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Incidence and outcomes of positive donor rim cultures and infections in the Cornea Preservation Time Study

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Abstract

Purpose—Assess donor rim culture results and outcomes of ocular infections in the Cornea Preservation Time Study (CPTS).

Methods—Donor corneal rim cultures were optional. Donor characteristics were assessed for association with positive cultures using Fisher exact test and Poisson regression analyses. Incidence rates of ocular infections were estimated and 95% confidence intervals were calculated.

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Results—Cultures were performed in 784 (58.9%) of the 1330 cases. For the 0–7 day vs. 8–14 day PT groups, respectively, positive fungal growth occurred in 10 of 397 (2.5%) vs. 5 of 387 (1.3%) corneas ($p=0.30$), while positive bacterial cultures occurred with 6 of 397 (1.5%) vs. 4 of 387 (1.0%) corneas ($p=0.75$). Surgeon-prepared tissue remained a significant risk for positive fungal cultures [RR of surgeon- vs eye-bank-prepared, 2.9; 95% CI (1.02, 7.98)], while younger donors [RR per year of age, 0.96; 95% CI (0.93, 1.00)] and accidental death donors [RR of accident vs disease, 3.7; 95% CI (1.36, 10.13)] were at greater risk for positive bacterial cultures. Fungal infection (*Candida glabrata*) developed in 1 (6.7%) of 15 recipients with a positive fungal culture, and no recipient infections occurred with positive bacterial culture. With one additional fungal keratitis (*Candida albicans*) and one bacterial endophthalmitis (*E. coli*) with no rim culture performed, a total of 2 of 1330 eyes (0.15%) fungal and 1/1330 eyes (0.08%) bacterial post-keratoplasty infections occurred.

Conclusion—Longer PT was not associated with a higher rate of positive donor rim cultures. The overall rate of infection across the entire cohort was low.

Keywords

infectious keratitis; endophthalmitis; endothelial keratoplasty

INTRODUCTION

Infectious keratitis and endophthalmitis are rare but devastating complications after keratoplasty. Risk factors include contaminated donor corneal tissue or late inoculation due to sutures, epithelial defects, and use of topical corticosteroids.^{3,4} Despite the use of aseptic conditions for corneal graft procurement, irrigation of the donor ocular surface with antiseptics (e.g. povidone-iodine) or saline prior to procurement, and the use of antibiotic-containing storage solutions, the incidence of corneal graft contamination has been reported to be as high as 20%, driven by bacterial contamination of donor rims.⁵ The risk of fungal keratitis has been reported to be more than three times that of bacterial keratitis for corneas preserved for four or more days.⁶ Fungal contamination of donor rims occurs in 1–2% of cases while establishment of fungal infections in eyes that received contaminated tissue may occur in up to about 7% of cases.^{7–10} Case reports and series highlight the risk of receiving fungal contaminated tissue and the belief that patients receiving donor tissue with fungal positive rim cultures should be prophylactically treated with antifungal therapy.^{11,12} Positive donor corneal rim fungal cultures at the time of keratoplasty may be more predictive of infectious keratitis and endophthalmitis as compared to positive bacterial rim cultures.⁷ Finally, a trend towards more post-keratoplasty fungal infections after Descemet's stripping automated endothelial keratoplasty (DSAEK) with eye-bank-processed donor tissue compared to surgeon-processed donor tissue keratoplasty was noted in an unpublished report by the Medical Advisory Board Subcommittee of the Eye Bank Association (EBAA) (Aldave, personal communication, December 2017), and a 2017 publication showing that there is an increased risk of positive fungal rim cultures in tissue processed by eye banks for endothelial keratoplasty (EK) compared to unprocessed tissue.¹⁰

The Cornea Preservation Time Study (CPTS) was designed to examine graft success and endothelial cell loss and its relation to preservation time (PT) following DSAEK in a multi-

center, randomized clinical trial.¹ Given the concern at the outset of the study in 2011 of a potentially higher risk for fungal keratitis with longer PT in part due to the lack of anti-fungal agents in the storage solution, the development of infectious keratitis or endophthalmitis was closely monitored in the CPTS. Subsequently, with multiple reports,¹³ (Aldave, personal communication, December 2017) raising concerns regarding a possibly higher fungal infection rate in eye-bank prepared donor tissue, further analysis of bacterial and fungal culture results obtained in the CPTS was warranted. We hypothesized that fungal contamination and infection would not be more prevalent in the longer PT group. The purpose of this study is to report the incidence and predictive factors of positive donor corneal rim bacterial and fungal cultures and recipient infectious keratitis and endophthalmitis in the CPTS.

METHODS

Details of the CPTS protocol and primary results related to graft success and endothelial cell loss following DSAEK have been described previously.^{1,2,14} The protocol was approved by institutional review boards at each investigational site, and individual participants gave written informed consent to participate in the study. In brief, participants were enrolled by 70 surgeons across 40 clinical sites, and donor corneas were provided by 23 eye banks across the United States between May 2012 and April 2014. CPTS participants were 42 to 90 years old (median age 70 years) with a corneal disease associated with endothelial dysfunction (94% Fuchs dystrophy and 6% pseudophakic/aphakic corneal edema). 1330 eyes were assigned to receive a donor cornea with PT of 0–7 days (“0–7d PT group”) or 8–14 days (“8–14d PT group”); in participants with both eyes eligible, the first eye was randomly assigned and the second eye was assigned to the other group. Assigned corneas were from donors 12 to 75 years old (median age 61 years), with an eye-bank measured central ECD with a minimum of 2300 cells/mm², a median PT of 6 days in the 0–7d PT group and 11 days in the 8–14d PT group, and 74% were eye-bank prepared (versus surgeon-prepared) for DSAEK. Eye-banks utilized their standard routines for procuring and preparing the tissue. Clinical investigators and participants were masked to all characteristics of the donor cornea including age and PT. Preoperative management, surgical technique, and postoperative care, including prescription of medications, were provided according to each investigator’s standard care.

