A Mathematical Model to Predict Endothelial Cell Density Following Penetrating Keratoplasty With Selective Dropout From Graft Failure

Tonya D. Riddlesworth,¹ Craig Kollman,¹ Jonathan H. Lass,² Sanjay V. Patel,³ R. Doyle Stulting,⁴ Beth Ann Benetz,² Robin L. Gal,¹ and Roy W. Beck¹

¹Jaeb Center for Health Research, Tampa, Florida, United States

²Case Western Reserve University and University Hospitals Eye Institute, Cleveland, Ohio, United States

³Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota, United States

⁴Woolfson Eye Institute, Atlanta, Georgia, United States

Correspondence: Tonya D. Riddlesworth, Jaeb Center for Health Research, 15310 Amberly Drive, Suite 350, Tampa, FL 33647, USA; cds@jaeb.org.

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PURPOSE. We constructed several mathematical models that predict endothelial cell density (ECD) for patients after penetrating keratoplasty (PK) for a moderate-risk condition (principally Fuchs' dystrophy or pseudophakic/aphakic corneal edema).

METHODS. In a subset ($n = 591$) of Cornea Donor Study participants, postoperative ECD was determined by a central reading center. Various statistical models were considered to estimate the ECD trend longitudinally over 10 years of follow-up. A biexponential model with and without a logarithm transformation was fit using the Gauss-Newton nonlinear least squares algorithm. To account for correlated data, a log-polynomial model was fit using the restricted maximum likelihood method. A sensitivity analysis for the potential bias due to selective dropout was performed using Bayesian analysis techniques.

RESULTS. The three models using a logarithm transformation yield similar trends, whereas the model without the transform predicts higher ECD values. The adjustment for selective dropout turns out to be negligible. However, this is possibly due to the relatively low rate of graft failure in this cohort (19% at 10 years). Fuchs' dystrophy and pseudophakic/aphakic corneal edema (PACE) patients had similar ECD decay curves, with the PACE group having slightly higher cell densities by 10 years.

CONCLUSIONS. Endothelial cell loss after PK can be modeled via a log-polynomial model, which accounts for the correlated data from repeated measures on the same subject. This model is not significantly affected by the selective dropout due to graft failure. Our findings warrant further study on how this may extend to ECD following endothelial keratoplasty.

Keywords: penetrating keratoplasty, endothelial cell density, mathematical model

The Cornea Donor Study (CDS) was designed to determine the effect of donor age on the outcome of penetrating keratoplasty (PK) in eyes with a diagnosis associated with a moderate-risk of failure (principally Fuchs' dystrophy and pseudophakic or aphakic corneal edema [PACE]). Longitudinal central endothelial imaging, performed as part of the Specular Microscopy Ancillary Study (SMAS), showed that there was substantial endothelial cell loss in successful grafts over the 10 years of follow-up, irrespective of donor age. In particular, the SMAS showed that lower endothelial cell density (ECD) at 6 months, 1 and 5 years were associated with endothelial graft failure after 10 years of follow-up.¹ Therefore, it is important to understand the decay of the ECD over time to better understand this mechanism of graft failure. Previous clinical studies have analyzed the effect of donor, recipient, postoperative, and operative factors on ECD loss.^{2–10} The focus of this study is to develop a mathematical model to describe the endothelial cell loss over time after PK.

Redmond et al.¹¹ proposed an exponential decay model for endothelial cell loss after PK in which an initial rapid rate of

cell loss was followed by a reduced rate at later times. The bimodal rate of cell loss has been discussed by many investigators, including Armitage et al.,¹² Patel et al.,¹³ and Böhringer et al.,¹⁴ who concluded that a single exponential model does not adequately describe cell loss after PK. Armitage et al.¹² proposed a mixture of two exponentials with different decay rates (a "biexponential" curve). However, none of these previous analyses accounted for the correlated data from repeated measures on the same subjects in longitudinal follow-up or the selective dropout from subjects with lower ECDs who are more likely to experience graft failure. The latter is particularly problematic because selective dropout can result in a slower rate of cell loss with time if there is a higher rate of cell loss in grafts that fail earlier. In this study, we evaluate various mathematical models for describing ECD over 10 years of follow-up after PK accounting for correlated data and the possible bias due to the selective dropout so that we may better understand the decay of the ECD post-PK.

