ABSTRACT

PURPOSE: To describe an outbreak of diffuse lamellar keratitis (DLK) and provide a hypothesis about the etiology.

METHODS: A retrospective analysis was carried out on 328 eyes of 220 patients who underwent LASIK over 9 months. The occurrence of DLK using two different methods of cleaning and sterilizing surgical instruments and an autoclave reservoir were analyzed. Microbial analyses were carried out by two laboratories on samples obtained from the original autoclave reservoir and tubing. A chi-square test was used to compare qualitative values. The Student t test was used to compare numerical values.

RESULTS: Forty-six (24.5%) of 188 cases of DLK were diagnosed. Sphingomonas paucimobilis and Burkholderia pickettii were isolated in the reservoir of the steam sterilizer. Electron microscopy revealed gram-negative microbes on the tubing walls. After changing the reservoir of the steam sterilizer and implementing a new cleaning and sterilization protocol based on air-drying the instruments and draining and drying the reservoir of the sterilizer, the occurrence of DLK stopped. No statistically significant correlation was noted between the occurrence of DLK and gender, age, or volume of tissue removed.

CONCLUSIONS: Data obtained during this DLK outbreak support the theory that a bacterial endotoxin, which can survive short-cycle steam sterilization, could be responsible for an outbreak of DLK. We recommend cleaning and sterilization protocols based on air-drying surgical instruments and leaving the reservoirs completely dry at the end of each surgical day. [J Refract Surg. 2007;xx:xxx-xxx.]

With the increased volume of LASIK surgeries performed worldwide, a small but progressively higher number of complications are being reported. One of the most common is diffuse lamellar keratitis (DLK) or Sands of Sahara syndrome. Diffuse lamellar keratitis is characterized by a diffuse white, granular, culture-negative lamellar keratitis occurring within the first week after LASIK. Since the first published report in 1998, there have been an increasing number of reports of DLK in the medical literature. Lineberger et al proposed four stages of clinical classification taking into account the severity and location of the lamellar interface inflammation. To successfully treat DLK, it is imperative to identify DLK as soon as possible and initiate treatment with topical steroid drops (eg, prednisolone acetate 1%) hourly and steroid ointment (eg, dexamethasone 1%) at bedtime. If DLK is diagnosed as stage 3 or progresses to stage 3, surgical intervention in addition to steroid therapy is warranted. Surgical intervention involves lifting the flap and irrigating the bed and the undersurface of the flap with balanced salt solution (BSS). Untreated or severe cases may progress to flap melt with the attendant risk of significant vision loss.

A number of etiologies have been proposed for DLK such as deposits from microkeratome blades, particles from the eye drape, oil, wax, silicates, bacterial endotoxins, epithelial defects, meibomian secretions, and laser/contaminant interaction. All of these proposed etiologies suggest multifactorial causes of DLK.
Diffuse lamellar keratitis can occur sporadically or as an epidemic. Sporadic cases seem to be related to limited factors such as epithelial defects or meibomian secretions. However, larger outbreaks or clusters of this potentially sight-threatening complication present a more challenging issue due to clinical and medical implications.

Some reports have related epidemic DLK to antigenic bacterial cell-wall breakdown products in sterilization units. The hypothesis that an outbreak of DLK is an immunologic reaction to a heat-stable toxin introduced under the corneal flap has been proposed by Holland et al. They postulate that a bacterial lipopolysaccharide (endotoxin) released from gram-negative biofilms in sterilizer reservoirs can survive short-cycle steam sterilization. This toxin incites a polymorphonuclear reaction in susceptible individuals resulting in DLK.

We describe an outbreak of DLK, which, in our opinion, supports the theory that one of the main causes of epidemic DLK is the endotoxin released from gram-negative microorganisms that survive steam sterilization.

**PATIENTS AND METHODS**

Over a 9-month period, 328 eyes of 220 patients (135 women and 85 men) underwent LASIK for myopia or compound myopic astigmatism using the Summit Apex Plus excimer laser (Summit Corp, Waltham, Mass). A DLK outbreak occurred at our center over 7 months of this 9-month period. All surgeries were performed by an experienced LASIK surgeon (A.V.). Average patient age was 31.3 ± 7.2 years (range: 22 to 45 years). Preoperative examination included uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), slit-lamp microscopy, corneal topography (Zeiss-Humphrey, Jena, Germany), ultrasound pachymetry, manifest refraction, cycloplegic refraction, and a dilated fundus examination. All patients underwent bilateral or unilateral surgery based on age, occupation, lifestyle demands, and surgeon and patient preference.

