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Factors Associated with Graft Rejection in the Cornea Preservation Time Study

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The comprehensive list of participating CPTS clinical sites, investigators and coordinators; eye bank investigators; members of the Operations, Executive, Eye Bank Advisory, Data and Safety Monitoring Committee; Coordinating Center, Cornea Image Analysis Reading Center (CIARC), and Data Management and Analysis Center Staff; and the National Eye Institute staff have been previously published (*Cornea* 2015;34:601–608; *JAMA Ophthalmology* 2017;135:1401–09)

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Abstract

Purpose: To identify factors related to graft rejection following Descemet stripping automated endothelial keratoplasty (DSAEK) in the Cornea Preservation Time Study (CPTS).

Design: Cohort study within a multicenter randomized clinical trial.

Methods: 1330 eyes of 1090 subjects undergoing DSAEK were randomized to receive a donor cornea with preservation time (PT) of 0–7 days (N=675) or 8–14 days (N=655) and followed for three years. Central endothelial cell density (ECD) was determined by a central image analysis reading center. Multivariable Cox models adjusted for PT, recipient diagnosis, and surgeon effect were used to identify factors associated with rejection.

Results: Cumulative probability of definite graft rejection was 3.6% (99% CI 2.5% to 5.3%). Younger recipient age was associated with graft rejection [p<0.001; HR: 0.53 (0.33, 0.83) per decade]. PT, donor-recipient gender mismatch, recipient diagnosis, recipient race, graft size, discontinuation of topical corticosteroids, and immune-modulators, prior immunizations within three months, and prior glaucoma surgery were not associated with rejection (p>0.01). Among clear grafts with an ECD measurement at baseline and 3 years (N=913), endothelial cell loss (ECL) was greater in eyes that experienced a rejection episode (N=27) than in those that did not (N=886) (48% vs 38%, p=0.03). Twelve of 44 eyes (27%) with definite graft rejection subsequently failed, comprising 15% of the 79 failures in the CPTS.

Conclusions: Graft rejection is uncommon after DSAEK and more likely with younger age, in a study cohort mostly > 50 years old. Rejection increases ECL, but it is not a leading cause of DSAEK failure.

Introduction

Over the last decade, Descemet stripping automated endothelial keratoplasty (DSAEK) has become the most common surgical procedure for the management of corneal endothelial cell failure in the United States.[1] Its acceptance has been driven by excellent single-site studies that established the efficacy and safety of DSAEK, advanced and simplified surgical

techniques[2,3] and provided useful outcome data.[4–9] However, these studies provided limited information about the association of donor, recipient, operative, and postoperative factors with the success of the procedure. There also have been conflicting results on the rejection rate of this procedure (ranging from 0–45%[10–17]) and the factors influencing this rate.

The Cornea Preservation Time Study (CPTS) was a large, prospective, randomized, double-masked clinical trial designed to examine the relationship between donor preservation time (PT), graft success, and endothelial cell loss.[18] The CPTS established that there was no difference in graft success or endothelial cell loss for donor tissue preserved up to 11 days.[19,20] Secondary analyses demonstrated that donor diabetes, recipient diagnosis of pseudophakic/aphakic corneal edema (PACE), and operative complications were associated with lower graft success and greater endothelial cell loss.[21,22]

In addition, because of the varying reported rejection rates with DSAEK,[10–17] another pre-planned secondary outcome of the CPTS was the occurrence of rejection episodes. These data were collected with the *a priori* objective of identifying donor, recipient, and operative factors that might be associated with graft rejection, as well as the consequences of these rejection episodes on subsequent graft failure and endothelial cell loss from the largest prospective study of DSAEK to date.

Methods

The protocol was approved by institutional review boards for participating clinical sites and eye banks (e-Table1; supplemental material at AJO.com), and each participant provided written informed consent. Enrollment occurred between April, 2012, and February, 2014, and follow-up ended in June, 2017 with 1,330 cases (1,090 subjects) entered into the CPTS, performed across 40 sites involving 70 surgeons. Study oversight was provided by an independent data and safety monitoring committee. Details of the study methods have been reported previously,[18–20] and methods relevant to this paper are summarized below. Briefly, eyes with primary endothelial dysfunction, Fuchs endothelial corneal dystrophy (FECD) or PACE, were randomized to receive a donor cornea from either the “0–7d PT group” (PT ≤7 days) or the “8–14d PT group” (PT 8–14 days) which was stratified by surgeon. Surgeons used multiple surgical techniques and corticosteroid regimens. The full protocol is available at https://clinicaltrials.gov/ct2/show/NCT01537393.