TABLE 1. Availability of ECD Data Over Time ($n = 591$ subjects)

Time (Window)	Cumulative # Graft Failures*	Cumulative # Withdrawals*,†	# Still Active*	# Completed Visit‡	# Gradable Images‡
6 Mo (0–273 d)	N/A	N/A	591	591	301
1 Y (274–547 d)	3	3	585	584	377
2 Y (548–912 d)	10	13	568	559	354
3 Y (913–1277 d)	23	34	534	517	313
4 Y (1278–1643 d)	30	45	516	476	290
5 Y (1644–2008 d)	43	71	477	467	345
7–8 Y (2375–3103 d)	59	155§	377	374	188
10 Y (3287–4383 d)	84	194§	313	313	176

* At the beginning of the indicated window.

† Includes patient deaths and lost to follow-up.

‡ During the indicated window.

§ Includes 51 subjects who chose not to participate in the 10-year extension (original consent was for a 5-year study).

METHODS

This study adhered to the tenets of the Declaration of Helsinki. Details of the CDS and SMAS protocols have been reported previously.^{15–19} Between January 2000 and August 2002, 1090 eligible subjects between 40 and 80 years had a PK for corneal disease associated with endothelial decompensation and moderate risk of failure. Eligible corneas were from donors aged 10 to 75 years that met Eye Bank Association of America standards for transplant. Clinical investigators and participants were masked to all characteristics of the donor cornea. Preoperative management, surgical technique, and postoperative care and perioperative medications, were provided according to each investigator's routine. The definition of graft failure, based on the definition used in the Collaborative Corneal Transplantation Studies (CCTS),^{20,21} was a regraft or, in the absence of regraft, a cloudy cornea in which there was loss of central graft clarity sufficient to compromise vision for a minimum of three consecutive months.

A subset of patients ($n = 612$) participated in the SMAS ancillary study and $n = 591$ had at least one gradable central endothelial image at one of the follow-up visits. As previously reported, baseline characteristics were similar in the CDS subjects who participated in SMAS and those who did not participate in SMAS.¹⁹ Images were obtained at 6 months, annual follow-up visits through year 5, at years 7 to 8 and at year 10 for a total of $n = 2344$ data points from 591 subjects (Table 1). Images recorded after graft failure (including those due to graft rejection) were omitted from this analysis. Mean ($\pm$ SD) age was 69 ± 9 years for recipients and 57 ± 15 years for donors; 398 (67%) had Fuchs' dystrophy, 173 (29%) PACE, and 20 (3%) other corneal pathology. At 10 years, the overall graft failure rate ($\pm$ 95% confidence interval) was $19\% \pm 4\%$ with 176 gradable images remaining at 10 years from eyes without graft failure.

Images were evaluated for quality and ECD by a central reading center, the Cornea Image Analysis Reading Center (CIARC; formerly the Specular Microscopy Reading Center) at

Case Western Reserve University, Department of Ophthalmology and Visual Sciences, and University Hospitals Eye Institute (Cleveland, Ohio, USA), using a variable frame analysis method. Details of CIARC procedures have been described previously for donor and postoperative images, 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.¹⁷ Images were only available before graft failure or censor date due to regraft or loss to follow-up.

Various statistical models were considered to estimate the ECD trend curve longitudinally over 10 years of follow-up (Table 2). A biexponential model, $ECD_t = p_1 \cdot \exp(\alpha_1 \cdot t) + p_2 \cdot \exp(\alpha_2 \cdot t) + \text{error}$ (where ECD_t is the cell density at time t , p_1 and p_2 are constants, and α_1 and α_2 are exponential decay rates), was fit. Since residual values were not normally distributed, a second biexponential model was fit using a logarithm transformation ("log biexponential"): $\log(ECD_t) = \log[p_1 \cdot \exp(\alpha_1 \cdot t) + p_2 \cdot \exp(\alpha_2 \cdot t) + \text{error}]$. Both models were fit by nonlinear least squares using the Gauss-Newton algorithm. Attempts to account for the correlated data in the nonlinear regression led to models that did not converge. Therefore, the biexponential models were fit assuming (incorrectly) independent observations for purposes of comparison.

To account for correlated data, an alternate model was considered where the logarithm of the ECD value was assumed to be a polynomial in time ("log-polynomial"): $\log(ECD_t) = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot t^2 + \beta_3 \cdot t^3 + \text{error}$. The four parameters $\{\beta_i\}_{i=0}^3$ were assumed to be random effects varying between subjects each according to a normal distribution. This model was fit using restricted maximum likelihood (REML). Results are presented using the back transformation $ECD_t \sim \exp(\beta_0 + \beta_1 \cdot t + \beta_2 \cdot t^2 + \beta_3 \cdot t^3)$.

