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Clinical Trials

Utility of a Model to Predict Endothelial Cell Density of Donor Corneas to Determine Suitability for Transplantation

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Purpose: In Germany, approximately one-third of the harvested donor corneas are not suitable for transplantation, mostly due to insufficient endothelial cell density (ECD). The ECD can only be reliably determined after harvesting and processing of the cornea. Our group has previously developed a predictive model for corneal ECD:

$$Predicted\ ECD = 2919 - 6^{*}\ age\ [years] - 189\ [if\ male] - 7^{*}\ death\text{-}to\text{-}explantation\ interval\ [hours] - 378\ [if\ pseudophakic]\ cells/mm^2$$

Methods: A total of 2.999 consecutive donor corneas harvested between 2017 and 2021 from the Eye Bank of Rhineland-Palatinate in Mainz, Germany, were included. An actual ECD of >2000 cells/mm^2 was defined as the cutoff value. To evaluate the clinical utility of the prognostic model as a screening instrument for transplant eligibility in an independent cohort, we performed a decision curve analysis.

Results: The median predicted ECD was 2061 cells/mm^2 (interquartile range [IQR] = 1834 to 2221), whereas the median actual ECD was 2377 cells/mm^2 (IQR = 1907 to 2624). There was a positive correlation between predicted and actual ECD (correlation coefficient = 0.411; $P < 0.01$). Our predictive model for ECD is a strong predictor for an actual ECD greater than 2000 (odds ratio = 1.374, 95% confidence interval [CI]) per 100 cells; $P < 0.001$, area under the curve [AUC] of 0.73). Decision curve analysis showed that the predictive model yielded a positive net benefit in clinical settings.

Conclusions: Decision curve analysis demonstrated a positive net benefit of the ECD predictive model in clinical settings with limited eye bank resources.

Translational Relevance: In possible scenarios where a choice between corneal grafts is required, or in countries with limited eye bank infrastructure and staff, the initial estimate of ECD from the formula may be beneficial.

Introduction

In many countries, the number of corneal transplantations is increasing every year. In the last decade, the number of keratoplasties in Germany has doubled to approximately 9000 in 2020.[1] The increasing number of keratoplasties is accompanied by a corresponding demand for suitable donor corneas. Despite a leading share of donor corneas of about 90 percent

of all donated tissues and an increasing number of donors each year, the growing demand for corneal tissue cannot be fully met.[2] In Germany, approximately one third of donor corneas harvested are not suitable for transplantation.[3,4] The most common reason for corneal exclusion is a low endothelial cell density (ECD).[5–7] Further reasons for exclusion include lack of valid blood sample, exceeding the maximum death-to-explantation interval (DEI) limited to 72 hours in Germany, inaccessibility of relatives, lack of consent for tissue donation, logistical problems, and others. Previous studies reported on an association between advanced donor age and pseudophakic lens status,[7–13] cause of death, such as cardiovascular disease,[14] cancer,[8] or sepsis,[15] and an increased postmortem interval[16] with endothelial cell loss, but some other studies could not confirm these associations.[5,13,17–22] Usually, the ECD of the cornea is measured between the third and fifth day after explantation of the cornea and organ culture.

Our group previously investigated the influence of certain donor characteristics on corneal suitability for transplantation in a large donor cohort of 2032 consecutive donor corneas harvested at the Eye Bank Rhineland-Palatinate in Mainz, Germany, between 2014 and 2016. Factors of interest were age, sex, lens status, cause of death, cardiopulmonary resuscitation (CPR), DEI, and the influence of these factors on the proportion of discarded donor corneas.[23] DEI was calculated for each corneal donor using the time of death from the donor's death certificate and the eye bank's records of processing dates and times. Lens status (phakic, pseudophakic, or aphakic) was assessed at explantation. They developed a predictive model for the expected ECD from predefined potential predictors:

$$Predicted\ ECD = 2919 - 6^{*}age\ [years] - 189\ [if\ male] - 7^{*}DEI\ [hours] - 378\ [if\ pseudophakic]\ cells/mm^2$$

The aim of this study was to test the hypothesis that the ECD prediction based on our model has a positive net benefit in a clinical setting.

Materials and Methods

The Eye Bank Rhineland-Palatinate, Mainz, Germany, recruits donors within the University Medical Center of the Johannes Gutenberg University Mainz and from eight cooperating hospitals and institutions in Rhineland-Palatinate. The maximum radius of the 8 corresponding hospitals and institutions that cooperate with the Eye Bank Rhineland-Palatinate is approximately 85 km. According to the guidelines for tissue donation in Germany, there is no upper age limit for the donation of ophthalmologic tissue. There is a restriction for donors under 3 years of age.[24] All data in this retrospective study were obtained from deceased donors; therefore, no ethics vote was obtained in this study.

