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Donor age and factors related to endothelial cell loss ten years after penetrating keratoplasty: Specular Microscopy Ancillary Study

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Abstract

Objective—To examine the effect of donor age and other perioperative factors on long term endothelial cell loss after penetrating keratoplasty (PKP)

Design—Multi-center, prospective, double-masked clinical trial

Participants—176 participants from the Cornea Donor Study cohort who had not experienced graft failure 10 or more years after PKP for a moderate risk condition (principally Fuchs' dystrophy or pseudophakic/aphakic corneal edema)

Methods—Corneas from donors 12 to 75 years old were assigned to participants using a randomized approach, without respect to recipient factors. Surgery and post-operative care were performed according to the surgeons' usual routines. Images of the central endothelium were

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*A full listing of the Cornea Donor Study Research Group is available at http://aaojournal.org.

Conflict of Interest: Jonathan H. Lass, MD serves as a board member for Midwest Eye Banks and the Cleveland Eye Bank. Edward J. Holland, MD is employed by the Cincinnati Eye Bank, serves as a consultant and receives lecture fees from Bausch & Lomb, and serves as a board member for the ASCRS. Christopher Chow, MD has received lecture fees from Bausch & Lomb. Kristen McCoy is employed by Midwest Eye Banks. Francis Price, Jr., MD receives lecture fees from Bausch & Lomb.

obtained preoperatively and at intervals for ten years postoperatively. Images were analyzed by a central image analysis reading center to determine endothelial cell density (ECD).

Main Outcome Measure—Endothelial cell density at 10 years

Results—Among study participants with a clear graft at 10 years, the 125 who received a cornea from a donor 12 to 65 years old experienced a median cell loss of 76%, resulting in a 10-year median ECD of 628 cells/mm^2 (interquartile range, 522-850), whereas the 51 who received a cornea from a donor 66 to 75 years old experienced a cell loss of 79%, resulting in a median 10-year ECD of 550 cells/mm^2 (interquartile range, 483-694) (*P* adjusted for baseline ECD=0.03). In addition to younger donor age, higher ECD values were significantly associated with higher baseline ECD (P<0.001) and larger donor tissue size (P<0.001). Forty-two (24%) of the 176 participants had an ECD below 500 cells/mm^2 at 10 years and only 24 (14%) had an ECD above 1,000 cells/mm^2.

Conclusions—Substantial cell loss occurs in eyes with a clear graft 10 years after PKP, with the rate of cell loss being slightly higher with older donor age. Higher pre-operative ECD and larger donor tissue size are associated with higher ECD at 10 years.

Trial Registration—NCT00006411

INTRODUCTION

The Cornea Donor Study (CDS) evaluated the relationship between donor age and graft survival following penetrating keratoplasty (PKP) in eyes with a moderate risk condition (principally Fuchs' dystrophy or pseudophakic/aphakic corneal edema). At 5 years, there was no difference in graft survival with corneas from donors 12 to 65 years old compared with corneas from donors 66 to 75 years.[1] However, in the Specular Microscopy Ancillary Study (SMAS), a slight association was detected between increasing donor age and greater endothelial cell loss at five years, with substantial cell loss in successful grafts irrespective of donor age (median cell loss preoperatively to 5 years = 69% for donors 12 to 65 years old and 75% for donors 66 to 75 years).[2] Along with older donor age, smaller graft size and male donor gender also were associated with a lower central endothelial cell density (ECD) at 5 years.[3] Important factors not associated with differences in ECD included method of tissue retrieval, donor cause of death, history of diabetes, and time from death to preservation or to surgery. Interestingly, the preoperative ECD was not associated with graft failure by 5 years, while the 6 month ECD was[3], suggesting that early cell loss is influenced by recipient factors such as corneal diagnosis and perioperative factors.

The finding of slightly greater cell loss with older donors (66 to 75 years) raised the question as to whether graft failure would remain unassociated with donor age over longer-term follow up. To investigate this possibility, the CDS was extended for an additional 5 years for those grafts that had not failed by 5 years. In this article, we report on the relationship between donor age and other donor, recipient, operative and postoperative factors with ECD after PKP among grafts that survived for at least 10 years.

METHODS

Details of the CDS and SMAS protocols have been published[1, 2, 4, 5]; pertinent aspects are briefly described. The protocol was approved by institutional review boards at each investigational site, and individual participants gave written informed consent to participate in the study. CDS participants were between 40 and 80 years old with a corneal disease associated with endothelial dysfunction [principally Fuchs' dystrophy and pseudophakic/aphakic corneal edema (PACE)]. Assigned corneas were from donors 12 to 75 years old with an eye-bank measured central ECD from 2300 to 3300 cells/ mm^2. Clinical

investigators and participants were masked to all characteristics of the donor cornea including age and ECD. Preoperative management, surgical technique, and postoperative care, including prescription of medications, were provided according to each investigator's routine. Central corneal thickness measurements, using an ultrasonic pachymeter by the investigator's usual routine, were optional at 6 and 12 month follow-up visits and annually through year 10. Participation in the SMAS was optional for eye banks, clinical sites and participants. Of the 1,090 eligible participants enrolled in the CDS, the SMAS included 609 participants at 46 clinical sites (a listing of the Cornea Donor Study Research Group is available at http://aaojournal.org). Donor corneas were assigned to the SMAS participants by 31 of 43 participating CDS eye banks. A gradable endothelial image at 10 years was available for 176 of the eligible, consented 609 participants. Among the other 433 participants, 105 had a graft failure, 100 died, 103 completed less than 10 years of CDS follow up, and 125 did not have a 10-year image for other reasons. Among the 176 participants with a 10-year image, 141 (80%) participants also had a gradable image at 5 years and 35 (20%) participants did not.

Endothelial Cell Density Determination

The baseline ECD was determined by the Cornea Image Analysis Reading Center (CIARC, Case Western Reserve University and University Hospitals Case Medical Center, Cleveland, Ohio), formerly the Specular Microscopy Reading Center, from a single image of the central endothelium of each study donor cornea submitted by the eye banks participating in the SMAS. The time from death to image capture was not recorded. For this study of clear grafts at 10 years, the eye bank determined the baseline ECD for 53 (30%) corneas assigned by eye banks not participating in the SMAS and the CIARC determined the baseline ECD for the other 123 (70%). In a sensitivity analyses, results were similar when the cohort was restricted to the 123 (70%) cases with a CIARC-determined preoperative ECD (data not shown).