Finally, a selection model using Bayesian analysis was fit to account for the nonrandom dropout due to graft failure. The probability of graft failure was modeled as a logistic function of the (potentially unobserved) ECD value. Other sources of

TABLE 2. Summary of Models Fit to the ECD Data

Model	Logarithm Transformation	Accounts for Correlated Data	Accounts for Selective Dropout	Estimated Regression Equation for ECD_t , t Denotes Years From Surgery
Bi-exponential	No	No	No	$2246 \cdot \exp(-0.299 \cdot t) + 439 \cdot \exp(2.98 \cdot 10^{-2} \cdot t)$
Log bi-exponential	Yes	No	No	$2066 \cdot \exp(-0.412 \cdot t) + 604 \cdot \exp(2.01 \cdot 10^{-3} \cdot t)$
Log-polynomial	Yes	Yes	No	$2678 \cdot \exp(-0.365 \cdot t + 2.81 \cdot 10^{-2} \cdot t^2 - 6.00 \cdot 10^{-4} \cdot t^3)$
Bayesian MCMC	Yes	Yes	Yes	$2664 \cdot \exp(-0.361 \cdot t + 2.66 \cdot 10^{-2} \cdot t^2 - 5.30 \cdot 10^{-4} \cdot t^3)$

FIGURE 1. Endothelial cell density over time for all subjects ($n = 591$) and restricted to subjects with a surviving graft and a gradable image at 10 years ($n = 176$). Vertical axis is on a logarithmic scale. In each box, the black dot indicates the geometric mean, horizontal lines inside the box indicate medians, and the bottom and top of the boxes indicate the 25th and 75th percentiles, respectively.

missing data (e.g., missed visits, ungradable images) were assumed to be missing at random. The ECD value itself was modeled as a log-polynomial with an unstructured covariance matrix to account for correlated data. Noninformative prior distributions were assumed for the logistic function parameters and for the $\{\beta_i\}_{i=0}^3$ parameters of the log-polynomial as defined above. An inverse Wishart prior distribution was used for the covariance matrix. Markov chain Monte Carlo (MCMC) was used to estimate the posterior distribution for each of the parameters. Multiple chains were run from various starting points to verify that results were similar. The ECD trend curve was then taken by using the posterior mean for each parameter.

Separate analyses were done for subjects with a diagnosis of Fuchs' dystrophy ($n = 398$) and subjects with a diagnosis of PACE ($n = 173$).

A summary of these four models is given in Table 2. All models were fit using SAS version 9.3 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The logarithm of ECD decreased nonlinearly in time suggesting that a simple exponential decay model would not be a good fit to the ECD data (Fig. 1). A similar flattening of the rate of decline was observed for the entire cohort (Fig. 1A) and for cases with a surviving graft at 10 years (Fig. 1B). Among the 176 patients with a surviving graft and a gradable image at 10 years, the median ECD (25th, 75th percentiles) was 611 (502, 769) cells/mm² ranging from 275 to 2674, and the median cell loss from baseline was 76% (70%, 82%). The rate of cell loss per year was approximately 13% over the first 5 years, slowing to approximately 6% from years 5 to 10.

For both of the biexponential models, the estimated value of the second decay parameter (α_2 as described in Methods) was slightly positive denoting growth instead of decay (Table 2). In both models, the value was very close to zero so that the resulting curve in each case was basically a single exponential decay shifted up by a constant value. Figure 2 shows the estimated trend curves from the four models. At 3 years and later, the biexponential model without a logarithm transformation tended to estimate the mean values whereas the other three models with a logarithm transformation tended to estimate the medians (Fig. 2; Table 2). The log-polynomial model accounting for correlated data tended to be approximately 30 to 70 cells/mm² lower compared to the log biexponential model that assumed independent values. Accounting for selective dropout from graft failure did not meaningfully affect the estimated ECD as the Bayesian MCMC and the log-polynomial curves in Figure 2 were virtually identical.

When further analyzing the data for those recipients with Fuchs' dystrophy and those with PACE, we found a similar pattern of the cell loss curve with the PACE group having lower ECDs for the first 5 to 6 years, but slightly higher 10-year ECDs (Fig. 3).