Donor corneas met current Eye Bank Association of America (EBAA) standards for DSAEK with a minimum endothelial cell density (ECD) ≥2,300 cells/mm^2.[23] Eligibility criteria for participants have also been previously published.[18] Recipients could be 30 to 91 years old with eyes undergoing DSAEK for FECD or PACE, and eyes were excluded if they had complicated histories including previous penetrating keratoplasty (PKP) or DSAEK, tube shunts, uncontrolled glaucoma, anterior chamber intraocular lenses or significant anterior synechiae. Participants could enroll both eyes, if eligible. Preoperatively, a history of ocular surgery (particularly glaucoma surgery), diabetes, smoking, and FECD, other corneal dystrophies, and current medications (including systemic and topical corticosteroids, anti-glaucoma medications and systemic or topical immune-modulators) was obtained. Eye banks or surgeons prepared the donor corneas according to their customary technique.

Preoperative care, surgical technique, and postoperative care were provided according to each investigator's routine.

The Corneal Image Analysis Reading Center at Case Western Reserve University and University Hospitals Eye Institute (CIARC, Cleveland, OH), served as the reading center for endothelial image analysis to determine ECD and also was responsible for quality control measures at the eye banks and clinical sites. Details of the image quality, certification procedures, and endothelial imaging devices for each of the study eye banks and clinical sites have been previously published.[18,20,24] Details of image capture by the eye banks and clinical sites, and determination of the ECD by the CIARC are also detailed in these publications.[18,20,24] The CIARC-determined ECD using a variable frame method conducted by two independent masked readers, along with adjudication if the two readings differed by ≥ 5%.[18,20,25]

Follow-up examinations were performed at 1 day, 1 week, and 1, 6, 12, 24, and 36 months postoperatively. For participants who consented to extended follow-up, visits were also completed at 48 months and if possible within the study period, a 60-month visit was completed. Central recipient stroma clarity was assessed at every visit and graded on a validated three-point scale[18]: clear, equivocal, and cloudy. Each study visit also included documentation of topical medications, including systemic and topical corticosteroids, and systemic or topical immune-modulators. Whether the participant received any immunizations in the last 3 months prior to the appointment and, if yes, the type of immunization (e.g. influenza, tetanus, herpes zoster, pneumococcus, unknown vaccines, or other) was also recorded. Data from visits between scheduled study examinations were captured if a corneal abnormality was noted, including a suspected rejection episode. If active rejection was diagnosed, data from subsequent visits tracking resolution or worsening of the rejection episode were also captured.

Graft rejection was assessed using a modification of the Collaborative Corneal Transplantation Studies (CCTS) classification.[26–28] The rejection types are summarized in Table 1. All principal investigators at each clinical site were trained on assessing both definite and probable/possible rejection signs at an investigator meeting prior to commencement of study. Graft rejection episodes were managed at the discretion of each investigator. Criteria for determining the principal cause of graft failure have been previously published.[18,19]

Statistical Analysis

All eyes that underwent DSAEK were included in the analysis (N=1330). Results were analyzed by eyes, rather than patients. The outcome was time to the first definite graft rejection episode occurring up to three years after DSAEK. In all models, time was categorized based on intervals corresponding to the study examinations required by the protocol. Mild and severe definite graft rejection episodes were combined in the analysis. Data were censored at the time of non-rejection failure or the time of the last visit. No adjustments for multiple comparisons were made. Cumulative incidence plots based on

Kaplan-Meier estimates[29] of the distribution of time to the first graft rejection episode were constructed for each PT group.

The association of factors with graft rejection was evaluated using Cox proportional hazard models with the discrete logistic model option applied. A base model for evaluating candidate predictive factors included PT and the recipient diagnosis. All models included surgeon as a random effect to accommodate the potential correlation in graft rejection among DSAEKs performed by the same surgeon ("surgeon effect"). All candidate factors that were considered are listed in Table 2 and e-Table 2 (supplemental material at AJO.com). Each factor was first evaluated by adding the factor to the base model. Candidate factors were selected for inclusion in a final multivariable model in two stages. In stage 1, baseline recipient and donor factors associated with $p < 0.10$ were included in a multivariable backward model selection procedure.[30] In stage 2, all factors selected in stage 1 were retained and operative and postoperative factors associated with $p < 0.10$ were included in another multivariable backward model selection procedure to yield a final model.

Time dependent Cox models were also built for discontinuation of topical corticosteroid use, discontinuation of topical immune-modulator use, whether any prior immunizations were received over the past three months, whether any influenza immunizations were received over the past three months, and whether any pneumococcus immunizations were received over the past three months. All analyses above were repeated with the inclusion of possible rejection episodes.

Descriptive summary statistics were calculated for the subset of eyes experiencing a definite rejection episode. A Cox model adjusting for recipient diagnosis and a random surgeon effect was constructed to compare failure rates between PT groups among eyes that ultimately failed due to any cause after experiencing a rejection episode. Student's two sample t-test was used to compare pre-operative ECD between eyes that failed and those that survived.