Images of the central endothelium were obtained at 6 and 12 months after PKP, annually through 5 years, and at years 7-8 and 10 as long as a participant remained in follow up without graft failure or regraft. Up to three endothelial images were obtained at each visit utilizing a contact or non-contact specular or confocal microscope from one of 5 different companies (BioOptics Inc., Portland, OR; CooperVision (no longer manufactured); Konan Medical Inc., Irvine, CA; Tomey Corporation USA, Phoenix, AZ; and Nidek Inc., Fremont, CA). All microscopes were calibrated for magnification using images of a known magnification provided by the manufacturer to minimize any differences in image characteristics between microscopes that could have impacted the image analysis by CIARC.

Assessments of image quality and ECD were made by the CIARC using standardized procedures. Details of CIARC procedures have been previously described for donor and post-operative images[2, 3, 6], including reader training and certification, image quality grading, image calibration, variable frame analysis for ECD determination, and adjudication procedures for image quality and ECD determination. Pertinent information is summarized here. The ECD of all analyzable images was independently determined by two readers using the variable frame analysis method. If the ECDs determined by the two readers differed by ≥5.0%, a third independent determination of ECD was made by an adjudicator. Final ECD was the average of all ECDs that were within 5% of each other. Readers were masked to all information about the donor corneas and study participants. Throughout the study, the CDS Coordinating Center selected clinical images for repeat, masked image quality grading and ECD determination to assess both intra- and inter-observer variability. Quality control results were similar to previously published findings of quality control analyses for donor

and clinical endothelial images[2]; there was excellent intra-observer and inter-observer agreement for image quality assessment and ECD measurement.

Statistical Methods

Analyses were restricted to 176 participants with gradable endothelial images at 10 years (108-144 months). If a participant had multiple images during that time frame, only the image closest to 120 months was included in the analyses. Only images obtained prior to graft failure were included. The analyses, therefore, were conditional on graft survival. The relative difference (referred to as "percent change or loss") between baseline and 10-year ECD was calculated by subtracting the baseline ECD from the 10-year ECD and then dividing by the baseline ECD. This difference is expressed as a percent relative to the baseline ECD, with negative numbers corresponding to cell loss. Pre-specified donor age groups were 12 to 65 vs. 66 to 75 years. Post-hoc analyses were replicated in four data-derived (not pre-specified) donor age groups (12 to 33, 34 to 52, 53 to 71, and 72 to 75 years) following an inspection of graft outcomes across the entire donor age distribution, which showed relatively constant success rates within each of the three age ranges.

The 10-year ECD values were not normally distributed; therefore, they were compared by donor age groups based on ranks. The rank scores were transformed to have a normal distribution (van der Waerden scores).[2] The resulting values were used as the dependent variable in Analysis of Covariance (ANCOVA) models adjusting for baseline ECD. The relationships between the 10-year ECD and percent cell loss with donor age as a continuous variable were assessed by the Spearman correlation coefficient; a partial correlation coefficient was used to adjust for the baseline ECD.

The relationship between the 10-year ECD and corneal thickness values were assessed by the Spearman correlation coefficient. The relationships between baseline (donor, recipient, and operative) factors and 10-year ECD values were explored in univariate and multivariate analyses. Cross-sectional regression models were used to evaluate change in ECD from baseline to 10 years, and longitudinal regression models that accounted for correlated values from the same participant were used to evaluate ECD from 6 months through 10 years of follow-up. Several factors were not analyzed in the cross-sectional regressions because there were too few cases: non-white or Hispanic recipient race (n=6), non-white or Hispanic donor race (n=7), positive smoking status (n=9) and glaucoma history (n=7). The model was fit with the rank-normalized transformation (van der Waerden scores), and adjusted for baseline ECD. Additional covariates with $P<0.10$ were included in the multivariate model generated through stepwise selection to control for any potential confounding factor; however, only covariates with $P<0.01$ were considered statistically significant. The large number of statistical comparisons increases the likelihood of a false positive and no attempt was made to control the overall type I error probability in these exploratory analyses. A threshold of $P<0.01$ was used to define statistical significance as a compromise to balance the risks of type I vs. type II errors. All reported p-values are two-sided. Statistical analyses were conducted using SAS versions 9.3 software (SAS Institute Inc., Cary, NC).

RESULTS

Mean age (±SD) at the time of PKP of the 176 participants was 66 ± 9 years; 114 (65%) of the participants were female, and 170 (97%) were white, non-Hispanic. Indications for PKP included Fuchs' dystrophy in 145 (82%), PACE in 27 (15%), and other causes in 4 (2%). Forty nine (28%) were pseudophakic and 7 (4%) aphakic prior to PKP. Post-PKP, 53 (30%) eyes were phakic, 121 (69%) pseudophakic, and 2 (1%) aphakic. A cornea from a donor 12 to 65 years old was assigned to 125 (71%) of the participants and a cornea from a donor 66

to 75 years to 51 (29%). Participant characteristics were similar in the older and younger donor age groups (Table 1).

Among the 176 participants without graft failure, the median donor corneal ECD was 2695 cells/mm^2 (interquartile range: 2498 to 2890) at baseline and 611cells/mm^2(interquartile range: 502 to 769) at 10 years, representing a median decrease from baseline to 10 years of 76% (interquartile range: 82% to 70%). Forty-two (24%) participants had an ECD below 500 cells/mm^2 at 10 years and only 24 (14%) had an ECD above 1,000 cells/mm^2. Ten-year ECD was not related to the baseline ECD (r= +0.06, 95% confidence interval −0.09 to +0.21, Figure 1A), but among the 141 participants who also had a 5-year image there was a positive association between 5-year and 10-year ECD values (r= +0.34, 95% confidence interval +0.19 to +0.48, Figure 1B).