Donor corneal rim bacterial and fungal culture results were collected when performed by 43 of the 70 (61.4%) surgeons at 26 of the 40 (65.0%) clinical sites as part of the surgeon’s routine and were not a protocol requirement. Donor corneas that were cultured were provided by 17 of the 23 (73.9%) eye banks in the CPTS. Sites provided data on specimen type (donor rim tissue and/or storage solution), culture results (positive growth or no growth), and treatment initiated based on positive cultures. We did not include data if only storage solution (without rims) was cultured. If growth was positive, for each specimen cultured, the name of each organism and the quantification of growth were obtained from the laboratory report. Cases of ocular infections were identified and data were collected regarding characteristics of the adverse reactions.

Statistical Analysis

The incidence of positive cultures in each PT group was computed as the number of positive cultures (bacterial or fungal) divided by the total number of cultures. Potential risk factors including death to preservation, PT, type of storage solution, refrigeration, donor age, donor history of diabetes, cause of death, and preparer of lamellar dissection (eye bank technician or surgeon) were assessed for association with incidence of positive cultures using Fisher exact test and Poisson regression analyses adjusted for correlation in culture results between the eyes of donors contributing two corneas using generalized estimating equations (GEE).¹⁵ Factors from univariable analysis with $p < 0.10$ were evaluated in a backward multivariable model selection procedure, retaining factors with $p < 0.05$ in the model. Sensitivity analyses were performed to check whether results changed meaningfully when excluding all cultures from one surgeon who performed 27.6% (216/784) of the surgeries in the culture cohort. The agreement between two corneas from the same donor of the incidence of a positive culture was assessed using the Kappa statistic.

95% confidence intervals (CIs) for the proportion of eyes developing post-DSAEC infections were calculated using exact methods for binomial proportions.¹⁶ Evaluation of potential risk factors for infection was limited by the low number of infections. Statistical analyses were conducted using SAS software (version 9.4, SAS Inc., Cary, NC).

RESULTS

Of the 675 study eyes assigned to the 0–7d PT group and 655 assigned to the 8–14d PT group, donor rim cultures were performed in 397 (58.8%) and 387 (59.1%) eyes, respectively. Of the 836 corneas transplanted by the 43 surgeons who provided culture results on 1 or more corneas, 784 (93.8%) had donor rim cultures performed. The donor characteristics were balanced between PT groups in this ancillary cohort (Table 1). 743 of the 784 donor corneas (94.8%) with donor rim cultures performed were stored in Optisol GS (Bausch and Lomb, Irvine, CA), while 41 (5.2%) were stored in Life 4°C (Numedis, Isanti, MN). Of the donor corneas with donor rim cultures, 523 (66.7%) had lamellar dissection performed by eye bank technicians, and 261 (33.3%) had lamellar dissection performed by the surgeon. A listing of all organisms identified and the quantified growth as specified by the microbiology laboratory are tabulated in Table 2.

Positive fungal cultures were obtained in 10 (2.5%) donor rims in the 0–7d PT group and 5 (1.3%) donor rims in the 8–14d PT group; positive bacterial cultures were obtained in 6 (1.5%) donor rims in the 0–7d PT group and 4 (1.0%) donor rims in the 8–14d PT group with no significant effect of PT on incidence of positive cultures ($p = 0.30$ for positive fungal cultures; $p = 0.75$ for positive bacterial cultures, Table 3). There was also no significant effect of PT on positive bacterial or fungal cultures when evaluated as continuous days ($p = 0.46$ for positive fungal cultures; $p = 0.38$ for positive bacterial cultures). The incidence of positive fungal cultures was 1.1% (6/523) in the eye-bank-prepared group, and 3.4% (9/261) in the surgeon-prepared group [relative risk of surgeon-prepared vs eye-bank-prepared of 2.9; 95% confidence interval (1.02, 7.98, Table 3). The only factor remaining in the multivariable model for positive fungal culture was the preparer of the lamellar dissection (higher if surgeon prepared). No association of positive fungal culture with PT was detected after

adjustment for the preparer of the dissection. For positive bacterial cultures, in univariate analyses, younger donors and donors with death due to accident were at greater risk. Donor age [relative risk per year of age of 0.96; 95% confidence interval (0.93, 1.00)] and cause of donor death [relative risk of accident vs disease of 3.7; 95% confidence interval (1.36, 10.13)] remained significant and were each included in the final multivariable model for positive bacterial culture. No association with PT was detected after adjustment for these factors. Results were similar in the sensitivity analyses when the data of a single high enrolling surgeon were removed.

Overall there were 60 donor mates (120 tissues) in this culture cohort. Of those 120, five corneas had positive culture results. Among those 5 corneas, 2 were from the same donor. No statistically significant association within donor mates were found ($p=0.10$).