The subset of eyes that did not experience graft failure were evaluated comparing ECD at three years and percent change in ECD from baseline to 3 years between eyes with and eyes without definite rejection episodes using two sample Wilcoxon tests. Two sample Wilcoxon tests were used to compare ECD at three years and percent change in ECD from baseline to three years between eyes with severe and mild rejection episodes. All statistical analyses were conducted using SAS, version 9.4 (SAS Inc).[31] All reported p-values are two-sided.

Results

The donor and recipient cohort have previously been described.[18] The mean recipient age of the 1090 DSAEK recipients at the time of enrollment was 70 ± 9 years (mean ± SD). 663 (61%) were female, and 983 (90%) were white. 1015 (93%) of the subjects had FECD, and the remaining 75 (7%) had PACE.

Definite Rejection

The three-year cumulative probability of a definite graft rejection episode was 3.6% (99% C.I.; 2.5% - 5.3%). During the follow-up period, 44 of the 1330 eyes (3.3%) in 44 of the 1090 subjects (4.0%) experienced one or more definite graft rejection episodes. Of the 44 definite graft rejection episodes, 22 (50%) were mild and 20 (45%) were severe. Severity was not determined for the remaining 2 episodes (5%), but these two grafts subsequently failed due to rejection. 22 (50%) of the 44 definite graft rejection episodes occurred prior to the one-year visit, whereas 16 (36%) occurred in between the one and two year visits, and 6 (14%) occurred in between the two and three year visits. The risk of definite rejection did not differ between the two PT groups (log rank p=0.85; Figure 1).

In a univariate analysis, there was a higher risk of definite rejection with female donors, non-white race, and younger recipient age. Those three factors were selected as candidate variables to be included in the final multivariable model (Table 2); however, donor gender and recipient race were removed from the final model during the backwards selection process. For every decade increase in recipient age, the hazard ratio (99% CI) was 0.53 (0.33, 0.83), indicating a decreased risk for rejection for older recipients compared to younger ones. In similar analyses, longer PT, gender mismatch between recipient and donor, recipient diagnosis, graft size, and recipient race all were found not to be associated with rejection (e-Table 2; supplemental material at AJO.com). No significant associations were detected for the immunization and topical corticosteroid or immune-modulator variables (Table 3). Among the 445 eyes with follow-up beyond 3 years that had not experienced a rejection episode, no eyes had a definite rejection episode in the 4th year.

Combined Definite, Probable/Possible Rejection

The three-year cumulative probability of a definite or probable/possible rejection episode was 6.0% (99% CI; 4.5% - 8.0%). Seventy-four of the 1330 eyes (5.6%) met the definition of definite or probable/possible rejection episode. Results of a multivariable analysis evaluating factors associated with definite, probable, or possible graft rejection episodes were similar to those of definite graft rejection episodes only (e-Table 3 through e-Table 5; supplemental material at AJO.com), with younger recipient age being the only significant factor ([HR: 0.63 (0.44, 0.89) per decade); p<0.001). Among the 445 eyes with follow-up beyond 3 years that had not experienced a graft rejection episode, 2 had a possible rejection episode in the 4th year.

Subset of Eyes with Definite Rejections

Twelve of 44 eyes (27%) that experienced definite rejection episodes subsequently failed, while the remainder cleared with treatment. These 12 failures comprised 15% of the 79 failures for all causes (Table 4) in 1% (12 of 1330 eyes) of all the eyes in the study. Four of 22 (18%) grafts with rejection episodes in the 0–7 day PT group failed, while 8 of 22 (36%) of grafts with rejection episodes in the 8–14 day PT group failed (p=0.20). Of the 42 eyes that had active definite rejection episodes, 28 (67%) were on topical corticosteroids at the time of rejection, 2 (5%) were on topical immune-modulators at the time of rejection, 1 (2%) was on both topical corticosteroids and immune-modulators, and the remainder 11 (26%) were not on either topical corticosteroids or immune-modulators.

Among 3 eyes with rejection episodes that ultimately failed, ECD (when available) was reduced by 1356 ± 984 cells/mm^2 (mean ±SD). For 29 eyes with rejection episodes that ultimately remained clear and had an ECD determination available after rejection, the mean (±SD) was a loss of 658 cells/mm^2 (± 632) (Table 4 and Figure 2). Similarly, the median (IQR) number of days between ECD determination occurring before and after rejection was 175 days (173, 716) for grafts that went on to failure. For grafts that did not fail, it was 321 days (196, 378). The median (IQR) ECL from the measurement before rejection to after rejection among all eyes experiencing rejections with an ECD determination available after rejection was 26% (13%, 60%). The mean (±SD) eye bank-determined preoperative ECD for grafts that had rejection and failed was 2799 cells/mm^2 (± 325), while the mean (±SD) eye bank-determined preoperative ECD for those that had rejection and survived was 2709 cells/mm^2 (± 260) (t-test p=0.35).