Cell loss was substantial in both the older and younger donor age groups (Figure 2). At 10 years younger donor age was associated with higher ECD values (r adjusted for baseline ECD = −0.30, 95% confidence interval −0.43 to −0.16, Figure 3A), and reduced cell loss from baseline to 10 years (r adjusted for baseline ECD = −0.31, 95% confidence interval −0.44 to −0.17, Figure 3B). Participants who received a cornea from a donor 12 to 65 years old experienced a median cell loss of 76% resulting in a 10-year median ECD of 628 cells/mm^2(interquartile range: 522 to 850), while participants who received a cornea from a donor 66 to 75 years old experienced cell loss of 79% resulting in a median 10-year ECD of 550 cells/mm (interquartile range: 483 to 694, P adjusted for baseline ECD = 0.03). In an exploratory analysis, there was slightly less cell loss in corneas from donors at the youngest end of the range of donor ages (Table 2, Figure 4). For instance, the 26 participants who received a cornea from a donor 12 to 33 years of age had a median 10-year cell loss of 67% compared with 77% in the 150 participants who received a cornea from a donor aged 34 to 75 years (P adjusted for baseline ECD <0.001).

Among 159 participants who had a 10-year corneal thickness measurement, the 10-year ECD values were not related to the 10-year corneal thickness values (r= −0.10, 95% confidence interval −0.25 to +0.06). In the multivariate cross-sectional analysis at 10 years, in addition to younger donor age (P<0.001), PACE baseline diagnosis (P=0.001) was significantly associated with higher ECD at 10 years (Table 3, Table 4, available at http://aaojournal.org, includes all of the variables evaluated in the analysis). The model also adjusted for trends towards higher ECD at 10 years with larger donor tissue (P=0.02) and in younger recipients (P=0.07) that did not meet our threshold for statistical significance with multiple comparisons. One hundred forty five participants diagnosed with Fuchs' dystrophy at baseline experienced a median cell loss of 78% (interquartile range: 82% to 72%), resulting in a median 10-year ECD of 601 (interquartile range: 499 to 732) cells/mm^2, while 27 participants with PACE experienced a median cell loss of 72% (interquartile range: 78% to 58%), resulting in a median 10-year ECD of 760 (interquartile range: 589 to 1174) cells/mm^2.

In the multivariate longitudinal analysis incorporating measurements from 6 months through 10 years, results were similar to those from the 5-year data.[3] Higher ECD values were significantly associated with higher baseline ECD (P<0.001), larger donor tissue size (P<0.001), and younger donor age (P<0.001). Female donor and no history of glaucoma also tended toward higher ECD values and were included in the final multivariate model to account for potential confounding (P<0.10), but did not meet the criterion for statistical significance (P≥0.01). The association of follow-up ECD with baseline ECD weakened over time (interaction P<0.001).

DISCUSSION

The Cornea Donor Study[1] and the Specular Microscopy Ancillary Study[2] were extended from 5 to 10 years in part because even though graft survival at 5 years was comparable at 86% in both donor age groups (<66 years and ≥66 years to 75 years), there was a trend toward greater endothelial cell loss in the older donor age group (75% loss vs. 69%). With the extended follow up, we found that higher donor age (analyzed as a continuous variable) was in fact associated with slightly lower graft success after the first 5 years (P<0.001), although a comparison of graft success with corneas from donors 12 to 65 years old and corneas from donors 66 to 75 years old was not significant (77% versus 71%, (P=0.11). Similar to the graft success analysis[7], we found a significant association (P<0.03) between older donor age and greater cell loss in the grafts that were clear at 10 years. However, the association was slight and appeared to be related primarily to a lower rate of cell loss with donors 12 to 33 years old. In this young age group, median cell loss at 10 years was 67% compared with 77% for donors 34 to 75 years.

While some authors have found no association between donor age and cell loss[8, 9], Bohringer et al found greater cell loss with increased donor age[10], and Ing et al found less cell loss with increased donor age[11]. The differing results of the Ing et al study from ours and Bohringer et al may be influenced by: 1) differences in the corneal diseases included in the studies; only endothelial failure conditions in our cohort versus a mixed population with endothelial failure and normal endothelium cases (e.g. keratoconus) in other cohorts; 2) unavailability of long-term data in cases with graft failure; 3) varying follow up; and 4) the range of donor ages. In our study the youngest donor age group (12 to 33 years old) had a significant impact on our results. Why age may have its greatest effect on endothelial survival at this extreme is unclear. However, one explanation may be an overall decline in high energy tissue metabolism with age (change in the ratio of high-energy phosphates/low-energy phosphates); a study examining donor corneas ranging from < 1 to 79 years of age suggested that the youngest corneas have the most ability to withstand stress (surgery)[12]. Notably, we found that no other donor factors influenced long term cell loss (i.e. death to preservation time, preservation time, cause of death), similar to Langenbucher et al[9] and Ing et al[11].

In regard to the degree of cell loss we observed compared with other studies, Bourne et al[13] observed less cell loss at 5 years (59%) and a slower rate of loss between years 3 and 5 in 187 of 393 eyes with 5-year follow up after PKP in a mixed cohort of diseased endothelium (bullous keratopathy and Fuchs' dystrophy) and normal endothelium (keratoconus). A subset of 129 patients with no rejection episodes and annual visits for the entire follow up period also experienced 59% cell loss at 5 years with a 13% loss between 3 and 5 years. Subsequently in this same cohort, 119 eyes at 10 years experienced an even slower rate of loss between 5 and 10 years (4% per year) with 67% loss by 10 years.[11] Attrition was a factor with this study as less than 50% of the cohort had 10-year follow up data available because of graft failures (n=68), deaths (n=80), and loss to follow up.[11]

Similar to the Bourne cohort, the SMAS cohort experienced a marked flattening of the rate of endothelial cell loss between 5 and 10 years in the surviving clear grafts in both donor groups; the rate of loss overall was 5% per year in our cohort over this time period. However, our overall cell loss through 10 years was higher at 76% than the cell loss reported for the mixed disease cohorts of Ing et al[11] (67%) and Borderie et al[14] (61%). This is understandable given that none of our participants had a normal recipient endothelium to provide a reservoir of cells to support the dwindling donor endothelial population over time.

