OPEN

Primary Graft Failure, Infection, and Endothelial Cell Density in Corneal Transplants With Increased Death-to-Preservation Time

Michelle J. Sun, MD, MPH, Andrew T. Duong, BA,* Khoa D. Tran, PhD,† Megan M.W. Straiko, PhD,† Christopher G. Stoeger, MBA, CEBT,† and Christopher S. Sales, MD, MPH‡*

Purpose: To ascertain whether death-to-preservation time (DPT) is associated with donor endothelial cell density (ECD), primary graft failure (PGF), and infection.

Methods: Donor corneas aged older than 10 years with ECD 2000 to 4500 cells/mm^2 were procured between 2011 and 2018 by a single eye bank. Donor corneas were analyzed retrospectively for the main outcome measures of PGF, infection, and ECD. Means and proportions of study parameters were compared between corneas with long and short DPT, defined as greater or less than 14 hours, respectively, excluding corneas with a history of intraocular surgery or diabetes. Multivariate analyses were performed using logistic regression, adjusting for donor age at time of death, history of diabetes mellitus, and history of cataract surgery.

Results: Among 12,015 corneas, those with long DPT had a statistically but not clinically significant higher ECD than that of corneas with short DPT (2754 vs. 2724 cells/mm^2, $P < 0.01$). There was no difference in PGF and infections in corneas with long versus short DPT (0.28% vs. 0.26%, $P = 0.86$; 0.43% vs. 0.29%, $P = 0.51$, respectively).

Conclusions: Longer DPT is not associated with a clinically meaningful reduction in donor ECD, PGF, or infection.

Key Words: Descemet membrane endothelial keratoplasty, eye bank, tissue processing, death-to-preservation, primary graft failure

(*Cornea* 2021;40:1462–1465)

Surgeon preferences for corneal transplants have not always been evidence-based and, thus, may unnecessarily limit use of tissues. Although the Corneal Preservation Time Study (CPTS) showed that nearly three quarters of respondent ophthalmologists indicated a maximum preservation time (PT) preference of 8 days, there was no evidence that a PT between 8 and 11 days resulted in greater endothelial cell loss or graft failure after 3 years.[1] Although tissues from older donors may have lower endothelial cell densities that may increase risk for graft failure, donor age itself has not been shown to increase risk for graft failure,[2,3] although surgeons often request younger tissues.

Additional donor characteristics of race, sex, and graft size have not been found to be associated with increased graft failure.[2–4] Interestingly, although earlier studies did not show donor diabetes mellitus to affect graft failure,[3,5] the CPTS found that a donor history of diabetes resulted in a more than 2-fold increase in risk for primary or early graft failure after 3 years in Descemet-stripping automated endothelial keratoplasties (DSAEKs),[2] suggesting that there may be other disease-specific factors at play. Factors found to contribute to graft failure include operative complications,[2] recipient history of anterior synechiae,[6] and in some cases, recipient glaucoma surgery and/or use of glaucoma drops.[5,7]

This study examines the impact of death-to-preservation time (DPT) specifically on primary graft failure (PGF) and infection after corneal transplantation. DPT is an important selection criterion that often limits potential transplant tissue and places constraints on tissue recovery. Studies to date have not found an association between increased DPT and graft failure, endothelial cell count, or infection; however, these studies have been limited by sample size and range of DPTs and look at graft failure broadly as opposed to PGF specifically, as we do in this study.[3,5,6,8–10] Our study, although limited in its retrospective nature, allows for comparison of a large number of transplants with a longer DPT cutoff. The Cornea Donor Study (CDS) used a cutoff of 12 and 8 hours and the CPTS, which was not powered for the DPT variable, used a cutoff of 20 and 10 hours, for

Received for publication October 3, 2020; revision received January 11, 2021; accepted January 12, 2021. Published online ahead of print March 12, 2021.

From the *Department of Ophthalmology, Weill Cornell Medicine, New York, NY; †Lions VisionGift, Portland, OR; and ‡Department of Ophthalmology and Visual Sciences, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA.