DISCUSSION

Chronic loss of endothelial cells following PK has been shown to be associated with graft failure.^{1,5,22,23} A model predicting the decay of ECD following PK, therefore, could help better predict the risk of endothelial graft failure. Any model for such cell loss must account for the distribution and timing of cell loss following the PK. Clinical studies have been limited because longitudinal endothelial imaging is restricted to the central endothelium of grafts due in part to the limitations of

FIGURE 2. Endothelial cell density over time with modeled curves – untransformed scale (A) and a logarithmic scale (B) for the vertical axis. In each box, the black dot indicates the mean (A) or geometric mean (B), horizontal lines inside the box indicate medians, and the bottom and top of the boxes indicate the 25th and 75th percentiles, respectively.

FIGURE 3. Endothelial cell density over time with modeled curves – Fuchs' dystrophy diagnosis (A), PACE diagnosis (B), and the Bayesian MCMC models for Fuchs' dystrophy and PACE (C). In each box in (A) and (B), the black dot indicates the mean, horizontal lines inside the box indicate medians, and the bottom and top of the boxes indicate the 25th and 75th percentiles, respectively.

specular or confocal microscopy technology. Traditionally, hypotheses to explain the change in endothelial cell density with time after corneal transplantation have focused on the factors affecting cell damage and loss, the role of donor cell migration, enlargement of remaining healthy donor endothelial cells to compensate for the damage and loss, and the role of the recipient endothelium outside the donor bed to contribute to the reparative process. Clinical studies have speculated that the progressive cell loss observed centrally following PK is due to donor factors (e.g., sex, age, preparation, corneal preservation, lens status), recipient factors (e.g., diagnosis, pre-existing glaucoma, anterior chamber IOL, glaucoma shunt tube), postoperative factors (graft rejection, glaucoma), and operative factors (e.g., trephination, suturing).²⁻¹⁰

Recent vital dye and histologic studies provide direct evidence as to the major contributors to the clinically observed central endothelial cell loss. In a donor trephination study, Alqudah et al.² showed 25% cell loss over the entire area of the donor endothelium attributable to the areas of trephination and suture placement. A study by Regis-Pacheco and Binder⁸ found endothelial cell migration from the higher to the lower density across the PK wound over time, accounting for the progressive central endothelial cell loss necessary to repair the significant peripheral damage at the wound. This impact of peripheral damage is most prominent in PACE where there is no recipient endothelium to contribute to maintenance of the endothelial population of the graft. However, cell loss is most likely moderated in Fuchs' dystrophy by contribution of the recipient endothelium not affected by the disease,²⁴ particularly if there is excellent posterior wound apposition between the recipient bed and the donor. The end result observed clinically is profound central endothelial cell loss. Ing et al.⁵ described changes in ECD over 10 years of follow-up from 394 subjects who underwent PK for any indication and reported a mean cell loss of 67% at 10 years among the 119 with a surviving graft at that point. Observed cell loss was similar in the CDS, with a median cell loss of 76% among 176 with a surviving graft at 10 years. Cell loss was slightly higher in the CDS compared to the study by Ing et al.,⁵ possibly because only cases of endothelial dysfunction were included in the CDS, whereas Ing et al.⁵ included a large number of keratoconus cases where recipient endothelium may have moderated the central endothelial cell loss by assisting in the repair of the peripheral donor endothelial cell damage.

A number of studies have proposed specific mathematical models for the decline of ECD following PK.^{11,12,14,25} Armitage et al.¹² proposed a biexponential model with two separate rates of exponential decay, postulated to reflect distinct modes of endothelial failure from trauma at the time of the surgery versus later chronic endothelial cell loss. Böhringer et al.¹⁴ ran simulations assuming that graft failure occurs when the ECD drops below 500 cells/mm². Data from the CDS have shown that many grafts still can survive even when the ECD drops below 500 cells/mm², but the risk of graft failure clearly increases with declining ECD.^{1,4,19} This indicates that one cannot simply specify a minimal ECD required to maintain a functioning graft.