Remarkably, 42 of our 176 participants with clear grafts at 10 years (24%) had an ECD below 500 cells/mm^2. Maintaining corneal clarity at such a low ECD is a testament to the ability of the endothelium to maintain its tight junctions, increase the number of pump sites, and shift its metabolism of glucose from the tricarboxylic acid cycle to the hexose monophosphate shunt for the production of nicotinamide adenine dinucleotide phosphate needed for membrane repair and to minimize the effect of oxidative stress.[15]

Regarding recipient factors that affected cell loss at 10 years in the clear grafts, surprisingly, the Fuchs' dystrophy grafts, whether phakic or pseudophakic, by multivariate analysis showed significantly greater cell loss (overall median of 601 cells/mm^2) compared with the PACE group (760 cells/mm^2) (P = 0.001). We believe this finding, while statistically significant, is most likely due to the substantially higher graft failure rate in PACE eyes prior to 10 years, leaving a small group of clear surviving grafts (27) that statistically had lower cell loss than the greater number of surviving Fuchs' grafts. In fact, the Fuchs' grafts had an 80% survival rate at 10 years, as opposed to a 63% survival rate for the PACE grafts. This higher failure rate in PACE grafts has been previously observed by the CDS at 5 years[16] and by other authors[17-20]. The 5-year analysis of the CDS cohort found a 4 fold increased risk of graft failure for the PACE group compared with the Fuchs' dystrophy group (27% vs. 7%). In regards to cell loss for these two disease groups, Ing et al[11] did not find a difference in long term cell loss, but their analyses may be limited by graft failures and drop out. At two years, Langenbucher et al[21] found that the Fuchs' group had significantly less cell loss when compared with the PACE group.

The strengths of the SMAS study include its large sample size, masking of surgeons to donor age, standardization of specular microscopy imaging techniques at eye banks and clinical sites, and use of a central reading center with standardized quality-controlled procedures for ECD measurements. As reported previously, CDS participants in SMAS had similar baseline characteristics compared with CDS participants not in SMAS.[2] A weakness which is inherent in all studies evaluating ECD post-PKP over time is that grafts that fail are no longer available for continued endothelial imaging. Several methods have been explored for addressing this[14, 22, 23] although no method can fully eliminate the bias that may occur. We will explore this using our data in separate analyses.

Our observations and those of other investigators regarding long term cell loss only apply to PKP for endothelial disease and not to the rapidly evolving endothelial keratoplasty procedures, Descemet stripping endothelial keratoplasty (DSEK) or Descemet membrane endothelial keratoplasty (DMEK). These two procedures have now supplanted PKP for the surgical management of the great majority of endothelial disease cases in the United States. Of the 21,547 keratoplasties performed in the United States in 2012 for Fuchs' dystrophy and PACE, endothelial keratoplasty was the procedure of choice in 16,477 (76%) cases.[24] With substantial long-term experience, we are now well aware that the degree and pattern of cell loss with endothelial keratoplasty is quite different from PKP, as observed in the CDS and the SMAS. Following DSEK[25-28] and DMEK[29] the central ECD decline in the first 6 months is greater than observed with PKP, but then the rate of decline is significantly lower by the third year, and by the fifth year, cell loss is less than observed with PKP[25]. The reasons for this difference remain unclear, but most likely represent a difference in the type of endothelial trauma sustained at the time of surgery. In DSEK, there is more central damage than peripheral damage from surgery and this is reflected 6 months postoperatively in the central endothelial area with a lower central ECD than PKP.[30] But with less peripheral damage overall in DSEK than PKP, there is less stimulus for central endothelial migration, and so the long term ECD of DSEK is relatively high. With PKP, the damage is primarily peripheral in the areas of trephination and suturing, reflected 6 months postoperatively by a central ECD that is higher than that of DSEK.[31] However, there is then a continued drop in

central ECD in PKP over time as most likely the central endothelial cells migrate to repair the more extensive peripheral damage (Terry, M. personal communication). Lower rates of graft rejection with endothelial keratoplasty compared with PKP may also contribute to less long term cell loss.[32] Finally, the larger donor diameter with endothelial keratoplasty versus PKP may contribute to the higher ECD long term, although two independent studies on varying donor diameters with DSEK have not found a relationship between cell loss over time and graft diameter.[33, 34]