This investigation was supported under the National Institutes of Health (NIH) by grant UL1TR002384 of the Clinical and Translational Science Center at Weill Cornell Medical College. The sponsor or funding organization had no role in the design or conduct of this research.

The authors have no conflicts of interest to disclose.

Correspondence: Michelle J. Sun, MD, MPH, Weill Cornell Department of Ophthalmology, 1305 York Ave, 11th floor, New York, NY (e-mail: mis2060@nyp.org).

refrigerated and unrefrigerated tissues, respectively.[2,11] Both looked at graft failure rather than PGF primarily, although there was also a subanalysis of the CPTS that showed no association of increased DPT to PGF.[2,11] A large number of transplants is necessary for evaluation of this rare outcome, which our study provides. We hypothesize that longer DPT does not impact PGF or infection. The utilization of corneas with longer DPT may increase availability of tissue for transplantation.

METHODS

Study Protocol

A retrospective review of the Lions VisionGift eye bank database of donor corneas procured in Oregon for penetrating keratoplasty (PK), DSAEK, and Descemet membrane endothelial keratoplasty between January 1, 2011, and December 31, 2018, was performed. The research performed adhered to the tenets set forth by the Declaration of Helsinki. The Legacy Research Institute Institutional Review Board reviewed and approved the use of Lions VisionGift eye bank database studies where no patient identifying information is accessed or used. Only transplanted corneas with donors older than 10 years at the time of death and those with preoperative eye bank–determined endothelial cell densities between 2000 and 4500 cells/mm^2 were included in our study.

The Lions VisionGift database contains demographic and health characteristics of donors and baseline tissue characteristics. The Lions VisionGift eye bank follows existing standards proposed by the Eye Bank Association of America. Recovered corneas are evaluated by slit lamp biomicroscopy, and endothelial cell counts are obtained by specular microscopy just before processing. For each distributed tissue, the eye bank solicits follow-up information through surveys mailed to surgeons 3 months after tissue transplantation. PGF is defined as a cloudy cornea with loss of central graft clarity, resulting in decrease in vision for at least 3 months postoperatively, with failure attributed to the donor tissue. PGF and infection are reported by the surgeon, and identified instances of either entity are confirmed on investigation by Quality Assurance staff and chart review by the medical director. The time from donor death to corneal preservation in Optisol-GS storage media (Bausch and Lomb, Rochester, NY) was recorded as DPT. All corneas were excised in the field as corneoscleral rims. The upper limit of DPT used was 24 hours.

TABLE 1. Characteristics of Donor Corneas Grouped by DPT, Defined as Less Than and Greater Than or Equal to 14 Hours

	DPT <14 h (n = 9831)	DPT ≥14 h (n = 2184)
DPT (hours)		
Mean (SD)	8.7 (2.6)	17.3 (2.5)
Donor demographics		
Age (ys, SD)*	60.5 (12.6)	56.8 (15.2)
Female sex (%)*	40.4	36.1
Race*		
White (%)	92.1	83.3
Black (%)	1.2	1.8
Hispanic (%)	1.9	2.0
Other (%)	3.6	4.1
Unknown (%)	1.2	8.8
Donor history		
Diabetes mellitus (%)	29.7	29.8
Phakic (%)*	87.3	89.5

*P value < 0.05.

TABLE 2. Outcomes in Corneas With Long DPT, Defined as Greater Than or Equal to 14 Hours, Compared With Those With Shorter DPT in Donors With No History of Diabetes or Cataract Surgery

	DPT <14 h	DPT ≥14 h	*P*
PGF (%)	0.26	0.28	0.86
Infection (%)	0.29	0.43	0.51
ECD (cells/mm^2)	2724	2754	<0.01