Of the 15 recipients of corneas with positive donor rim fungal cultures, 8 received no additional prophylactic medical treatment (including Case 1 below initially), 3 received oral fluconazole, 1 received topical amphotericin B and natamycin, and 3 received topical voriconazole. Only one of these eyes (Case 1 below) went on to develop infectious keratitis or endophthalmitis. Therefore, the incidence of post-keratoplasty infection in eyes with a positive donor rim fungal culture was 1/15 (6.7%) with 95% CI 0.2% to 32.0%. Of the 10 recipients of corneas with positive donor rim bacterial cultures, none had an alteration of their standard post-operative antibiotic therapy. None of the eyes developed infectious keratitis or endophthalmitis.

In addition to fungal keratitis developing in one of the 15 recipients receiving a donor tissue with a positive fungal culture, one eye developed fungal keratitis and another developed bacterial endophthalmitis following transplantation of a donor cornea for which a donor rim culture was not performed. This totals 2 of 1330 CPTS surgical eyes (0.15% with 95% CI 0.02% to 0.54%) that developed infectious keratitis (one secondary to *Candida albicans* and one secondary to *Candida glabrata*), with both cases in the 8–14d PT group while one of the 1330 eyes (0.08% with 95% CI 0% to 0.42%) developed endophthalmitis (secondary to *E. coli*), which was in the 0–7 d PT group. Donor rim cultures were obtained only in the infectious keratitis case secondary to *Candida glabrata*.

Although the incidence of post-keratoplasty infections is low, the three infection cases encountered in the CPTS are described below to illustrate varying clinical course and management approaches.

Case reports

Case 1—A 76-year-old man with bilateral pseudophakia and Fuchs dystrophy underwent uneventful DSAEK of the right eye. The donor tissue was stored in Optisol GS and prepared by the eye bank with a PT of 13 days. Perioperative treatment included betadine 5% preparation and postoperative treatment with ofloxacin 0.3% and prednisolone acetate 1% four times per day for 1 week, at which point the ofloxacin was discontinued. The graft was attached at 1 day and 1 week with clearing recipient stromal edema. The donor rim culture was positive for moderate growth of *Candida glabrata* at 1 week; neither topical nor systemic anti-fungal treatment was initiated at the time of receipt of the positive culture results.

One month after surgery, an opacity at the peripheral donor-recipient interface measuring 1mm was noted with no edema or anterior chamber inflammation (Figure 1a). The patient was continued on prednisolone acetate 1% four times per day with close observation. Three weeks later, a larger superotemporal infiltrate in the recipient-donor interface extending into the recipient corneal stroma peripheral to the donor, diffuse keratic precipitates and cells in the anterior chamber were observed (Figure 1b). Topical amphotericin 0.15% and oral fluconazole 150 mg were started. Four days later, the graft was removed (but not replaced) along with injection of intracameral amphotericin 5 µg/0.1 ml. Bacterial and fungal cultures of the removed graft, posterior stromal surface curettage, and aqueous humor grew *Candida glabrata*, sensitive to fluconazole and voriconazole. Histopathologic examinations showed active inflammation with fungal yeast forms identified by special stain. The topical amphotericin and oral fluconazole were continued during follow-up while the cornea remained edematous; however, there was no recurrence of infection. Seven weeks after removal of the DSAEK graft, the patient underwent an uneventful replacement with a second DSAEK graft. Three years after re-graft, the cornea is clear with best-corrected visual acuity of 20/25. No complications were reported with the mate cornea, which was not enrolled in CPTS and for which rim cultures were not reported.

Case 2—A 52-year woman underwent uncomplicated DSAEK for Fuchs dystrophy with cataract extraction and intraocular lens placement in the left eye. The donor tissue was stored in Optisol GS and prepared by the eye bank with a PT of 9 days. Post-operative medications included topical besifloxacin 0.6%, difluprednate 0.05%, cyclogyl 1%, and sodium chloride 5%. There was no history of trauma post-operatively.

On the one-day postoperative visit, the graft was attached with an expected degree of mild post-operative inflammation. Eleven days postoperative on the same medications, the eye was still mildly injected with mild cells in the anterior chamber. At the interface between the host and donor cornea were several opacities, but no obvious recipient stromal infiltrate.

Thirteen days postoperative the graft was in place, the recipient and donor stroma were equivocally cloudy with no progression of the interface opacities, but the inflammation had not diminished. The opacities were not near any of the incisions. A diagnosis of fungal keratitis was suspected and the patient was started on topical voriconazole 1% and moxifloxacin 0.5% (in the event of an atypical mycobacterial infection).

The following week, the recipient and donor stroma remained equivocally cloudy as defined in the CPTS criteria for stroma clarity,¹ excessive (2+ cells/flare) intraocular inflammation was present, and increasing corneal inflammation and presumed infectious interface infiltrates were noted. Nineteen days postoperative a repeat DSAEK and cultures of the explant were performed. The explanted tissue was positive for *Candida albicans* sensitive to voriconazole and amphotericin. Histopathologic examination showed PAS positive budding organisms (consistent with fungi).

Twenty-five days after the initial DSAEK surgery, inflammation persisted (Figure 2) and a penetrating keratoplasty (PKP) was subsequently performed. Postoperative antimicrobials included topical voriconazole and amphotericin. The explanted tissue was positive for

Candida albicans. Histopathologic examination from the PKP showed multiple fungal elements with budding forms, hyphae, and pseudomycelium in the donor graft/host cornea interface. An intraocular pressure elevation was treated with topical aqueous suppressants and oral acetazolamide. One week after the PKP the intraocular pressure normalized and there were no further complications. The patient recovered well with no recurrence of infection as monitored by the Coordinating Center up to one year following the PKP.

