized before each patient’s procedure. During hyperopic LASIK, the three masks are held with a pair of sterile jeweler’s forceps at approximately 1 cm above the cornea, for approximately 10 seconds each, in succession from smallest to largest, as the laser is applied to the cornea. All surgical equipment, with the exception of the trephines used to create the masks, is the same for both myopic and hyperopic LASIK.

The surgeon reported using a new contact lens for each case patient, but the same masks were used for both eyes of each patient. Masks were placed on a sterile tray between procedures on the first and second eyes. Contact lenses from two manufacturers were used during the month of April 2001. All eyes undergoing hyperopic LASIK during the period April 11–14, 2001 were masked with contact lenses from a single manufacturer. The surgeon had received five contact lenses from that manufacturer at the beginning of April 2001. The surgeon reported using four of these five lenses to create masks for the four case patients. The fifth lens was discarded by the surgeon before use, and was not available for examination. All other hyperopic LASIK procedures were performed using masks created from contact lenses obtained from the other manufacturer, but none of these cases were performed during the period April 11–14, 2001.

The surgeon routinely wears sterile gloves while performing LASIK, but does not change instruments or gloves between eyes of the same patient. Surgical staff was constant throughout the month of April 2001, with the same individuals being involved in all surgeries. They reported that all surgical instruments, including trephines, had been sterilized between use on each patient. Other products, including gentian violet corneal ink, sterile gloves, and topical medications, were used in all procedures throughout the month. The brands of balanced salt solution, distilled water, and dish soap were switched on approximately April 18, 2001.

Tap water from the refractive surgery center sink grew M. gordonae, a common contaminant. All other environmental cultures, as well as cultures of ground contact lenses and lens package fluids, were negative. PCR tests of ground contact lenses and lens package fluid were negative for mycobacteria. In all tests, the office sterilizer worked effectively, as all spore strips that underwent sterilization failed to grow B. subtilis during culture, while both control spore strips did grow B. subtilis during culture.

**DISCUSSION**

**CORNEAL INFECTION IS AN UNCOMMON COMPLICATION OF LASIK SURGERY,** with reported rates of 1 to 2 cases per 1000 eyes.22 Isolated case reports have described corneal infections with a variety of organisms, including Staphylococcus aureus, Aspergillus sp., Nocardia sp., and Mycobacteria sp. Nontuberculous mycobacterial keratitis is one of the most common infections to be reported. Among 22 culture-positive cases of post-LASIK infectious keratitis in 18 publications since 1995,1–10 Mycobacterium sp. were identified in six cases (five cases of M. chelonae keratitis1,4,5,9,16 and one case of M. fortuitum keratitis5). We were unable to identify any previous reports describing a cluster of infections from a single refractive surgery center.

There are approximately 50 species within the genus *Mycobacterium*, including *M. tuberculosis*, *M. leprae*, and numerous nontuberculous mycobacterial species. The *R. pylori* classification system categorizes nontuberculous mycobacteria into four categories, one of which (group IV) contains the rapidly growing mycobacteria (so named because these species usually grow in vitro within seven days),24 including *M. fortuitum*, *M. chelonae*, and *M. abscessus*. Nontuberculous mycobacteria are ubiquitous in soil, animals, milk, foodstuffs, municipal tap water, and laboratory water.25–28 *R. pylori* group IV organisms multiply readily in distilled water, and can remain viable for a year. They are also resistant to chemical disinfectants, such as chlorine.25 Nontuberculous mycobacterial infections are not transmitted by human-to-human contact.25,29

*M. chelonae* and *M. fortuitum* are the most common causes of nontuberculous mycobacterial ocular disease. Other species reported to cause keratitis include *M. gordonae*, *M. marinum*, *M. avium-intracellulare*, *M. nonchromogenic*, and *M. asiaticum*.21,25,30–32

The occurrence of multiple cases of *M. chelonae* keratitis in a cluster provides an opportunity to understand clinical features, disease course, and response to therapy. Clinical features of these four cases were similar. All patients
developed findings suggestive of diffuse lamellar keratitis in the immediate postoperative period, but findings persisted despite intense topical, and in some cases oral, corticosteroid therapy, and patients eventually developed focal, dry-appearing opacities. Clinicians should be alert to the possibility of nontuberculous mycobacterial keratitis in patients with persistence of apparent diffuse lamellar keratitis, and in those who develop infiltrates at the level of the interface.