Statistical Analysis

Short DPT was defined as <14 hours, and long DPT was defined as ≥14 hours. The CPTS used a 14-hour cutoff in their subanalysis of DPT, and this is a longer time point than that used in other analyses.[11] In our study, low endothelial cell density (ECD) was defined as less than 2300 cells/mm^2 based on the lower limit for transplant use in the CDS and CPTS.[2,11] Baseline characteristics were compared between transplants with short and long DPT using *t* tests for continuous variables and χ^2 tests for categorical variables. Univariate analysis excluded corneas with a donor history of diabetes mellitus and/or cataract surgery and used 2-sided *t* tests, Fisher exact, and χ^2 tests to compare outcomes between the short and long DPT groups. Multivariate analyses used logistic regression and adjusted for donor age at time of death, history of diabetes mellitus, and history of cataract surgery. In these multivariate analyses, DPT was further categorized into varying time intervals and compared with a reference of less than 7 hours, as has been used in previous studies.[11] The percentage of transplant tissues with PGF, infection, and low ECD in each DPT interval were reported and compared with the same reference of tissues with less than 7 hours using χ^2 tests to determine whether a significant difference existed among groups. A *P* value of less than 0.05 was considered statistically significant. All statistical analysis was performed using STATA version 16 (StataCorp LP, College Station, TX).

RESULTS

Among 12,015 corneas transplanted for penetrating and endothelial keratoplasties, 9831 (81.6%) had short DPTs and 2184 (18.2%) had long DPTs. Median DPT in the short DPT group was 8.7 hours with a range from 1.8 to 13.9 hours.

TABLE 3. Odds of Outcomes of Corneal Grafts With DPT Greater Than or Equal to 7 Hours Compared With Those With DPT Less Than 7 Hours, Adjusted for Age, Diabetes, Cataract Surgery, and Race*

DPT (h)	Odds Ratio	95% CI	*P*
PGF			
<7	1.00	Ref	Ref
7 to <10	0.95	0.33–2.78	0.93
10 to <14	1.22	0.42–3.55	0.71
≥14	2.32	0.83–6.44	0.11
Infection			
<7	1.00	Ref	Ref
7 to <10	0.67	0.26–1.72	0.41
10 to <14	0.76	0.31–1.89	0.56
≥14	0.74	0.26–2.08	0.57
Low ECD†			
<7	1.00	—	—
7 to <10	0.92	0.78–1.08	0.29
10 to <14	0.96	0.82–1.13	0.64
≥14	0.79	0.64–0.96	0.02

*Adjusted factors obtained from PGF model.
†Low ECD defined as ECD between 2000 and 2300 cells/mm^2.

Median DPT in the long DPT group was 17.3 hours with a range from 14.0 to 23.9 hours. The mean donor age at time of death was 60.5 ± 12.6 years and 56.8 ± 15.8 years in the short and long DPT groups, respectively. There were more female donors in the short DPT group, with most donors reported as white in both (Table 1). In both groups, there were similar percentages of patients with diabetes but a statistically greater percentage of patients with history of cataract surgery in the long DPT group (Table 1). Of mailed posttransplant surveys, 69.9% were returned (n = 8393), with the assumption that those not returned did not develop graft failure or infection.

Overall, 32 (0.27%) transplanted corneas developed PGF. Nearly 30% of donors in both groups had a history of diabetes mellitus, and more than 10% had a history of cataract surgery and were either pseudophakic or aphakic (Table 1). After excluding tissues with donor history of diabetes or cataract surgery, there was no difference in PGF or infection rates between tissues with short versus long DPT (0.26% vs. 0.28%, $P = 0.86$; 0.29% vs. 0.43%, $P = 0.51$) (Table 2). There was a statistically but not clinically significant difference in ECD (2724 cells/mm^2 vs. 2754 cells/mm^2, $P < 0.01$) (Table 2). Similar results were seen using multivariate analysis including all tissues and adjusting for age, race, donor history of diabetes mellitus, and previous cataract surgery in the donor (Table 3). Tissues with longer DPTs showed no difference in PGF or infections rates compared with those with shorter DPTs (Table 4). There was a lower odds of having a lower endothelial cell count, defined as 2000 to 2300 cells/mm,^{2} in tissues with DPT greater than or equal to 14 hours, compared with tissues with DPT less than 7 hours (Tables 3 and 4).