In conclusion, the extension of the SMAS to ten years has shown continued loss of endothelial cells albeit at a slower rate than observed in the first 5 years. The study also has demonstrated that in some cases grafts can remain clear after 10 years even at cell densities below 500 cells/mm^2. The SMAS has shown that donor age does influence ECD long term, although this finding was primarily influenced by a small group of the youngest donors (12 to <34 years of age) that had the least cell loss and the best graft survival. Nevertheless, as with our graft survival findings[7], the great majority of clear grafts had similar cell loss over 10 years, which should be reassuring to our patients still undergoing PKP for endothelial diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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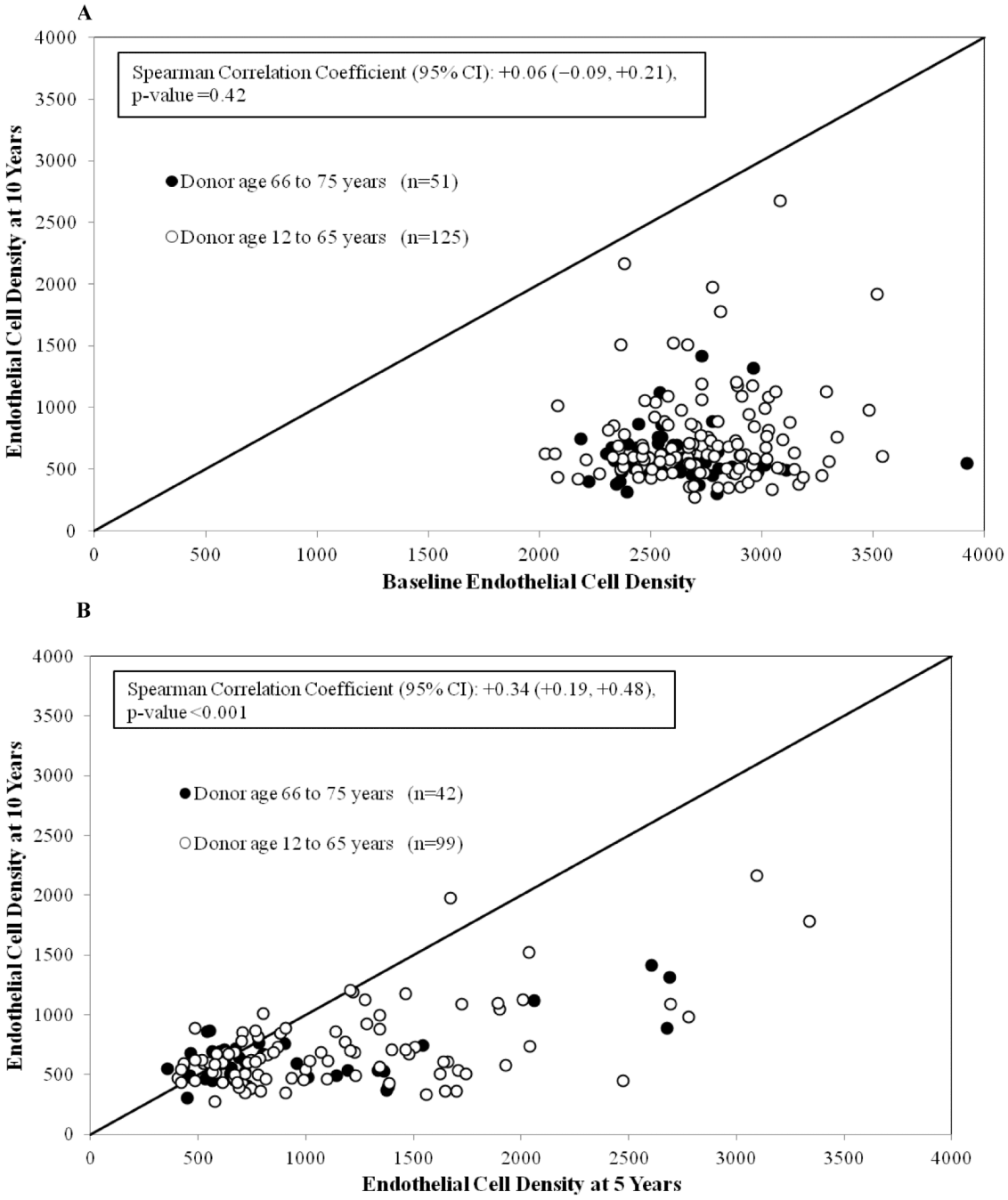

Figure 1. A. Endothelial Cell Density at Ten Years versus Baseline for Eyes with a Successful Graft at 10 Years (N=176)

CI = confidence interval.

B. Endothelial Cell Density at Ten Years versus Five Years for Eyes with a Successful Graft at 10 Years (N=141)

CI = confidence interval.

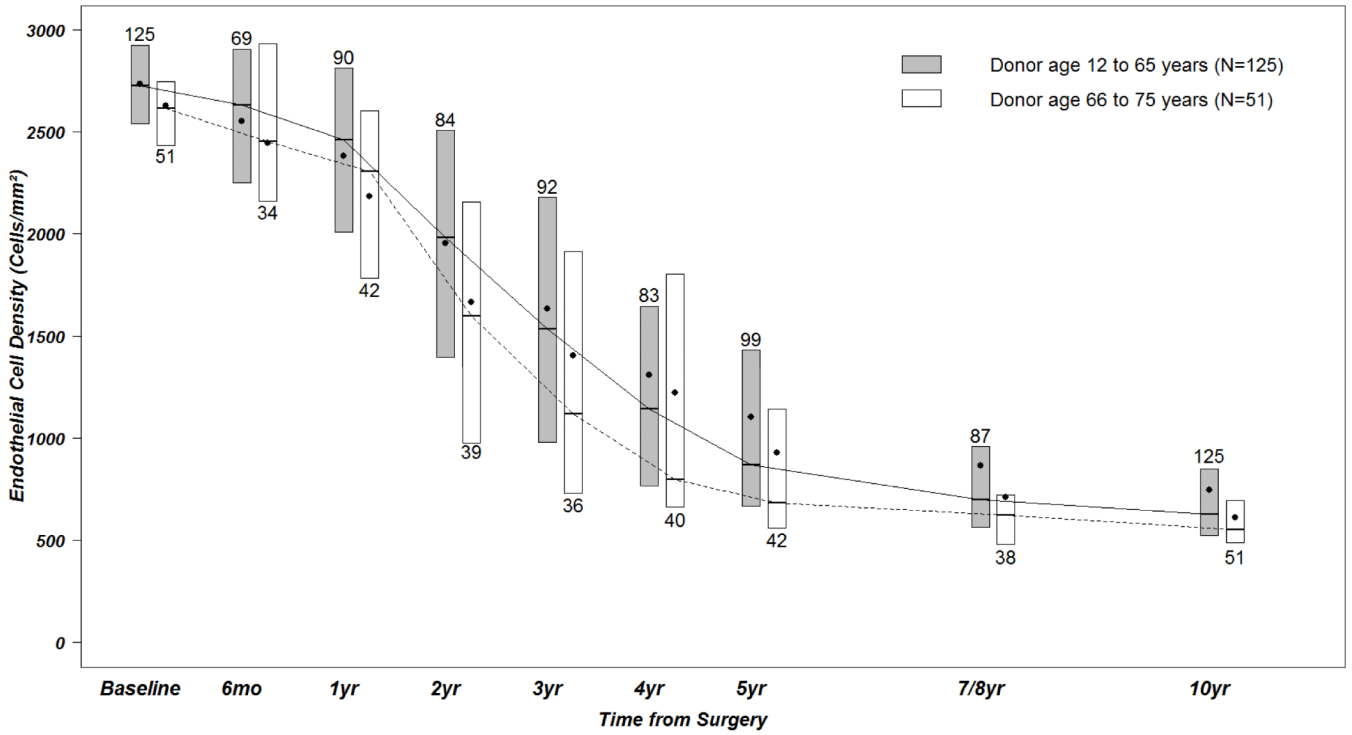

Figure 2. Endothelial Cell Density Over Time for Participants with a Successful Graft at 10 Years According to Donor Age

Boxplot of endothelial cell density (ECD) according to 2 donor age groups 12 to 65 and 66 to 75 years). Black dots denote mean values, horizontal lines in the boxes are medians, and the bottom and top of the boxes represent the 25th and 75th percentiles.