With regard to diagnostic testing, elevation of the lamellar keratectomy flap appears to be necessary for obtaining culture material. Organisms will grow rapidly on specific media, such as Middlebrook 7H11 media, but rapidly growing mycobacteria will also grow on blood agar. Acid-fast bacteria may not be seen in smears of material obtained by scraping of the interface, however.

The course of infection, and late findings were also similar between patients. Late changes in the corneas include diffuse and focal anterior scarring, superficial vascularization, and variable amounts of tissue loss, especially from the flap.

Whether the common characteristics in our patients are unique to the specific isolate in this cluster or reflect the nature of nontuberculous mycobacterial keratitis associated with LASIK (because of unique anatomic considerations) cannot be determined. The cases reported are similar to other previously reported cases of nontuberculous mycobacterial keratitis after LASIK, however.1,4,5,9,16 In contrast, findings are different than those associated with nontuberculous mycobacterial infections after penetrating injury, radial keratotomy, or penetrating keratoplasty; such cases are characterized by deep densely opaque infiltrates, frequently with severe necrosis and tissue loss.13–19 These differences probably reflect the different types of incisions made. Following LASIK, infiltrates develop at the level of the interface and inflammatory material spreads along the plane of incision. Ford and associates21 have speculated that organisms will remain superficial unless introduced into deeper layers mechanically.

Amikacin has been the traditional drug of choice for treatment of nontuberculous mycobacterial keratitis, and topical ciprofloxacin has been shown to be an effective treatment in animal studies.40–42 A recent clinical report suggests that up to 60% of nontuberculous mycobacterial keratitis cases are nonresponsive to amikacin, and an even higher percentage are nonresponsive to ciprofloxacin.21 An animal model has also been used to show that clarithromycin may be effective for treatment of nontuberculous mycobacterial keratitis.42,43 Based on limited experience, Ford and associates21 suggested that topical clarithromycin should now be considered the drug of choice for nontuberculous mycobacterial keratitis. Topical clarithromycin has been used to treat several cases of M. chelonae keratitis after LASIK.1,5,9 There is no commercially available intravenous formulation of clarithromycin from which fortified eye drops can be made; and dilution of a commercially available suspension, marketed for oral use, has been described for use as an eye drop.21 Our patients found this topical preparation of clarithromycin to be irritating, which prohibited its extended use. This irritation has also been reported previously.21

Azithromycin was chosen as an alternative macrolide for continued treatment of our cases. Although clarithromycin appears to be more active in vitro than other macrolides, susceptibility testing may not reflect its relative activity in vivo.20 In contrast, azithromycin is concentrated in tissues; thus, it has the potential for being more effective than clarithromycin in vivo.20 Furthermore, the fact that clarithromycin must be given as a suspension may limit its efficacy as a topical preparation.

There are no standards for in vitro susceptibility testing with azithromycin and we found no published reports describing its use topically against M. chelonae keratitis. The concentration of azithromycin was chosen in part for its known stability, and in part on the basis of in vitro susceptibility testing with clarithromycin, which is believed to reflect susceptibility to azithromycin. It is known that dilutions of intravenous azithromycin to 2 mg/ml are stable for 7 days with refrigeration (package insert; Pfizer Inc., New York, NY); the stability of higher concentrations has not been shown. Because it is concentrated in tissues, we believed that a solution of 2 mg/ml would achieve higher tissue concentration than the 10 mg/ml suspension of clarithromycin and would be effective against an organism with an MIC < 0.5 μg/ml.