For those eyes (N = 28) that had at least one definite rejection episode but remained clear with 3-years follow-up and had an analyzable image at the exam compared to those eyes (N = 917) that did not experience any rejection episode or other postoperative complications and had clear grafts with an analyzable image, the central ECD at 3 years was 1349 ± 752 cells/mm^2 vs. 1693 ± 623 cells/mm^2, respectively (p=0.01). Among eyes with rejection episodes that had a central ECD determination available at 3 years, the mean (±SD) central ECD at 3 years was 1268 cells/mm^2 (±814) for eyes with severe rejections vs. 1419 cells/mm^2 (±714) for eyes with mild ones (p=0.45). Among eyes that had an ECD measurement at baseline and 3 years (N=913), ECL for the eyes that had a rejection (N=27) but had clear grafts at 3 years was 48% compared to 38% for those eyes that had clear grafts at the same time point but no prior history of rejection (N=886) (p=0.03). ECL for eyes with severe rejections at 3 years was 48% compared to 49% for eyes with mild rejections.

Discussion

We found an overall low cumulative probability of a definite rejection episode for DSAEK of 3.6% and of a possible, probable or definite rejection of 6.0% in the CPTS with 50% of the definite rejection episodes occurring within the first postoperative year. This rate is lower than rates reported in many previous studies of DSAEK[10–17] and well below the rejection rate of 17% for PKP in one large series.[16] Immunologic graft rejection of corneal transplants is less likely than that of solid organs, presumably because the cornea is normally avascular, isolating it from the immune system. Theoretically, rejection of DSAEK grafts should be less likely than that of penetrating grafts because DSAEK grafts are smaller, containing less foreign antigen, and are more isolated from the vascular system, lymphatic system, and peripheral Purkinje processing cells. DSAEK grafts also present allo-antigens via the anterior chamber, which tends to induce tolerance, rather than rejection (anterior chamber-associated immune deviation, ACAID).[32,33]

The incidence of rejection episodes is dependent on the definition that is used for a rejection episode. In general, the definition used in this study is broader than that used for other studies, which often require the presence of keratic precipitates. For example, Allan et al[10] reported 7.5% rejection in the first two years after deep lamellar endothelial keratoplasty (DLEK) and DSAEK based on defining rejection as “any anterior chamber inflammatory

episode with keratic precipitates on the transplanted endothelium needing an unscheduled increase in topical steroid medication". Hjordtal et al[34], reported a rejection rate of 5% through 5 years after DSAEK (although all episodes occurred in the first two years), based on defining rejection as "precipitates on the corneal graft but not on the peripheral recipient cornea, either scattered or in the form of a Khodadoust line along with an increase in central corneal thickness". Finally, Ezon et al[35], also reported a post DSAEK rejection rate of 5% with a median follow-up of 29 months, based on defining rejection as the presence of keratic precipitates on the endothelium, a rejection line, or sub-epithelial infiltrates. Considering that our definition was broader (i.e. allowed for other signs of inflammation and corneal thickness changes as well as keratic precipitates) and we are reporting on rejection events up to 48 months after DSAEK, our lower 3.6% cumulative probability of a definite rejection episode across multiple surgeons and sites supports a much lower rate of rejection in DSAEK compared to that of PKP[16].

The rejection rate in the CPTS, although nearly double the mean rejection rate of 1.9% for DMEK reported by the American Academy of Ophthalmology Ophthalmic Technology Assessment[36], was comparable to the rates in several DMEK studies utilizing topical corticosteroid therapy[37–40] and lower than the 6% rate when corticosteroids were discontinued.[41] Meta-analyses either support no significant difference in the rejection rates between the two procedures[42] or a slight advantage to DMEK.[43]

After a comprehensive multivariable analysis of the association of 25 donor, recipient, operative, and postoperative factors with the occurrence of a definite rejection episode, the only statistically significant factor identified was recipient age; for every decade increase in recipient age, the risk for rejection decreased (HR: 0.53; 99% CI (0.33, 0.83). However, it is important to note that there were only 15 recipients <50 years old; thus, this finding principally applies to recipients over 50 years old. A higher risk for rejection of penetrating grafts has been well recognized in the pediatric population.[44] To our knowledge this is the first report that among recipients older than 50 years old, graft rejection following DSAEK is more likely in younger recipients, as has been noted with high rejection risk PKP cases.[27] This may be the result of the immune system becoming less active with increasing age,[45]