**SURGICAL PROCEDURE**

The eyelids were cleaned using a Betadine (Purdue Frederick Co, Norwalk, Conn) scrub. Subsequently, the eye undergoing surgery had two drops of proparacaine instilled and a sterile drape was used to isolate the surgical field. A lid speculum was inserted to allow maximum exposure of the globe. A wet Merocel sponge (Medtronic ENT, Jacksonville, Fl) was used to clean the fornices of any debris. The Summit Krumeich-Barraquer microkeratome (SKBM; Alcon Laboratories Inc, Ft Worth, Tex) was used to create the flap. Proper alignment of the eye with the laser was achieved by aligning the microscope reticule cross-hairs and first Purkinje image of the red diode laser alignment beam in conjunction with cooperative patient fixation. The ablation was delivered to the cornea. Patients were required to fixate on a red fixation light, coaxial with the surgeons’ line of sight and the excimer laser beam, throughout the ablation. The flap was repositioned after irrigating the bed with BSS. Patients received topical fluoroquinolone antibiotic and corticosteroid drops to use four times per day for 5 days.

**POSTOPERATIVE EXAMINATIONS AND DLK TREATMENT PROTOCOL**

Postoperative examination was performed within 24 hours after LASIK. If grade 1 or higher DLK was diagnosed, patients were re-examined within the following 18 hours and daily until DLK had resolved. For stage 1 or 2 DLK, patients were treated with topical steroid drops (prednisolone acetate 1%) administered hourly and steroid ointment (dexamethasone 1%) administered at bedtime. For stage 3 DLK, the flap was relifted and the bed and undersurface of the flap were irrigated with BSS. For stage 4 DLK, a conservative treatment was initiated using prednisolone acetate 1% tapered over 2 months until BSCVA was ≥20/25 in both eyes.

**INSTRUMENT CLEANING PROTOCOL**

The cleaning and sterilization protocol prior to the outbreak of DLK involved cleaning the instruments with distilled water at the end of the surgical day, then the instruments were dried using compressed air. At the beginning of the next surgical day, the instruments were sterilized using the STATIM autoclave (STATIM 2000 S; SciCan, Toronto, Ontario, Canada). Between each patient, the surgical instruments were immersed in a peracetic acid bath (Perasafe; Biocordis, Lisses, France) for 10 minutes and then washed with BSS to remove any debris and residual Perasafe. At the end of the surgical day, the surgical instruments were not STATIM autoclaved and the SKBM handpiece was immersed in a box containing formaldehyde (solid presentation). At the beginning of the following surgical day, the SKBM handpiece was cleaned using a sterile towel. The STATIM reservoir was not drained or dried at the end of the surgical day. However, due to the volume of surgeries, and the rate of turnover of instruments, the STATIM reservoir was consistently refilled with tap water.

Just prior to the DLK outbreak, the SKBM handpiece had broken and surgeries were cancelled for 17 days prior to receiving a replacement handpiece. Five months later, service technicians cleaned the STATIM
unit, changed the reservoir, and changed the silicone tube that connected the reservoir to the autoclave unit. Samples for microbial analysis were taken from the old reservoir and the old tubing. The samples were transferred to sheep blood agar, chocolate poliViteX agar (Biomerieux, l’Etoile, France), and MacConkey agar media. The colonies of microorganisms that grew in these media were transferred to Thioglycollate broth medium. Half a milliliter of this medium was transferred onto Columbia CNA solid agar medium and the growth was measured in colony-forming unit per milliliter (CFU/mL). Half of the silicone tubes were sent to Simon Holland, MD, at the University of British Columbia (Vancouver, British Columbia, Canada) for independent microbial analysis and electron microscopy of the tubing.