The mate tissue, preserved for 6 days, was also used for EK on a non-study patient at another practice. Culture of the donor rim was not performed for either tissue. The recipient of the mate tissue also developed a presumed fungal keratitis with the explanted tissue positive for *Candida albicans*.

Case 3—A 78-year-old man with bilateral pseudophakia, Fuchs dystrophy and history of DSAEK in the contralateral eye underwent uneventful DSAEK using donor tissue that had been stored in Optisol GS and prepared by the eye bank with a PT of 4 days. Perioperative treatment included three doses of moxifloxacin 0.5%, betadine 5% preparation and tobramycin/dexamethasone ointment. Donor rim cultures were not performed. One day postoperative, the vision was count fingers at 2 feet, the donor graft was attached with equivocally cloudy recipient stroma, trace anterior chamber reaction and a 60% air bubble. Prednisolone acetate 1% QID, moxifloxacin 0.5% QID, tobramycin/dexamethasone ointment QHS, and methylprednisolone dose pack were initiated. The patient presented four days after surgery due to concern for increased redness with vision of 20/600, equivocally cloudy recipient stroma, 4+ cells and 0.5 mm hypopyon. A diagnosis of endophthalmitis was made followed by a vitreous tap and intravitreal injection of vancomycin 1 mg and ceftazidime 2.25 mg. At this visit moxifloxacin was increased to every 2 hours along with prednisolone acetate 1% every 2 hours while awake. Culture of the vitreous aspirate grew *E. coli* sensitive to cefazolin (MIC 8), tobramycin (MIC 2) and gentamicin (MIC 2) with resistance to ciprofloxacin (MIC >2) and levofloxacin (MIC >4). With this information, the topical antibiotic was switched to tobramycin 0.3% every 2 hours for two weeks and then reduced to 4 times daily for weeks and then stopped. One week after the surgery, the vision improved to 20/200 with trace cell, mild graft edema, and clear recipient stroma. Two and half years after surgery, the graft remains clear with vision of 20/25. The mate cornea was not transplanted due to scarring.

DISCUSSION

The CPTS is a multi-center, prospective study focused on assessing impact of donor tissue PT on graft success following DSAEK.¹ It has provided an opportunity to assess incidence and outcomes of positive rim cultures and infections after DSAEK. With the completion of the study and report of primary results,^{2,14} we have been especially interested in examining both the bacterial and fungal rim culture and infection rates related to PT with the longer PTs performed in this study beyond the current PT practice in the United States of 7 to 8 days. Given that Hassan and Wilhelmus reported a more than three-fold increased risk of fungal endophthalmitis compared to bacterial endophthalmitis among corneas with longer PT,⁶ our investigator group was pleased that in the CPTS there was no impact of PT on the incidence of either positive bacterial or fungal rim cultures or postoperative infections.

In the past 10 years EK has surpassed PKP as the most commonly performed keratoplasty procedure for endothelial dysfunction in the United States.¹⁷ This change in the surgical approach over the last decade was the main reason why DSAEK was the only keratoplasty procedure utilized in the CPTS. However, while fungal keratitis is a rare but potentially devastating cause of vision loss following keratoplasty, the incidence after EK is up to two times greater than after PKP.¹³ The incidence of fungal keratitis following EK has increased nearly 3-fold from 0.012% in 2007 to 0.039% in 2013.¹³ Potential factors that may be contributing to this increase include a lamellar interface that may entrap infectious organisms, increased awareness of interface opacities with higher reporting, prolonged death-to-PT, an increased incidence of morbidities in corneal donors, and prolonged time of donors on ventilator support.^{12,18,19}

Of note, in an unpublished 2015 study, a trend (which was not statistically significant) towards greater incidence of fungal keratitis and endophthalmitis after DSAEK was reported when the lamellar dissection was carried out by the eye bank compared to being performed by the surgeon (Aldave, personal communication, December 2017). In a 2017 study utilizing a 2013 database, donor rim cultures were three times more likely to be positive for fungi in EK-processed eyes than for other uses (1.14% vs 0.37%; $p=0.009$).¹⁰ The leading hypothesis for this increase with EK tissue processed by the eye bank is the need for extended time for tissue warming in order to perform the lamellar dissection and obtain a post-preparation endothelial cell image^{10,20}. In contrast, the CPTS data indicate a lower rate of positive fungal donor rim cultures with eye bank prepared tissue as compared to surgeon prepared tissue. Usual tissue processing was followed and tracked by each eye bank in the CPTS, but not defined by the study. A possibility is that this rigorous tracking may have promoted greater efficiency and attention to reduce warming times. Previous studies indicating increased donor culture rim positivity in eye bank prepared tissue were single center,^{8,9} multi-center/single eye bank,¹⁰ or survey-based¹³ unlike the prospective, multi-eye-bank and multi-surgeon CPTS¹. Additional multi-surgeon, multi-center and multi-eye bank studies are needed to further determine the impact of eye bank prepared DSAEK tissue on donor rim fungal culture rates and fungal infection after DSAEK.