One common characteristic of virtually all published reports of ECD after PK is a relatively rapid loss of cells in the first few years after surgery, followed by a lower rate of cell loss, with stabilization of ECD at approximately 500 cells/mm². This observation was confirmed in the CDS. A possible explanation of this stabilization phenomenon would be the selective loss of grafts that fail if the ECD drops below approximately 500 cells/mm². However, in the SMAS and other studies, many grafts survived with ECD below 500 cells/mm².^{3,19} Furthermore, the similarities in the ECDs for the entire cohort and for the cases with a surviving graft at 10 years

as seen in Figure 1 suggests that selective loss of failed grafts does not explain the stabilization in the rate of endothelial cell loss. Given the limited ability for cell division in the donated endothelial population, stabilization in ECD in at least the grafts for Fuchs' dystrophy may be as a result of the contribution of recipient endothelium over the donor bed.²⁴

Limitations of the previously mentioned analyses on endothelial cell loss are that they do not account for correlated data from repeated measures on the same subject or the selective dropout among subjects with lower ECDs who are more likely to experience graft failure. The latter is particularly important to capture because a model for such cell loss could be biased by the selective loss of eyes with low ECDs.^{12,26,27} In this study, we used models that accounted for the correlated data and the selective dropout. Our log-polynomial model with random patient effects allows the exponential rate of decline to vary continuously within a population (according to a bell-shaped curve) while also incorporating significant nonlinear (quadratic and cubic in time) rates of decline. When we fit the biexponential models for purposes of comparison, the second exponential decay parameter was actually positive in both models (denoting growth rather than decay), calling into question whether the decline in ECD can be characterized with only two distinct mechanisms and showing that our log-polynomial model may be a better description of cell loss after PK.

Another limitation to this study is the approximation of the ECD due to the sample selection. It is possible that nonrandom distribution of cells within a small area of the central graft where the specular microscopic images were taken could inflate the measurement error. To minimize measurement error, two separate graders selected samples from multiple frames and multiple images to determine the final ECD, with a third independent determination of ECD by an adjudicator when ECD between the graders varied by >5.0%. The fact that our measured ECD was highly predictive of subsequent endothelial graft failure²⁸ suggested that we have a reasonably good approximation of the true ECD.

The recipient diagnoses of Fuchs' dystrophy and PACE give similar cell loss models. However, it may be interesting to note that cell loss for those recipients with PACE was much faster in the first 5 to 6 years post-PK than those with Fuchs', but after 6 years the cell loss stabilizes much faster for PACE than for Fuchs, leading to a higher 10-year cell loss for the Fuchs' group (Fig. 3). Previous analyses of the SMAS data had similar results.³ This could be attributable to the substantially lower graft failure rate in the Fuchs' group at 10 years versus the PACE group (15% vs. 28%) which causes the selective loss of grafts with low ECD.

It is somewhat reassuring that the Bayesian MCMC model that accounts for selective dropout from graft failure obtained nearly identical results to the log-polynomial model that ignores this phenomenon. This suggests that the bias from selective dropout may be negligible in this dataset, perhaps due to the fact that the graft failure rate at 10 years was only 19%. In a higher risk population with a higher failure rate, the problem of selective dropout would presumably create a more significant bias. It is important to note the limitations of the Bayesian MCMC model that attempts to account for the selective dropout. This model makes a number of untestable assumptions concerning the dropout mechanism^{29,30} and assumes that other causes of missing ECD data (e.g., missed visits, ungradable images) were missing at random. Another limitation is that the MCMC procedure can give misleading results if it does not converge properly. Even with these few limitations, our analyses provide significant evidence that the log-polynomial model is a good model for cell loss after PK.

It is unknown how well these results from PK might extend to endothelial cell loss following endothelial keratoplasty (EK). Endothelial cell loss in the early postoperative period is higher after Descemet stripping endothelial keratoplasty (DSEK) than after PK, but thereafter, the rate of cell loss through 5 years after DSEK appears to diminish faster than after PK.^{31,32} The Cornea Preservation Time Study (CPTS) is currently following over 1300 subjects who have undergone DSEK and will report graft survival and endothelial cell data at 3 years. Extended follow-up of this cohort, which has similar donor parameters to the CDS cohort, would offer the best direct comparison of outcomes between DSEK and PK to date and may improve our understanding of the role of ECD in graft survival. In addition, because EK techniques and postoperative anatomy differ, it is possible that different mathematical models will predict ECD after Descemet membrane endothelial keratoplasty (DMEK),³³ where just the endothelium with Descemet membrane is transplanted, compared to DSEK.

In summary, we have shown that endothelial cell loss after PK can be modeled via a log-polynomial model which accounts for the correlated data from repeated measures on the same subject. This model is not significantly affected by the selective dropout due to graft failure and is compatible with a limited potential for endothelial cell division that equals the rate of cell loss when the ECD is approximately 500 cells/mm². Our findings warrant further study on how this may extend to endothelial cell loss following EK.

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