Notably, the following factors were not associated with a higher risk for a rejection episode: donor age, recipient gender, gender mismatch between donor and recipient, recipient diagnosis, recipient race, individuals with history of use of glaucoma medications or past less complex glaucoma surgery (e.g. trabeculectomy, laser trabeculoplasty), PT, graft size, and prior immunizations within three months of the rejection episode. The CDS, another major prospective clinical trial found that PACE, prior use of glaucoma medications, and glaucoma filtering surgery were associated with higher risk for graft rejection following PKP.[46,47] Female recipient gender following PKP in the CDS,[47] as well as donor-recipient gender mismatch following PKP (63% of cases) and endothelial keratoplasty (37%) from United Kingdom cornea transplant registry data[48] were also associated with a higher risk of graft rejection. However, both the CDS and the UK registry studies involved more complicated eyes with PACE and glaucoma, predominantly performing PKP. A recent study of DMEK also did not find donor-recipient gender mismatch to increase the risk of rejection.[49] Longer PT could theoretically lower graft rejection risk because of reduced antigen load

with time,[50] but the CPTS did not support this theory. Graft size has been considered a risk factor for rejection, but primarily for large grafts over 9 mm for therapeutic PKP keratoplasty.[51] PKP studies have not shown an effect for typical donor diameters between 7.5 and 8.5 mm.[52,53] The CPTS confirms the findings of Terry et al for DSAEK who found no significant impact on rejection rate for 8.0 mm and 8.5 mm grafts.[54] Race has also been considered a risk factor in keratoplasty.[55] However, 90% of the subjects in the CPTS were Caucasian (primarily because 94% of our cases were for the surgical management of FECD)[18] and we were not able to detect an effect of race on rejection. It has been hypothesized that recent immunizations might increase the risk of corneal graft rejection[56]; however, the CPTS was not able to identify immunization within 3 months as a risk factor.

Only 12 (27%) of the grafts in the CPTS that experienced a definite rejection episode went on to failure from any cause after 3 years of follow-up and only 8 of these demonstrated a clear path from rejection to failure. This compares with PKP in the CDS with 5 years of follow-up and more complicated PACE which showed a somewhat higher 37% (92 of 247 eyes) of eyes with a rejection going on to failure.[47] Thus, the rate of reversal of rejection is somewhat better for DSAEK than it is for PKP, with only 18% (8 of 44 eyes) of eyes with a rejection episode going on to failure. Additionally, the overall rate of failure of DSAEK from rejection was very low in the CPTS (8 out of 1330 eyes, 1%) and in the literature.[42,43]

A definite rejection episode caused a significant decrease in ECD in grafts that went on to fail. Also, grafts that survived at least one definite rejection episode and were clear at 3 years had a significantly lower ECD and greater percentage cell loss (48% vs. 38%) at 3 years compared to those grafts that had had an uncomplicated postoperative course. Price et al. has also shown a significant correlation between rejection episodes and ECD at 5 years after both DSEK and DMEK.[49]

The strengths of this report include its design as a prospective multi-center, multi-surgeon large study with the collection of major factors that could have been associated with graft rejection by trained observers, and use of multivariable analytical modeling to identify the influential factors that might influence the development of a graft rejection episode. Follow-up was sufficient to determine the impact of these rejection episodes on subsequent graft failure and endothelial cell loss.

The limitations include the fact that rejection episodes between study visits may not have been captured, particularly if the patient was not symptomatic or was managed elsewhere, outside the practice of the investigator, although we suspect that was rare. Although corticosteroid and immunomodulatory therapy was tracked, the specific agents utilized were not; thus specific management approaches to the rejection episodes cannot be evaluated. Although the reporting of 99% confidence intervals does not fully adjust for the multiplicity effect of evaluating 25 factors, the p value for the association of graft rejection and age was <0.001, making chance an unlikely explanation for this finding. For some factors the lack of finding a significant association with graft failure may have had inadequate precision from the small number of graft rejections observed. The lack of detection of an association of the discontinuation of topical corticosteroids and immune-modulators may be an example of this with the confidence intervals for the hazard ratios wide (Table 2). Finally, exclusion criteria

prevented enrollment of potentially more complicated PACE eyes with anterior chamber intraocular lenses, more extensive anterior synechiae, and eyes with more advanced glaucoma including tube shunts. The CPTS could not confirm the increased risk of rejection associated with PACE[47] and previous use of glaucoma medications and filtering surgery[46] as in the CDS because these eyes were excluded from the CPTS. Thus, our findings only apply to less complicated DSAEK cases performed for FECD and uncomplicated PACE.