Our study has shown that tissue from younger donors or donor death due to accidents may have higher probability of having positive donor rim bacterial cultures, but not fungal cultures. Conversely, the Eye Bank Association of America Online Adverse Reaction Reporting System shows higher association of fungal infections with older donor age and systemic disease.²¹ Although traumatic deaths are more common at a younger age, the impact of trauma on donor rim bacterial cultures in the CPTS was independent of age. The most common cause of traumatic deaths is reported to eye banks as anoxia which may be due to drowning and is associated with a higher incidence of bacterial contamination of the ocular surface.²²

Donor-to-host microbe transmission is approximately 12 to 22 times greater in cases of a positive donor cornea rim culture.^{23,24} The overall rate of positive donor rim fungal cultures in the CPTS was 1.9% with postoperative recipient fungal infections developing in 6.7% of corneas with positive cultures. This finding is similar to other studies with an incidence of 1.1–2.1% for positive donor rim fungal cultures resulting in a rate of up to 7.5%

postoperative recipient fungal infections if fungal contaminated tissue was received (Table 4).^{6–10,19,25–29} Because of the small number of treated infections in the CPTS, there are insufficient data to determine if treatment in response to a positive fungal or bacterial culture has impact in the prevention or amelioration of infection. These studies have prompted interest in decreasing the risk of donor-to-host transmission of fungal infection by adding antifungal medication to the corneal storage solution at 4–8°C.^{8,13,30,31} For example, Ritterband et al reported the use of voriconazole to significantly reduce the rate of positive fungal donor rim cultures.⁸ Layer et al found that the addition of amphotericin B significantly decreased contamination with *Candida* species.³⁰ In 2017, the Eye Bank Association of America requested and funded a number of research grant proposals on the safety, efficacy, and cost effectiveness of antifungal supplementation of intermediate-term cold storage solutions, which may result in the recommendation that an antifungal medication be added to intermediate-term cold storage solutions.

Fungal keratitis following EK may result in vision loss and has the potential of compromising the integrity of the globe.³² Due to the severity of these complications, first line treatment includes systemic, topical, intrastromal and intracameral antifungal medication.^{33,34} Failing medical therapy, surgical intervention, including lamellar graft exchange and penetrating keratoplasty, is warranted.^{32,33} Therapeutic keratoplasty aims to remove active or recalcitrant infection and restore the corneal surface.^{25,32} Anatomic cure after therapeutic keratoplasty ranges from 86%–100%; however, functional success rate varies from 13.3% to 60%.³⁵ In the CPTS, both fungal keratitis cases had their infections managed successfully with anti-fungal therapy and either removal of the infected donor tissue and subsequent DSAEK or with PKP.

Among several published reports of fungal keratitis after EK, the majority indicate that PKP is the definitive treatment.^{3,4,32,36–41} In a publication on interface infection following DSAEK, Nahum et al reported positive outcomes for 3 cases that underwent early PKP for treatment of fungal keratitis.³² Fewer authors report a preference of lamellar keratoplasty or intrastromal injections to PKP.¹² One reason is that lamellar keratoplasty may not effectively remove the full thickness of infected cornea, especially if the interface or recipient tissue is involved.^{32–34} However, the removal of the graft lenticule may reduce the size of the infection and therefore reduce the risk of endophthalmitis.^{4,36} Debridement of the posterior stroma may fully eradicate the infection after extraction of the infected donor tissue, but the timing of this approach is not well established. Intrastromal antifungal injection may be effective in late-onset fungal keratitis, where injection results in high and persistent stromal drug concentration and a maximal exposure of the antifungal agent to the interface lesion.⁴²

A limitation of this study is that corneal rim cultures were not required in all cases in the CPTS and the methodology for collection was not standardized. However, the possibility of bias from selection of corneas for culture is low because the surgeons who contributed results from culture of donor rims in the study did so for the vast majority (94%) of the corneas that they transplanted in the CPTS. The overall incidence of rim culture positivity and postoperative recipient infection was low in the CPTS and determining risk factors for either will require larger sample sizes for evaluation.

In summary, longer PT was not associated with a higher incidence of positive rim cultures. Although positive cultures were detected in about 3% of donor rims cultured, the overall rate of postoperative recipient infection was very low. Use of donor rim cultures especially in EK is recommended in order provide early assessment of and intervention in infections.