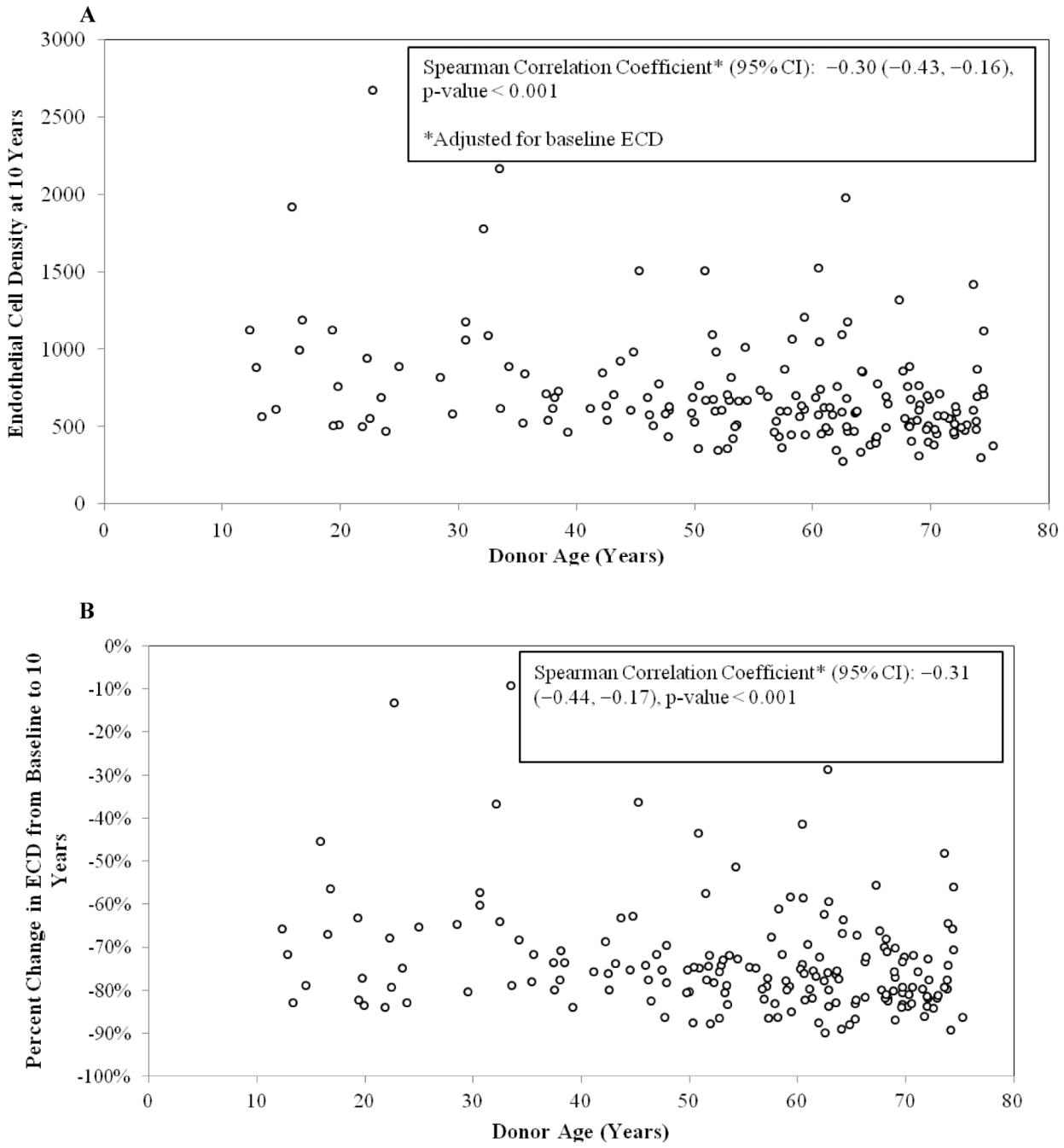

Figure 3. A. Ten-year Endothelial Cell Density by Donor Age for Eyes with a Successful Graft at 10 Years (N=176)

CI = confidence interval.

B. Percent Change in Endothelial Cell Density from Baseline to Ten Years by Donor Age for Eyes with a Successful Graft at 10 Years (N=176)

CI = confidence interval.