Once the reservoir and tubing had been replaced (after the DLK outbreak), a new cleaning and sterilization protocol was instituted—sterile water was used instead of distilled water to clean instruments, sterile water was used instead of distilled water in the reservoir, and the instruments were STATIM autoclaved between each patient. At the end of the surgical day, the reservoir was drained and any residual fluid was removed by aspiration or by using a wet-dry vacuum. At the end of the surgical day, the instruments were cleaned with sterile water and air-dried using compressed air and left in a dry autoclave cassette until the following surgical day. At the beginning of the next surgical day, the STATIM reservoir was filled with sterile water and the instruments were sterilized.

### Statistical Analysis

Statistical comparisons were done using SPSS software for Windows (SPSS Inc, Chicago, Ill). A chi-square test was used to compare qualitative values such as the presence of DLK with gender. The Student t test was used to compare numerical values such as the presence of DLK with age or the volume of corneal tissue removed due to laser ablation. A P value <.05 was considered statistically significant.

### Results

One hundred eighty-eight eyes of 135 patients (82 women and 53 men) underwent LASIK for myopia or compound myopic astigmatism during the 7-month period of DLK outbreak after replacement of the SKBM handpiece. Average patient age was 30.9±7.5 (range: 23 to 45 years). A total of 24.5% (46/188 eyes) of eyes were diagnosed with DLK. Seventeen cases were classified as stage 1, 15 cases were stage 2, 12 cases were stage 3, and 2 cases were stage 4. A number of cases with peripheral infiltrates that did not develop into DLK were seen and classified as DLK grade 0.5; however, these were not included in the statistical analysis. All cases were treated, based on the stage of DLK, as outlined in the Patients and Methods section. The Figure shows the distribution of the entire outbreak.

Microbial samples from the reservoir and tube yielded 960 CFU/mL of *Sphingomonas paucimobilis*. The University of British Columbia isolated *Burkholderia pickettii*. Scanning electron microscopy of the tube
sample carried out at the University of British Columbia found biofilm and gram-negative microorganisms on the tube walls.

No statistically significant association was noted between the occurrence of DLK and gender, age, or volume of tissue ablated. The microbial analyses were compared to those from an objective second party (University of British Columbia). The presence of gram-negative microorganisms and their endotoxins was demonstrated using microbial analyses and electron microscopy. Gender, age, and volume of tissue ablated were not associated with the occurrence of DLK. The results from this study are consistent with the hypothesis that endotoxin released from gram-negative microorganisms can cause an epidemic of DLK.

Diagnosis and treatment of any case of DLK is important as it is a potentially sight-threatening complication of LASIK. In addition to the clinical complications, there can be medicolegal consequences as well as economic consequences due to the negative publicity for the center involved and for refractive surgery in general. Hence, it is imperative to immediately diagnose, treat, and identify the sources responsible for outbreaks of DLK.

The outbreak of DLK at our center was likely due to stagnant water in the STATIM reservoir, which allowed gram-negative microbes to replicate during the 17-day hiatus from surgery. Our regular cleaning and sterilization routine prior to the DLK outbreak did not involve vacuuming any residual liquid at the end of the surgical day as there was constant turnover of water due to the number of procedures being performed on a regular basis. Seventeen days would have been ample time for the microbes to form biofilm and release toxins within the reservoir. By the time surgery resumed, the reservoir water was highly contaminated with gram-negative microbes from which biofilm and toxins were liberated during the sterilization process, coating the surgical instruments and subsequently causing the DLK. It was during the initial days when surgery resumed that we found the highest rates of DLK presenting at our center. Once surgery resumed to regular levels, the number of DLK cases reduced. This was likely due to constant turnover of water after every surgical day. This constant turnover acted to dilute the underlying contaminants. The observation that the majority of DLK cases presented at resumption of surgery after the 17-day hiatus is, in our opinion, evidence that the stagnant water was highly contaminated and that the contaminants are progressively cleared with each sterilization cycle.

We believe there is an individual susceptibility to develop DLK under the same stimulus. It has been shown, for example, that atopy is a risk factor in the development of DLK. Although we cannot definitively establish a cause of contamination of the STATIM reservoir, analyses of the tap water and sterile water found 4.42 CFU/mL of endotoxin in the tap water and <0.005 CFU/mL in the sterile water. It should be noted that we elected to refill the reservoir with tap water prior to the DLK outbreak.