In conclusion, we found that younger DSAEK recipients are more likely to reject their grafts than older recipients, with the caveat that few of the study participants were <50 years old. Within this age limit, the younger DSAEK patients may bear closer observation and consideration for more prolonged topical corticosteroid therapy. Studies are suggested for DMEK patients to determine if recipient age is a factor in these cases. Importantly, the CPTS found that the rejection rate was not increased for patients receiving immunizations for influenza or other infections. Therefore, physicians can feel confident in advising their DSAEK patients that they can proceed with immunizations without increasing their risk of graft rejection. Remarkably, the CPTS also found that other donor, recipient and operative factors were not associated with a higher rejection rate including donor age, PT, graft size, recipient gender, donor-recipient gender mismatch, and operative complications. Finally, the CPTS provides the best view of the rejection rate for DSAEK in a multi-center, multi-surgeon study. The rate was on the lower spectrum of reported rejection rates at approximately 3% of eyes and 4% of subjects, and only 1% of the grafts failed due to rejection. These data support the concept that DSAEK has made a major impact in lowering the failure rate of corneal transplants from immunologic rejection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- DSAEK rejection rates are low (3.6%) after 3 years in the CPTS.
- Younger recipient age is associated with a higher rejection rate.

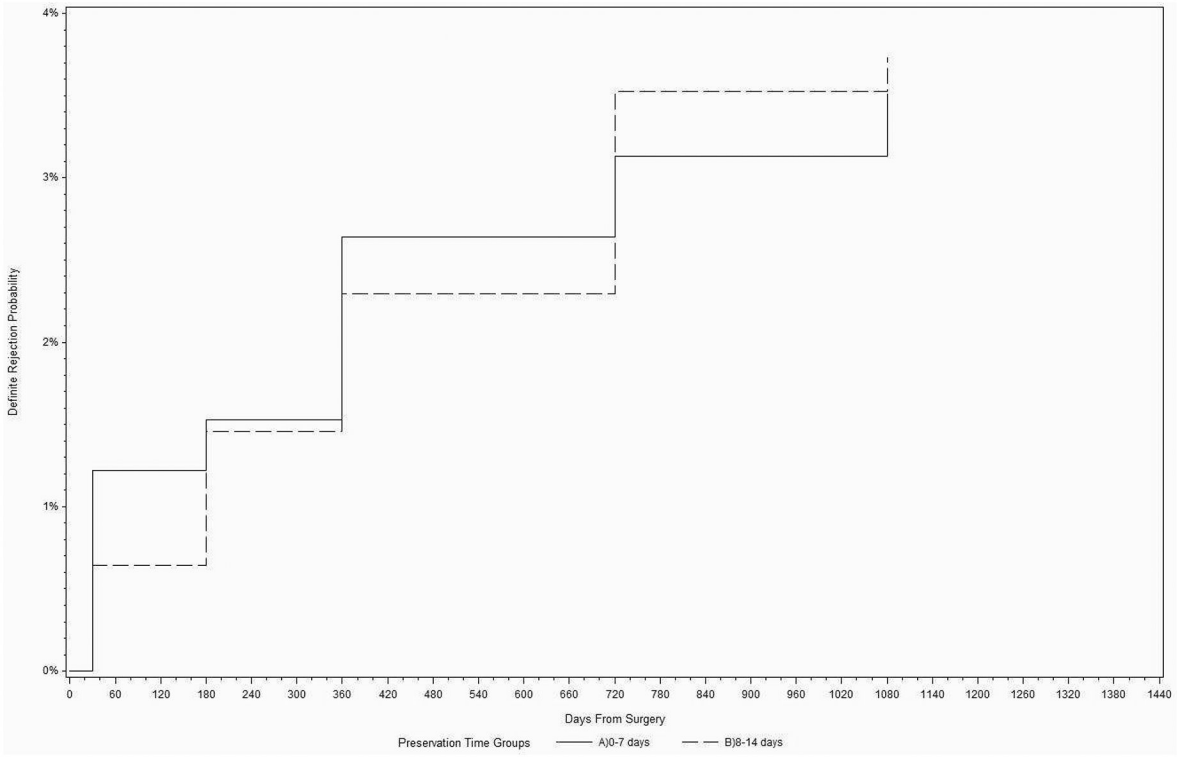

Figure 1.

Cumulative incidence for the first definite graft rejection episode in the Cornea Preservation Time Study