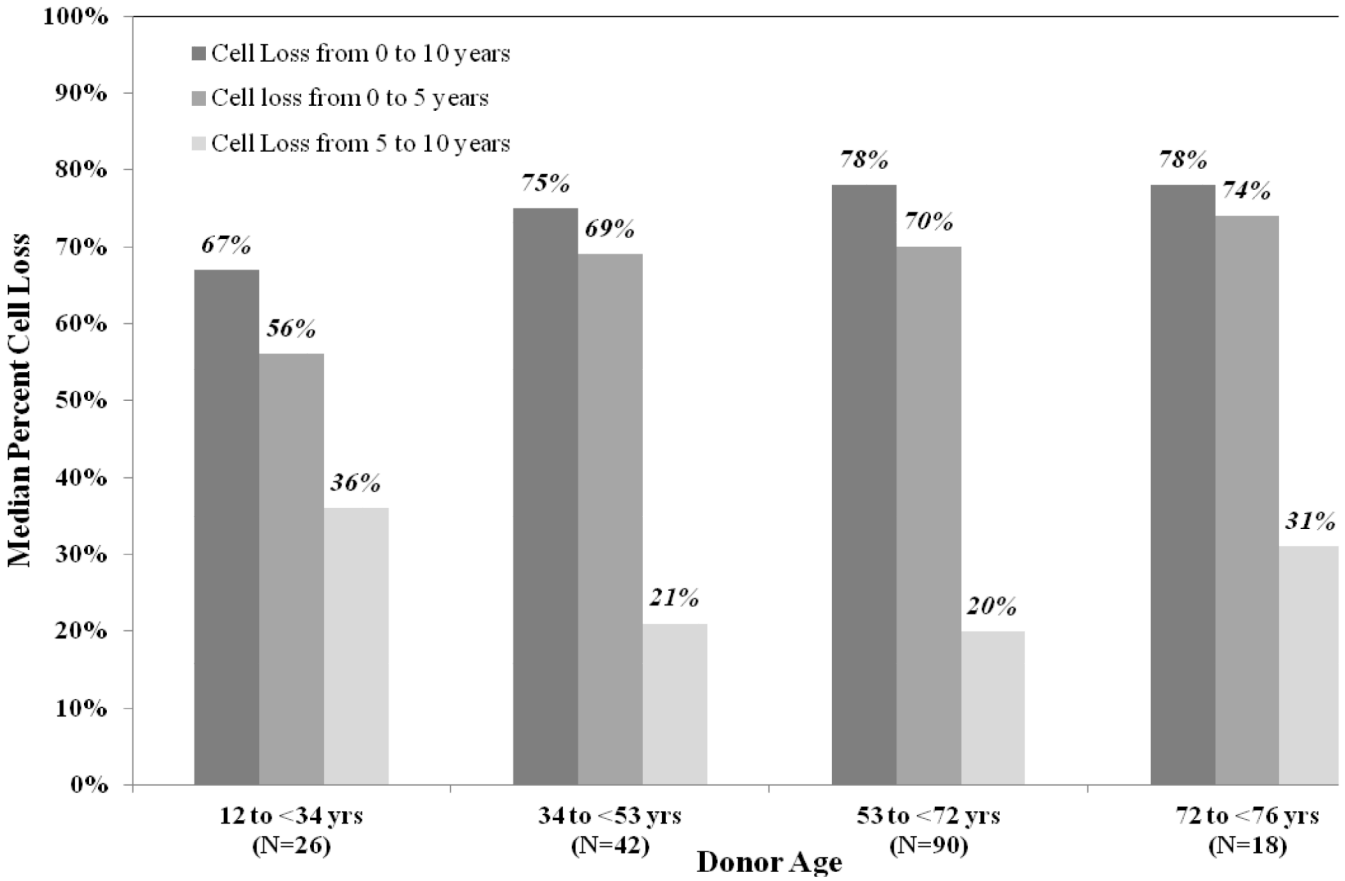

Figure 4. Median Endothelial Cell Loss from 0 to 5 years and 5 to 10 years in Eyes with Successful Graft at 10 Years

Table 1
Comparison of 10-Year Specular Microscopy Ancillary Study Participants by Donor Age

Baseline Characteristics:	Donor Age12 to 65 N=125	Donor Age 66 to 75 N=51
Recipient Age at Cornea Donor Study (CDS) entry (years)		
Mean (Standard Deviation)	65 (9)	67 (9)
Median (Interquartile Range)	68 (58, 72)	69 (60, 74)
Recipient Gender		
Female	79 (63%)	35 (69%)
Recipient Race		
Caucasian	121 (97%)	49 (96%)
African-American	4 (3%)	1 (2%)
Hispanic	0	1 (2%)
Recipient Cigarette Smoker at CDS entry	8 (6%)	1 (2%)
Recipient Use of Glaucoma Medications at CDS entry	4 (3%)	2 (4%)
Recipient Prior Glaucoma Surgery	2 (2%)	0
Recipient Diagnosis		
Fuchs' Dystrophy	102 (82%)	43 (84%)
Pseudophakic/Aphakic Corneal Edema	21 (17%)	6 (12%)
Other	2 (2%)	2 (4%)
Recipient Preoperative Lens Status		
Phakic	87 (70%)	33 (65%)
Pseudophakic	33 (26%)	16 (31%)
Aphakic	5 (4%)	2 (4%)
Recipient Postoperative Lens Status		
Phakic	38 (30%)	15 (29%)
Pseudophakic	85 (68%)	36 (71%)
Aphakic	2 (2%)	0
Recipient Bed Size (mm) *		
Mean (Standard Deviation)	7.92 (0.25)	7.82 (0.27)
Baseline Donor Endothelial Cell Density ** **(cells/mm^2)**		
Mean (Standard Deviation)	2735 (302)	2627 (281)
Median (Interquartile Range)	2726 (2542, 2924)	2617 (2426, 2747)
1708-2299	7 (6%)	2 (4%)
2300-2499	20 (16%)	15 (29%)
2500-2699	31 (25%)	14 (27%)
2700-2899	31 (25%)	14 (27%)
2900-3099	24 (19%)	4 (8%)
3100-3299	8 (6%)	1 (2%)
3300-3540	4 (3%)	1 (2%)

N(%) unless otherwise specified

eyes

*One participant with missing recipient bed size

**Includes baseline endothelial cell density from eye bank for 53 eyes and from reading center for 123 eyes

Table 2

Ten Year Endothelial Cell Density (ECD) in Eyes with Successful Transplant

Donor Age		Baseline ECD*	5-Year ECD	10-Year ECD	% Change from Baseline to 10 Years**	N (%) with 10-Year ECD<500 cells/mm^2	10-Year ECD P-value
	N	*Median (Interquartile Range)*					
Overall Donor Age (years):	176	2695 (2498, 2890)	786 (616, 1343)	611 (502, 769)	−76% (−82%, −70%)	42 (24%)	
12 to 65 years	125	2726 (2542, 2924)	870 (666, 1462)	628 (522, 850)	−76% (−80%, −68%)	25 (20%)	0.03
66 to 75 years	51	2617 (2426, 2747)	683 (560, 1142)	550 (483, 694)	−79% (−82%, −72%)	17 (33%)	
12 to 33 years	26	2950 (2731, 3082)	1309 (1021, 1624)	886 (584, 1126)	−67% (−79%, −60%)	1 (4%)	<0.001
34 to 52 years	42	2695 (2587, 2849)	792 (589, 1401)	654 (577, 764)	−75% (−78%, −72%)	5 (12%)	
53 to 71 years	90	2615 (2462, 2805)	748 (606, 1194)	581 (472, 698)	−78% (−82%, −72%)	30 (33%)	
72 to 75 years	18	2666 (2398, 2776)	719 (565, 1356)	563 (480, 706)	−78% (−82%, −71%)	6 (33%)	

P-values are from analysis of covariance models for the 2 and 4 level donor age groups. Both models were adjusted for baseline ECD. Models were fitted with van der Waerden scores of the ECD values at baseline and 10 years. While adjusting for baseline ECD, the p-value for the association between ECD at 10 years and continuous donor age was < 0.001.