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References

1. Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. *Cornea*. 2015; 34:601–608. [PubMed: 25850706]
2. Rosenwasser GO, Szczotka-Flynn LB, Ayala AR, et al. Effect of Cornea Preservation Time on Success of Descemet Stripping Automated Endothelial Keratoplasty: A Randomized Clinical Trial. *JAMA Ophthalmol*. 2017; 135:1401–1409. [PubMed: 29127431]
3. Koenig SB, Wirostko WJ, Fish RI, et al. Candida keratitis after descemet stripping and automated endothelial keratoplasty. *Cornea*. 2009; 28:471–473. [PubMed: 19411972]
4. Ortiz-Gomariz A, Higuera-Esteban A, Gutierrez-Ortega AR, et al. Late-onset Candida keratitis after Descemet stripping automated endothelial keratoplasty: clinical and confocal microscopic report. *Eur J Ophthalmol*. 2011; 21:498–502. [PubMed: 21240860]
5. Kiatos E, Armstrong JJ, Hutnik CM, et al. The value of corneal rim cultures in keratoplasty: a systematic review and cost-effectiveness analysis. *Clinicoecon Outcomes Res*. 2017; 9:459–474. [PubMed: 28848354]
6. Hassan SS, Wilhelmus KR. Medical Review Subcommittee of the Eye Bank Association of A. Eye-banking risk factors for fungal endophthalmitis compared with bacterial endophthalmitis after corneal transplantation. *American journal of ophthalmology*. 2005; 139:685–690. [PubMed: 15808165]
7. Wilhelmus KR, Hassan SS. The prognostic role of donor corneal rim cultures in corneal transplantation. *Ophthalmology*. 2007; 114:440–445. [PubMed: 17324694]
8. Ritterband DC, Shah MK, Meskin SW, et al. Efficacy and safety of voriconazole as an additive in Optisol GS: a preservation medium for corneal donor tissue. *Cornea*. 2007; 26:343–347. [PubMed: 17413963]
9. Vislisel JM, Goins KM, Wagoner MD, et al. Incidence and Outcomes of Positive Donor Corneal Rim Fungal Cultures after Keratoplasty. *Ophthalmology*. 2017; 124:36–42. [PubMed: 27817919]
10. Brothers KM, Shanks RMQ, Hurlbert S, et al. Association Between Fungal Contamination and Eye Bank-Prepared Endothelial Keratoplasty Tissue: Temperature-Dependent Risk Factors and Antifungal Supplementation of Optisol-Gentamicin and Streptomycin. *JAMA Ophthalmol*. 2017; 135:1184–1190. [PubMed: 28973097]
11. Al-Assiri A, Al-Jastaneiah S, Al-Khalaf A, et al. Late-onset donor-to-host transmission of Candida glabrata following corneal transplantation. *Cornea*. 2006; 25:123–125. [PubMed: 16331055]
12. Kitzmann AS, Wagoner MD, Syed NA, et al. Donor-related Candida keratitis after Descemet stripping automated endothelial keratoplasty. *Cornea*. 2009; 28:825–828. [PubMed: 19574899]
13. Aldave AJ, DeMatteo J, Glasser DB, et al. Report of the Eye Bank Association of America medical advisory board subcommittee on fungal infection after corneal transplantation. *Cornea*. 2013; 32:149–154. [PubMed: 23051906]

14. Lass JH, Benetz BA, Verdier DD, et al. Corneal Endothelial Cell Loss 3 Years After Successful Descemet Stripping Automated Endothelial Keratoplasty in the Cornea Preservation Time Study: A Randomized Clinical Trial. *JAMA Ophthalmol.* 2017; 135:1394–1400. [PubMed: 29127432]
15. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika.* 1986; 73:13–22.
16. Morisette JT, Khorram S. Exact binomial confidence interval for proportions. *Photogrammetric engineering and remote sensing.* 1998; 64:281–282.
17. Eye Bank Association of America. [Accesses August 2, 2017] 2016 Eye Banking Statistical Report. 2017. <http://restoresight.org/wp-content/uploads/2016/03/2015-Statistical-Report.pdf>
18. Rehany U, Balut G, Lefler E, et al. The prevalence and risk factors for donor corneal button contamination and its association with ocular infection after transplantation. *Cornea.* 2004; 23:649–654. [PubMed: 15448488]
19. Keyhani K, Seedor JA, Shah MK, et al. The incidence of fungal keratitis and endophthalmitis following penetrating keratoplasty. *Cornea.* 2005; 24(3):288–291. [PubMed: 15778600]
20. Tran KD, Clover J, Ansin A, et al. Rapid Warming of Donor Corneas Is Safe and Improves Specular Image Quality. *Cornea.* 2017; 36:581–587. [PubMed: 28257386]
21. Edelstein SL, DeMatteo J, Stoeger CG, et al. Report of the Eye Bank Association of America Medical Review Subcommittee on Adverse Reactions Reported From 2007 to 2014. *Cornea.* 2016; 35:917–926. [PubMed: 27158807]
22. Lindquist TD, Allen KA, Weber KR, et al. Ocular surface flora in drowning victims. *Cornea.* 1995; 14:418–422. [PubMed: 7671614]
23. Antonios SR, Cameron JA, Badr IA, et al. Contamination of donor cornea: postpenetrating keratoplasty endophthalmitis. *Cornea.* 1991; 10:217–220. [PubMed: 2055027]
24. Leveille AS, McMullan FD, Cavanagh HD. Endophthalmitis following penetrating keratoplasty. *Ophthalmology.* 1983; 90:38–39. [PubMed: 6402749]
25. Fontana L, Parente G, Di Pede B, et al. *Candida albicans* interface infection after deep anterior lamellar keratoplasty. *Cornea.* 2007; 26:883–885. [PubMed: 17667630]
26. Garg S, Said B, Farid M, et al. Prevalence of positive microbiology results from donor cornea tissue in different methods of corneal transplantation. *Cornea.* 2013; 32:137–140. [PubMed: 22580430]
27. Rauhen MP, Goins KM, Sutphin JE, et al. Impact of eye bank lamellar tissue cutting for endothelial keratoplasty on bacterial and fungal corneal donor rim cultures after corneal transplantation. *Cornea.* 2012; 31:376–379. [PubMed: 22410614]
28. Ritterband DC, Shah MK, Meskin SW, et al. Efficacy and safety of moxifloxacin as an additive in Optisol-GS a preservation medium for corneal donor tissue. *Cornea.* 2006; 25:1084–1089. [PubMed: 17133059]
29. Tsui E, Fogel E, Hansen K, et al. *Candida* Interface Infections After Descemet Stripping Automated Endothelial Keratoplasty. *Cornea.* 2016; 35:456–464. [PubMed: 26890665]
30. Layer N, Cevallos V, Maxwell AJ, et al. Efficacy and safety of antifungal additives in Optisol-GS corneal storage medium. *JAMA Ophthalmol.* 2014; 132:832–837. [PubMed: 24789459]
31. Duncan K, Parker J, Hoover C, et al. The Effect of Light Exposure on the Efficacy and Safety of Amphotericin B in Corneal Storage Media. *JAMA Ophthalmol.* 2016; 134:432–436. [PubMed: 26914028]
32. Nahum Y, Russo C, Madi S, et al. Interface infection after descemet stripping automated endothelial keratoplasty: outcomes of therapeutic keratoplasty. *Cornea.* 2014; 33:893–898. [PubMed: 25062335]
33. Xie L, Hu J, Shi W. Treatment failure after lamellar keratoplasty for fungal keratitis. *Ophthalmology.* 2008; 115:33–36. [PubMed: 17588665]
34. Xie L, Shi W, Liu Z, et al. Lamellar keratoplasty for the treatment of fungal keratitis. *Cornea.* 2002; 21:33–37. [PubMed: 11805504]
35. Sony P, Sharma N, Vajpayee RB, et al. Therapeutic keratoplasty for infectious keratitis: a review of the literature. *CLAO J.* 2002; 28:111–118. [PubMed: 12144228]