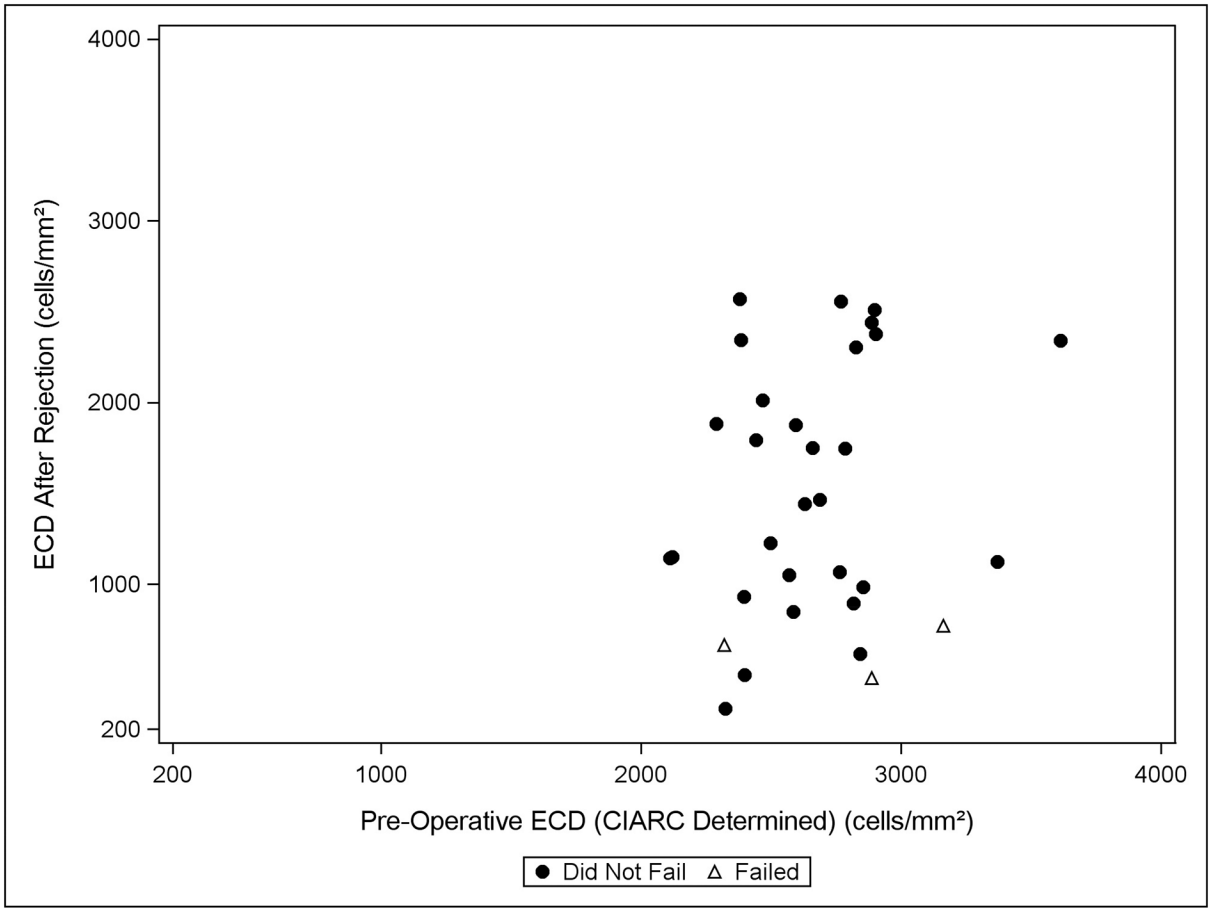

Figure 2.
Endothelial cell density after the first definite graft rejection episode vs. pre-operative endothelial cell density

Table 1.

CPTS Graft Rejection Classification

1	***Definite/Mild*:**
	a. Presence of one to five keratic precipitates or
	b. An increase in aqueous cells after the usual early postoperative period without clinically apparent change in stromal thickness from previous visit or clinically evident stromal and/or epithelial edema.
2	***Definite/Severe*:** presence of one or more of the following signs:
	a. More than five keratic precipitates,
	b. Inflammatory cells in the stroma,
	c. An endothelial rejection line,
	d. Moderate to severe increase in aqueous cells compared to previous visit,
	e. Any of the preceding with stromal and/or epithelial edema and a reduction in stromal clarity.
3	***Possible/Probable*:**
	a. Clinically apparent stromal edema impacting stromal clarity with the possible presence of new keratic precipitates.

Table 2.

Association between donor, recipient, and operative risk factors and definite graft rejection episode through 3 years in the Cornea Preservation Time Study