*Includes baseline ECD from eye bank for 53 eyes and from reading center for 123 eyes.

**Percent change from baseline to 10-year ECD = ECD at 10 years minus ECD at baseline divided by ECD at baseline and multiplied by 100%. A negative number indicates loss of cells.

Table 3

Endothelial Cell Density (ECD) at 10 Years by Baseline Recipient/Donor Factors Included in the Final Multivariate Model

Baseline Factors	*N*	Baseline ECD *Median*	10 year ECD *Median*	% Change in ECD* *Median*	Multivariate P -value for 10-year ECD**
Overall	176	2695	611	−76%	
RECIPIENT FACTORS					
Age (years)					0.07+
40 – <50	10	2794	700	−73%	
50 – <60	41	2690	595	−76%	
60 – <70	55	2673	618	−76%	
70 – 86	70	2709	607	−78%	
Baseline Diagnosis and Lens Status [1]					0.001++
Fuchs: pre/post phakic	52	2696	640	−76%	
Fuchs: pre phakic/post PA	64	2700	598	−78%	
Fuchs: pre/post PA	29	2668	603	−77%	
PACE: post PA	27	2636	760	−72%	
OPERATIVE FACTORS					
Donor tissue size					0.02+
7.00 –7.75 mm	39	2673	593	−78%	
8.00mm	24	2676	523	−79%	
8.25 – 9.00 mm	113	2718	632	−76%	
DONOR FACTORS					
Age (years)					<0.001+
12 – <34	26	2950	886	−67%	
34 – <53	42	2695	654	−75%	
53 – <72	90	2615	581	−78%	
72 – 75	18	2666	563	−78%	

PACE =Pseudophakic/Aphakic Corneal Edema PA = Pseudophakic or Aphakic

*10-Year ECD Loss is calculated as (10-Year ECD – Baseline ECD)/Baseline ECD and expressed as a percent

**Adjusted for baseline ECD

+P-values for continuous factor

++P-value for comparing PACE with overall Fuchs. The P-value for comparing the three Fuchs groups is 0.42.

1Excludes 4 cases with other diagnosis: posterior polymorphous dystrophy (n=2), interstitial keratitis (n=1), and perforating corneal injury (n=1).

Table 4
Endothelial Cell Density (ECD) at 10 Years by Baseline Recipient/Donor Factors

Baseline Factors	*N*	Baseline ECD *Median*	10 year ECD *Median*	% Change in ECD* *Median*	Multivariate P -value for 10-year ECD**
Overall	176	2695	611	−76%	
RECIPIENT FACTORS					
Age (years)					0.07+
40 – <50	10	2794	700	−73%	
50 – <60	41	2690	595	−76%	
60 – <70	55	2673	618	−76%	
70 – 86	70	2709	607	−78%	
Gender					
Male	62	2720	632	−75%	
Female	114	2693	603	−78%	
Baseline Diagnosis and Lens Status [1]					0.001++
Fuchs: pre/post phakic	52	2696	640	−76%	
Fuchs: pre phakic/post PA	64	2700	598	−78%	
Fuchs: pre/post PA	29	2668	603	−77%	
PACE: post PA	27	2636	760	−72%	
History of diabetes					
No	158	2700	607	−77%	
Yes	18	2630	661	−74%	
OPERATIVE FACTORS					
Donor tissue size					0.02+
7.00 – 7.75 mm	39	2673	593	−78%	
8.00mm	24	2676	523	−79%	
8.25 – 9.00 mm	113	2718	632	−76%	
Donor tissue–recipient bed size disparity [2]					
Graft (0.25-0.50mm) smaller	19	2666	664	−75%	
Same size	12	2761	615	−77%	
Graft 0.25 mm larger	109	2692	584	−78%	
Graft 0.50mm larger	35	2728	689	−72%	
Vitrectomy					
No	165	2700	607	−77%	
Yes	11	2636	628	−74%	
Post-operative Intraocular Pressure (mmHg)					
≤ 25 mm Hg	163	2700	607	−77%	
>25 mm Hg	13	2549	687	−74%	
DONOR FACTORS					

Baseline Factors	*N*	Baseline ECD *Median*	10 year ECD *Median*	% Change in ECD* *Median*	Multivariate P -value for 10-year ECD**
Age (years)					<0.001 +
12 – <34	26	2950	886	−67%	
34 – <53	42	2695	654	−75%	
53 – <72	90	2615	581	−78%	
72 – 75	18	2666	563	−78%	
Gender					
Male	122	2672	603	−77%	
Female	54	2779	652	−75%	
Cause of death					
Cardio/Stroke	104	2671	604	−77%	
Cancer	21	2628	613	−75%	
Trauma	22	2826	688	−75%	
Respiratory	17	2722	542	−80%	
Other	12	2557	865	−67%	
History of diabetes					
No	149	2692	622	−76%	
Yes	27	2700	570	−80%	
Recipient/Donor Gender					
Both Female	36	2743	652	−75%	
Both Male	44	2677	632	−75%	
Gender Mismatch	96	2699	594	−78%	
Tissue retrieval					
Enucleation	43	2636	632	−76%	
In situ	133	2700	606	−77%	
Tissue refrigerated					
No	30	2726	607	−76%	
Yes	146	2691	614	−77%	
Time from death to preservation					
0–<5 hrs	25	2729	606	−76%	
5–<9 hrs	98	2699	604	−77%	
9–<11 hrs	33	2665	673	−76%	
≥11hrs	20	2809	631	−76%	
Time from death to surgery					
0–<3 days	25	2673	636	−75%	
3–<5 days	93	2687	616	−77%	
5–<9 days	58	2710	579	−77%	

PACE =Pseudophakic/Aphakic Corneal Edema PA = Pseudophakic or Aphakic

*10-Year ECD Loss is calculated as (10-Year ECD – Baseline ECD)/Baseline ECD and expressed as a percent

**Adjusted for baseline ECD

[+] P-values for continuous factor

[++] P-value for comparing PACE with overall Fuchs. The P-value for comparing the three Fuchs groups is 0.42.

[1] Excludes 4 cases with other diagnosis: posterior polymorphous dystrophy (n=2), interstitial keratitis (n=1), and perforating corneal injury (n=1).

[2] One participant had a missing value for bed size.