Of the 32 total confirmed PGFs, 1 (3.1%) was a PK, 15 (46.8%) were DSAEKs, and 16 (50%) were Descemet membrane endothelial keratoplasties. A greater proportion of corneas transplanted for endothelial keratoplasties failed than that for PKs (Table 4). Indication for transplantation in recipients with PGFs was most commonly Fuchs endothelial dystrophy (n = 18, 56.3%), followed by uncategorized endothelial dysfunction (n = 5, 15.6%), postcataract surgery edema (n = 4, 12.5%), other or unknown causes (n = 4, 12.5%), and keratoconus (n = 1, 3.1%). Recipients with keratoconus were least likely to have PGF (Table 5).

TABLE 4. Percentage of Grafts With PGF, Infection, or Low ECD in Each DPT Interval, Compared With a Reference of DPT Less Than 7 hours

DPT (h)	Total N	%	*P*
PGF			
<7	2854	0.21	Ref
7 to <10	3624	0.22	0.93
10 to <14	3353	0.24	0.81
≥14	2184	0.46	0.12
Infection			
<7	1905	0.52	Ref
7 to <10	2411	0.33	0.32
10 to <14	2189	0.41	0.59
≥14	1468	0.48	0.85
Low ECD			
<7	2854	11.0	Ref
7 to <10	3624	10.6	0.60
10 to <14	3353	10.9	0.91
≥14	2184	8.0	<0.01

Infections were seen in 34 (0.28%) tissues with no significant difference in the likelihood of infection between corneas with short versus long DPT (0.29% vs. 0.43%, $P = 0.51$) (Table 2). This remained true in multivariable analyses

TABLE 5. Percentage of PGFs and Infections by Transplant Type and Recipient Diagnosis

	PGFs % (n)	Infections % (n)	All Transplants n
Transplant type			
PK	0.03 (1)	0.77 (26)	3360
DSAEK	0.32 (15)	0.13 (6)	4741
DMEK	0.46 (16)	0.06 (2)	3515
EK, unspecified	0.0 (0)	0.0 (0)	399
Recipient diagnosis			
Fuchs dystrophy	0.35 (18)	0.04 (2)	5157
Keratoconus	0.18 (1)	0.18 (1)	552
Other endothelial dysfunction	0.32 (5)	0.13 (2)	1565
Postcataract surgery edema	0.33 (4)	0.25 (3)	1198
Other*	0.07 (2)	0.90 (24)	2662
Unknown	0.23 (2)	0.23 (2)	881

*Trauma, ulcerative keratitis, congenital opacities, other causes of corneal dysfunction or distortion (nonendothelial), microbial changes, mechanical or chemical trauma, postrefractive surgery, and repeat corneal transplant.
EK, endothelial keratoplasty; DMEK, Descemet endothelial keratoplasty; DSAEK, Descemet-stripping anterior endothelial keratoplasty.

looking at tissues with longer and shorter DPT cutoff times (Tables 3 and 4). A greater proportion of transplants that were PKs developed infections compared with other transplant types, with 26 of 3360 (0.77%) PKs becoming infected (Table 5). Although the percentage of tissues that developed infections was still very low, a greater percentage of recipients with keratoconus and postcataract surgery edema developed infections than did those with Fuchs dystrophy (Table 5).