36. Yamazoe K, Den S, Yamaguchi T, et al. Severe donor-related *Candida* keratitis after Descemet's stripping automated endothelial keratoplasty. *Graefes Arch Clin Exp Ophthalmol*. 2011; 249:1579–1582. [PubMed: 21584763]
37. Lee WB, Foster JB, Kozarsky AM, et al. Interface fungal keratitis after endothelial keratoplasty: a clinicopathological report. *Ophthalmic Surg Lasers Imaging*. 2011; 42:44–48.
38. Sharma N, Agarwal PC, Kumar CS, et al. Microbial keratitis after descemet stripping automated endothelial keratoplasty. *Eye Contact Lens*. 2011; 37:320–322. [PubMed: 21451417]
39. Araki-Sasaki K, Fukumoto A, Osakabe Y, et al. The clinical characteristics of fungal keratitis in eyes after Descemet's stripping and automated endothelial keratoplasty. *Clinical ophthalmology (Auckland, N.Z.)*. 2014; 8:1757–1760.
40. Xie L, Dong X, Shi W. Treatment of fungal keratitis by penetrating keratoplasty. *The British journal of ophthalmology*. 2001; 85:1070–1074. [PubMed: 11520759]
41. Villarrubia A, Cano-Ortiz A. *Candida* keratitis after Descemet stripping with automated endothelial keratoplasty. *Eur J Ophthalmol*. 2014; 24:964–967. [PubMed: 24966030]
42. Tu EY, Majmudar PA. Adjuvant Stromal Amphotericin B Injection for Late-Onset DMEK Infection. *Cornea*. 2017; 36:1556–1558. [PubMed: 28976414]
43. Hassan SS, Wilhelmus KR. Quality assessment and microbiologic screening of donor corneas. *Cornea*. 2007; 26:953–955. [PubMed: 17721295]
44. Tsui JY, Goins KM, Sutphin JE, et al. Phakic descemet stripping automated endothelial keratoplasty: prevalence and prognostic impact of postoperative cataracts. *Cornea*. 2011; 30:291–295. [PubMed: 21099414]

Figure 1.

a: Slit-lamp photo of Case #1, 4 weeks after uncomplicated DSAEK with temporal interface opacity

b: Slit-lamp photo of Case #1, 7 weeks after DSAEK with enlarging temporal interface opacity and keratic precipitates

Figure 2.
Slit-lamp photo of Case #2, persistent inflammation after repeat DSAEK and prior to penetrating keratoplasty

Table 1Donor Characteristics Table by PT groups (N=784)^a

	Preservation Time Group	
	0–7 Days Group N= 397	8–14 Days Group N= 387
Donor Age (years) - n (%)		
<=30	18 (5)	24 (6)
31–50	75 (19)	62 (16)
51–60	121 (30)	96 (25)
61–70	142 (36)	144 (37)
71–75	41 (10)	61 (16)
Median (IQR)	59 (51, 67)	61 (52, 68)
History of diabetes: Yes- n (%)	101 (25)	113 (29)
Cause of death- n (%)		
Disease	327 (82)	307 (79)
Accident	52 (13)	56 (15)
Other	18 (5)	24 (6)
Method of retrieval- n (%)		
In situ	395 (99)	387 (100)
Whole globe	2 (<1)	0
Refrigerated / On ice: Yes- n (%)	357 (90)	333 (86)
Storage solution- n (%)		
Life 4°C	21 (5)	20 (5)
Optisol GS	376 (95)	367 (95)
Lamellar dissection performed by- n (%)		
Eye Bank	259 (65)	264 (68)
Surgeon	138 (35)	123 (32)
Death to preservation time – hours		
Median (IQR)	10 (7, 14)	11 (7,14)
[Min, Max]	[0, 20]	[0, 22]
Death to refrigeration time^b – hours		
Median (IQR)	3 (2, 4)	3 (2, 4)
[Min, Max]	[0, 9]	[0, 10]
Refrigeration to preservation time^b – hours		
Median (IQR)	7 (4, 11)	7 (4, 11)
[Min, Max]	[0, 18]	[0, 18]

^a. Donor characteristics for study eyes with donor rim culture performed.^b. Factors only available for cornea with refrigeration

TABLE 2

List of 27 organisms isolated from 24 positive donor corneal rim cultures, frequency and amount of growth