The features of this outbreak support the mechanism of DLK proposed by Holland et al. Taking into account the chronology of the entire outbreak, the microbes isolated, the location of the microbes in the reservoir and silicone tubing, and its termination with the implementation of a new cleaning and sterilization protocol is supportive of a bacterial lipopolysaccharide (endotoxin derived from the cell walls of gram-negative microorganisms, which can survive short-cycle steam sterilization) as the antigen that incites a polymorphonuclear reaction responsible for the clinical signs of DLK. The fact that our laboratory isolated different microorganisms (Sphingomonas paucimobilis) than the University of British Columbia (Burkholderia pickettii) is likely the result of the more stringent microbial analyses on the part of the University of British Columbia. Sphingomonas and Burkholderia belong to the same family—aerobic gram-negative bacilli, non-enterobacteriaceae, nonfermentative, catalase positive, oxidase variable.

Peters et al demonstrated that endotoxins are capable of inducing interface inflammation in a rabbit model and may therefore be a significant factor in epidemic DLK. They postulate two possible sources of bacterial contamination of the sterilization units: bacteria may arise from the water supplied directly into the unit or the steam sterilizer may be contaminated by residual bacteria that survive the cleaning of the LASIK instruments. Most of these bacteria are heat sensitive and would be destroyed by the sterilization process; the endotoxins, however, would survive and may be transmitted via steam onto the surgical instruments. Peters et al recommend air-drying the instruments prior to sterilization to lower the endotoxin load introduced into the STATIM. They also recommend draining the reservoirs after every surgical day to reduce the time in which the bacteria would be allowed to replicate inside the reservoir. Another study recommended that refractive surgery centers avoid stagnant fluids in their instrument cleaning and sterilizing protocols to
minimize the occurrence of DLK outbreaks.\textsuperscript{11} Based on these recommendations, we incorporated air-drying and draining the reservoir in our protocol after the DLK outbreak to reduce the likelihood of contamination.

Since we began the new cleaning and sterilization protocol, there have been no cases of DLK at our center ($P<.0001$). One criticism of this study may be the fact that we did not account for other factors that could cause DLK (ie, meibomian gland dysfunction, epithelial defect). However, our intention was to describe the DLK outbreak and to establish a likely hypothesis based on objective clinical data—that this outbreak initially originated due to a steam sterilizer (STATIM) whose reservoir was contaminated with gram-negative microorganisms. Further proof is required to definitively validate this hypothesis; however, our results confirm the observations of at least two separate but similar studies.\textsuperscript{6,10}

Although most of our treatment regimen was well within accepted guidelines, we elected conservative treatment of stage 4 DLK. Some surgeons advocate flap relift and irrigation if stage 4 DLK is present on day 4 to reduce the visual morbidity. However, in the stage 4 DLK cases that presented at our center, we believed an inherent risk of stromal volume loss existed due to the high density of central infiltrate, hence we treated with steroids tapered over 2 months until the final BSCVA was 20/25 in both eyes. A variety of treatments for the different stages of DLK have been described; however, at the time of our DLK outbreak, these treatments were not published or widely available.\textsuperscript{12-14}

We recommend the implementation of protocols that incorporate air-drying the microkeratome head and surgical instruments, draining the reservoir of the steam sterilizer, and “aspirating” the sterile water at the end of each surgical session. Given that any container storing water has the potential to develop gram-negative bacterial biofilms, Holland et al\textsuperscript{6} suggest that it would be beneficial to mechanically scrub the reservoir and to fill it with isopropyl alcohol (70\%) at the end of each surgical session. Although we agree with this suggestion, we believe that periodic scrubbing and isopropyl alcohol treatment, perhaps every 6 months, should suffice. If an outbreak of DLK is detected, we recommend canceling surgery, obtaining microbial samples from the reservoir and the silicone tubing between the reservoir and the autoclave, and replacing the reservoir and tubing immediately.

The data presented in this study indicate that one possible cause of epidemic DLK is the endotoxin released from gram-negative microorganisms.

**REFERENCES**