DISCUSSION

Although most of the tissues recovered and transplanted between 2011 and 2018 at Lions VisionGift had DPTs less than 14 hours, approximately 18% of those used for penetrating or endothelial keratoplasty had DPTs ≥14 hours. This is likely reflective of numerous factors, including efficiency in corneal procurement at this particular eye bank and bias toward use of tissues with shorter PTs. Although a few baseline characteristics showed statistical differences between both DPT groups, the groups were essentially similar and reflect published prevalence of diabetes in this population.[2,12]

Our study shows no significant difference in the likelihood of PGF in corneas with short versus long DPTs, and this remained true in the multivariable subanalyses comparing varying ranges of DPTs. There was a statistically but ultimately not clinically significant difference in ECD. The ECD was lower in the short DPT group, a trend also seen in the multivariable analysis. Given this is a retrospective cross-sectional study of transplanted tissues, this may be due to surgeon preference for ECD compensating for selection of tissues with a longer DPTs, rather than a true outcome. Furthermore, the overall percentage of grafts that developed infection was low and similar between the short and long DPT groups. As seen in other studies, there was no significant association of donor age, history of diabetes, or history of cataract surgery on PGF or infection rates.[2,6,13]

Our study used a large database of tissues, which allowed us to uniquely examine the association of DPT, specifically with the rare event of PGF. Other studies grouped PGF with other causes of graft failure in their outcomes or were not been able to adequately assess significance. However, limitations of our study include its retrospective and cross-sectional nature and its limitation in covariables analyzed. Notably, by assuming survey nonresponses did not develop graft failure or infection, we also potentially underestimate the prevalence of complications.

Our findings support the use of cornea transplant tissues with longer DPTs without concern for increased PGF or infection. Future studies may benefit from looking directly at viability of endothelial cells from tissues or control for postoperative ECD to isolate the impact of DPT from surgical manipulation. Addition of other covariates and detailed follow-up data would also be of interest. It is still unclear exactly how supply of tissues is affected by limitation of DPT; so, further examination of procedures and optimization of procurement strategies in line with current evidence will be important to increasing availability of corneal transplants.

ACKNOWLEDGMENTS

The authors thank Tom Horton, MS, for assistance exporting data for analysis.

REFERENCES

1. 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.
2. Terry MA, Aldave AJ, Szczotka-Flynn LB, et al. Donor, recipient, and operative factors associated with graft success in the cornea preservation time study. *Ophthalmology*. 2018;125:1700–1709.
3. Sugar A, Tanner JP, Dontchev M, et al. Recipient risk factors for graft failure in the cornea donor study. *Ophthalmology*. 2009;116:1023–1028.
4. Sugar J, Montoya M, Dontchev M, et al. Donor risk factors for graft failure in the cornea donor study. *Cornea*. 2009;28:981–985.
5. Price MO, Thompson RW, Price FW. Risk factors for various causes of failure in initial corneal grafts. *Arch Ophthalmol*. 2003;121:1087–1092.
6. Maguire MG, Stark WJ, Gottsch JD, et al. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. *Ophthalmology*. 1994;101:1536–1547.
7. Fasolo A, Capuzzo C, Fornea M, et al. Risk factors for graft failure after penetrating keratoplasty: 5-year follow-up from the corneal transplant epidemiological study. *Cornea*. 2011;30:1328–1335.
8. Mohamed A, Chaurasia S, Garg P. Outcome of transplanted donor corneas with more than 6 h of death-to-preservation time. *Indian J Ophthalmol*. 2016;64:635–638.
9. Patel D, Tandon R, Ganger A, et al. Study of death to preservation time and its impact on utilisation of donor corneas. *Trop Doct*. 2017;47:365–370.
10. Stulting RD, Lass JH, Terry MA, et al. Factors associated with graft rejection in the cornea preservation time study. *Am J Ophthalmol*. 2018;196:197–207.
11. Sugar A, Gal RL, Beck roy W, et al. Baseline donor characteristics in the cornea donor study. *Cornea*. 2005;24:389–396.
12. Lass JH, Riddlesworth TD, Gal RL, et al. The effect of donor diabetes history on graft failure and endothelial cell density 10 years after penetrating keratoplasty. *Ophthalmology*. 2015;122:448–456.
13. Cornea Donor Study Group. Donor age and corneal endothelial cell loss 5 years after successful corneal transplantation. *Ophthalmology*. 2008;115:627–632.e8.