Organism	Frequency	Growth (frequency of reporting)
Fungi		
<i>Alternaria</i> species	1	very sparse
Basidiomycete (<i>Irpex lacteus</i> or <i>Ceriporiopsis subvermispora</i>)	1	not reported
<i>Candida</i>	1	sparse
<i>Candida albicans</i>	4	rare (1) not reported (3)
<i>Candida glabrata</i>	1	moderate
<i>Candida parapsilosis</i>	4	sparse (3) light (1)
<i>Candida tropicalis</i>	1	sparse
<i>Penicillium</i> species	1	not reported
<i>Rhodotorula minuta</i>	1	rare
<i>Rhodotorula mucilaginosa</i>	1	not reported
yeast - not <i>Candida albicans</i>	1	moderate
Bacteria		
<i>Bacillus</i> species (not <i>B. anthracis</i>)	1	very sparse
coagulase-negative staphylococci	3	sparse(1) not reported (2)
coagulase-negative staphylococcus (methicillin resistant)	1	not reported
<i>Peptostreptococcus magnus</i>	1	isolate
<i>Propionibacterium</i> series	3	not reported (3)
<i>Propionibacterium Acnes</i>	1	isolate

Table 3

Candidate Risk Factors for Positive Culture Results (N=784)

	N	Positive Fungal Culture N=15	Univariable P value ^a	Positive Bacteria Culture N=10	Univariable P value ^a
Preservation Time (days)			0.30		0.75
0-7	397	10 (2.5)		6 (1.5)	
8-14	387	5 (1.3)		4 (1.0)	
Continuous time			0.46		0.38
Donor Age (years)			0.07		0.004
<=30	42	3 (7.1)		4 (9.5)	
31-50	137	3 (2.2)		2 (1.5)	
51-60	217	4 (1.8)		1 (0.5)	
61-70	286	4 (1.4)		1 (0.3)	
71-75	102	1 (1.0)		2 (2.0)	
History of diabetes: Yes- n (%)			0.77		0.30
No	570	12 (2.1)		9 (1.6)	
Yes	214	3 (1.4)		1 (0.5)	
Cause of death- n (%)			0.39		0.006
Disease	634	11 (1.7)		4 (0.6)	
Accident	108	4 (3.7)		5 (4.6)	
Other	42	0		1 (2.4)	
Refrigerated / On ice - n (%)			0.09		1.00
No	94	4 (4.3)		1 (1.1)	
Yes	690	11 (1.6)		9 (1.3)	
Storage solution- n (%)			0.56		1.00
Life 4°C	41	1 (2.4)		0	
Optisol GS	743	14 (1.9)		10 (1.3)	
Lamellar dissection performed by- n (%)			0.05		0.09

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	N	Positive Fungal Culture N= 15	Univariable P value ^a	Positive Bacteria Culture N=10	Univariable P value ^a
Eye Bank	523	6 (1.1)		4 (0.8)	
Surgeon	261	9 (3.4)		6 (2.3)	
Death to preservation time (hours)					
			0.26		0.48
<7 hours	212	6 (2.8)		2 (0.9)	
7–10 hours	178	3 (1.7)		1 (0.6)	
10–14 hours	193	3 (1.6)		4 (2.1)	
> 14 hours	201	3 (1.5)		3 (1.5)	

^a . Fisher's exact test, and Poisson regression models adjusted for potential correlation from eyes of same donors with generalized estimating equations (GEE) were used to calculate p-value for categorical and continuous factors, respectively. When continuous values were available, they were used in modeling process. Categories are shown for these variables for ease of interpretation only.

Table 4

Reports on the incidence of fungal corneal rim cultures with hypothermic storage and subsequent infection

Study	Time period	Eyes	Positive culture (%) (95% CI)	Recipient infection (95% CI)
CPTS ^{2,14}	2012–2014	784	1.9% (1.1%, 3.1%)	6.7% (0.2%, 32.0%)
Wilhelmus et al ⁷ (meta-analysis)	1983–2006	9484	1.4% (1.2%, 1.7%)	7.5% (3.7%, 13.4%)
Keyhani et al ¹⁹	1998–2003	2466	1.1% (0.8%, 1.6%)	14.3% (4.0%, 32.7%)
Fontana et al ²⁵	2000–2003	185	1.1% (0.1%, 3.9%)	0.0% (0.0%, 84.2%)
Hassan et al ⁴³	2003–2006	263	0.4% (0.0%, 2.1%)	0.0% (0.0%, 97.5%)
Rauen et al ²⁷	2003–2008	629	2.4% (1.3%, 3.9%)	13.3% (1.7%, 40.5%)
Ritterband et al ²⁸	published 2006	509	1.6% (0.7%, 3.1%)	0.0% (0.0%, 36.9%)
Ritterband et al ⁸	published 2007	533	1.3% (0.5%, 2.7%)	0.0% (0.0%, 41.0%)
Vislisei et al ⁹	1996–2015	3414	2.1% (1.6%, 2.6%)	5.6% (1.6%, 13.8%)
Garg et al ²⁶	2006–2010	544	0.7% (0.2%, 1.9%)	25% (0.6%, 80.6%)
Brothers et al ¹⁰	2013	1664 [*]	1.1% (0.7%, 1.8%)	Not available
Tsui et al ⁴⁴	2012–2014	99	7.1% (2.9%, 14.0%)	28.6% (3.7%, 71.0%)

^{*} Endothelial keratoplasty cases from sites that cultured >50% of donor rims

CI is confidence interval