			Base Model[a]		Multivariable Model[b]	
	N=1330	3-yr Graft Rejection (99% CI)	Hazard Ratio (99% CI)	p-value	Hazard Ratio (99% CI)	p-value
Significant Don or/Recipien t Factors						
Recipient Age				**<0.001**		**<0.001**
<51 years	15	16.7% (2.9%, 67.7%)				
51–60 years	178	7.2% (3.5%, 14.5%)				
61–70 years	459	3.8% (2.0%, 7.1%)	0.53 (0.33, 0.83) per decade		0.53 (0.33, 0.83) per decade	
71–80 years	502	2.4% (1.1%, 5.2%)				
81–90 years	173	1.9% (0.4%, 8.3%				
>90 years	3	0.0% (0.0%, 0.0%)				
Other Don or/Recipient Factors						
Donor Gender				0.03		0.02
Female	486	5.2% (3.1%, 8.8%)	1.93 (0.88, 4.24)		2.02 (0.92, 4.45)	
Male	844	2.7% (1.5%, 4.7%)	1 [Ref]		1 [Ref]	
Recipient Diagnosis				0.84		0.68
FECD	1255	3.6% (2.5%, 5.4%)	1 [Ref]		1 [Ref]	
PACE	75	3.1% (0.5%, 17.9%)	0.87 (0.13, 5.78)		1.35 (0.20, 9.38)	
Preservation Time Group				0.85		0.91
0–7 days	675	3.5% (2.0%, 6.0%)	1 [Ref]		1 [Ref]	
8–14 days	655	3.7% (2.2%, 6.4%)	1.06 (0.49, 2.31)		1.04 (0.47, 2.27)	
Recipient Race/Ethnicity				0.08		0.11
Black/African American	47	2.6% (0.2%, 29.6%)	0.77 (0.06, 10.78)		0.55 (0.04, 7.83)	
White	1207	3.3% (2.2%, 5.0%)	1 [Ref]		1 [Ref]	
Other	76	9.0% (3.2%, 23.8%)	2.90 (0.84, 9.96)		2.60 (0.75, 9.05)	

[a] Base models adjusted for PT, recipient diagnosis, random surgeon effect

[b] Multivariable model adjusted for preservation time, recipient diagnosis, random surgeon effect. Recipient age was retained in the final model via backward selection. For factors not retained in the final model, the hazard ratio and p-value were obtained from the final model with the factor of interest included.

CI = confidence interval.

Table 3.

Hazard ratios for Immunizations and steroid discontinuations of as time-dependent covariates for definite graft rejection episode through 3 years in the Cornea Preservation Time Study

Occurrence	N[a]	Hazard Ratio (99% CI)	p-value
Any Immunizations in Past Three Months	571	0.82 (0.24, 2.85)	0.67
Any Flu Immunizations in Past Three Months	509	0.94 (0.27, 3.29)	0.90
Any Pneumococcus Immunizations in Past Three Months	120	1.51 (0.11, 21.48)	0.69
Discontinued Topical Corticosteroid	86	1.16 (0.08, 16.86)	0.88
Discontinued Topical Immune-modulator	22	7.12 (0.46, 111.35)	0.06

CI = confidence interval.

[a]Number of eyes with the one or more occurrences during follow-up

Table 4.

Characteristics of 44 eyes with a definite graft rejection episode by graft failure status at 3 years in the Cornea Preservation Time Study

	Failed (N=12)	Did Not Fail (N=32)
Selected Donor/Recipient Factors		
n (%)		
Recipient Diagnosis		
FECD	10 (24%)	32 (76%)
PACE (without FECD)	2 (100%)	0 (0%)
Preservation Time Group		
0–7 Days	4 (18%)	18 (82%)
8–14 Days	8 (36%)	14 (64%)
Donor History of Diabetes		
No	7 (25%)	21 (75%)
Yes	5 (31%)	11 (69%)
mean ± SD		
Recipient Age (years)	67 ± 8	65 ± 10
Screening ECD (EB determined - cells/mm^2)	2834 ± 380	2713±291
Pre-Operative ECD (EB determined - cells/mm^2)	2799 ± 325	2709 ± 260
Pre-Operative ECD (CIARC determined - cells/mm^2)[a]	2769 ± 423	2667 ± 324
median (IQR)		
ECD before rejection (cells/mm^2)[a,b]	2000 (962, 2726)	2443 (1830, 2765)
ECD after rejection (cells/mm^2)[c]	665 (481, 772)	1453 (984, 2303)
mean ± SD		
Difference in ECD after and before rejection (cells/mm^2)[a,b,c]	−1356 ± 984	−658 ± 632
median (IQR)		
Days between ECD before and after rejection[a,b,c]	175 (173, 716)	321 (196, 378)
Days from last ECD before rejection to rejection[a,b]	147 (111, 267)	138 (82, 309)

[a] Excludes one eye that did not fail. This eye did not have a CIARC determined pre-operative ECD.

[b] For eyes with no ECD measurements at follow-up prior to rejection, the baseline value was used as ECD prior to rejection, and the first visit time was used as the time of measurement. One eye did not have a baseline ECD value or any ECD measurements before rejection. This eye was excluded from tabulations involving ECD before rejection.

[c] Nine eyes that failed and two eyes that did not fail did not have any ECD screenings after rejection. One eye that did not fail had an ECD screening after rejection but no screening before rejection. Only eyes with an ECD screening available before and after rejection were included in the tabulations of differences between before and after rejection. The difference in the number of days from lasted screening before rejection to the date of rejection could not be calculated for the eye with no measurement prior to rejection.

PACE = pseudophakic/aphakic corneal edema; FECD = Fuchs endothelial corneal dystrophy; ECD = endothelial cell density; EB = eye bank; CIARC = Cornea Image Analysis Reading Center