Disease was still active when topical azithromycin was added to the treatment regimens for three patients, and infection subsequently resolved in these cases. Azithromycin was not used as monotherapy, however, and thus its efficacy cannot be established conclusively from this series. It was better tolerated than topical clarithromycin. Corticosteroid use has been implicated as a contributing factor in the development of nontuberculous mycobacterial keratitis in both clinical33 and animal studies.44 The role of corticosteroids in our cases is uncertain; all patients had been treated with topical corticosteroids after surgery, and two patients were receiving topical corticosteroids at the time of diagnosis, including the patient who had areas of 90% stromal thinning in both eyes (case 3). The patient who developed infection in only 1 eye was treated with topical corticosteroids for only one week (case 1), although infection was severe in the involved eye. It is interesting to note that the patient still receiving oral corticosteroids at the time of diagnosis (case 4) was asymptomatic, while the other three patients were symptomatic.

It has been suggested that removal of the lamellar keratectomy flap may facilitate resolution of infection by improving the penetration of topical medications1 and by facilitating removal of loculated infiltrates at the level of the interface.5 Although keratitis improved in case 3 after flap removal, it cannot be determined whether improve-
treatment was due to flap removal, additional medical therapy, or both. Because the anterior stromal lamellae do not add to the structural integrity of the cornea after separation from the posterior stroma by the microkeratome, it is probably acceptable to remove the flap, if necessary for control of infection. In some cases, the flap becomes totally necrotic and nonadherent, as occurred in our case 1. In our patients with partial loss of flaps because of necrosis, there was diffuse opacification of necrotic flap tissue that had remained adherent and healed, while scarring and vascu-
larization of the interface bed appeared to be worse where overlying flap tissue had been lost.

Surgical extirpation by penetrating keratoplasty of infec-
tion has been advocated by several authors as treatment for nontuberculous mycobacterial keratitis. This procedure may be more applicable for deep corneal infections. Our series shows that resolution of infection, with good visual outcome in some cases, can result from medical therapy alone. Although recurrent infection after medical therapy has been described in cases of deep infection, we found no reports in the literature of recurrent infection in cases of post-LASIK M. chelonae keratitis that had apparently resolved with medical therapy alone.

Isolates were identical on pulsed-field gel electrophoresis testing, indicating a common source. Investigation found all infections to be associated with LASIK correction of hyperopia. The contact lens fragments used as masks during the procedures were implicated as the source of infection, although the means by which they were contaminated, and the route by which organisms were transferred from the lens fragments to the corneas, could not be determined. The association of infection with a specific shipment of contact lenses suggested the possibility of intrinsic contamination of the contact lenses, but mycobacteria could not be identified from five additional lenses with the same lot number. Laboratory evaluation of the contact lens lot was limited by the small sample size tested; average lots contain thousands of lenses, but additional lenses from the same lot were not available for testing at the time of this writing. It is also possible that the lens fragments were contaminated by the surgeon or surgical assistant, although observation of the surgeon’s hyperopic LASIK technique failed to identify a likely mechanism for preoperative or intraoperative contact lens contamination. Environmental cultures failed to reveal a source of the organisms, but cultures were not performed until more than 3 weeks after the case patients had surgery.

This cluster of nontuberculous mycobacterial keratitis cases is not unique. The CDC has received reports of at least three other recent, independent clusters of nontuber-
culous mycobacterial keratitis cases after LASIK; two occurred in the United States and one occurred in a South American country (CDC, unpublished data). Small clusters of M. chelonae keratitis have been associated with other ophthalmic procedures. Robin and associates described two cases following radial keratotomy performed by the same surgeon in the same outpatient facility. Newman and associates described three cases that occurred after a variety of invasive procedures performed in the same office. The source of infection was not determined for either cluster.

In summary, nontuberculous mycobacterial keratitis can occur in an epidemic manner after LASIK through contami-
nation of surgical materials. Although the specific source of infection was not identified in this cluster, nontuberculous mycobacteria are ubiquitous in the environment, and surgeons must remain meticulous in main-
taining sterile techniques during all aspects of LASIK and associated procedures. In addition, surgeons must be aware of the early signs of infection, which may be mistaken for diffuse lamellar keratitis. Early diagnosis, with immediate and aggressive antibiotic therapy, can lead to cure of infection. Azithromycin is an additional drug that may be useful for the treatment of nontuberculous mycobacterial keratitis, and should be investigated further.

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