In this study, we validated the previously developed linear mixed model for predicted ECD in an independent cohort consisting of consecutive donor corneas collected at the Rhineland-Palatinate Eye Bank in Mainz, Germany, as with the model. The main difference lies in the time span, as the corneas to test the model's utility were harvested between 2017 and 2021. We compared the predicted ECD with the actual measured ECD of the harvested corneal grafts using correlation coefficients. Using logistic regression, we determined the ability of the prognostic model to predict an ECD of more than 2000 cells/mm^2 as a clinically relevant cutoff value for transplantability. To evaluate the clinical utility of the expected ECD, we performed a decision curve analysis. For this, we estimated the corresponding net benefit of the expected ECD model in predicting if a harvested cornea is transplantable at a wide range of threshold probabilities. The threshold probability is defined as the minimum probability of an event at which a decision-maker would choose to harvest the cornea. Selection of a higher threshold probability would, for instance, imply limited eye bank resources, whereas selection of a lower threshold probability implies, for instance, a higher demand on transplantable corneas than available organ donors. The net benefit is defined as the proportion of correct positive classifications subtracted from the proportion of false negatives, weighted by the risk threshold. The decision curve analysis is presented as a graphical plot of net benefit against threshold probability.

Results

Data of 2999 consecutive donor corneas were analyzed. The median ECD predicted with the formula was 2061 cells/mm^2 (interquartile range [IQR] = 1834 to 2221) compared to the median measured ECD of 2377 cells/mm^2 (IQR = 1907 to 2624). The predicted ECD underestimates the actual measured ECD translating to a significant difference in the paired *t*-test. There was a positive correlation between the predicted and actual ECD (correlation coefficient = 0.411; $P < 0.01$; Fig. 1).

Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of the prognostic model for an

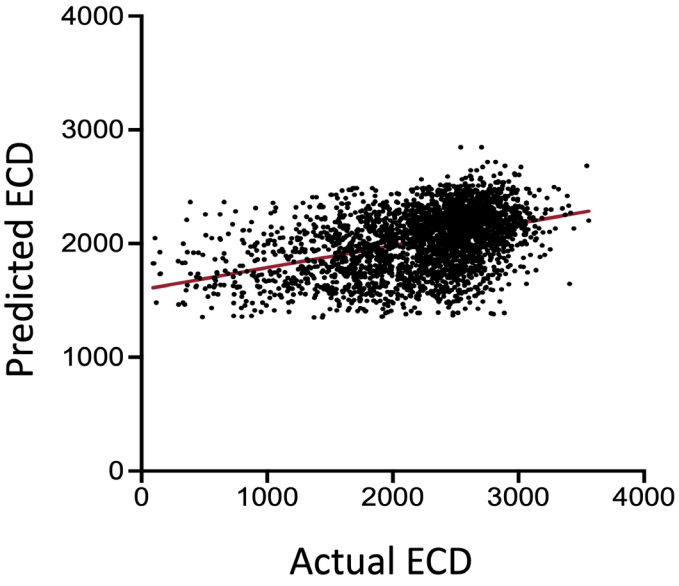

Figure 1. Scatterplot. The X-axis shows the actual ECD and the Y-axis the predicted ECD.

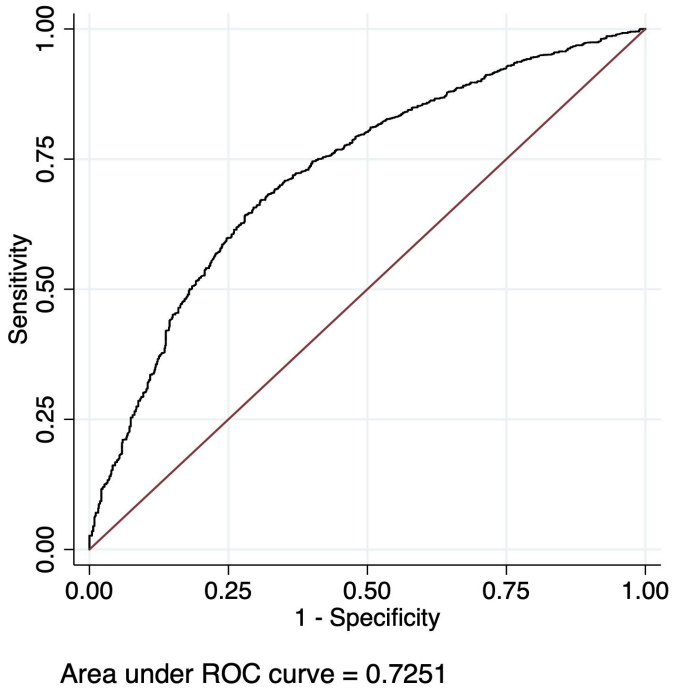

Figure 2. Receiver operating characteristic (ROC) curve analysis. The X-axis shows 1-specificity and the Y-axis the sensitivity with an AUC of 0.73.

ECD > 2000 cells/mm^2. The prognostic model significantly predicts probability of ECD > 2000 cells/mm^2 with an area under the curve (AUC) of 0.73 (odds ratio = 1.374, 95% confidence interval [CI] per 100 cells; $P < 0.001$; Fig. 2).

Furthermore, we performed a decision curve analysis. The results of our decision curve analysis are shown in Figure 3. The X-axis shows the predicted probability of a transplantable cornea, at which point the decision for harvesting and processing of the donor cornea would be made. The Y-axis represents the corresponding net benefit, which is the proportion of correct

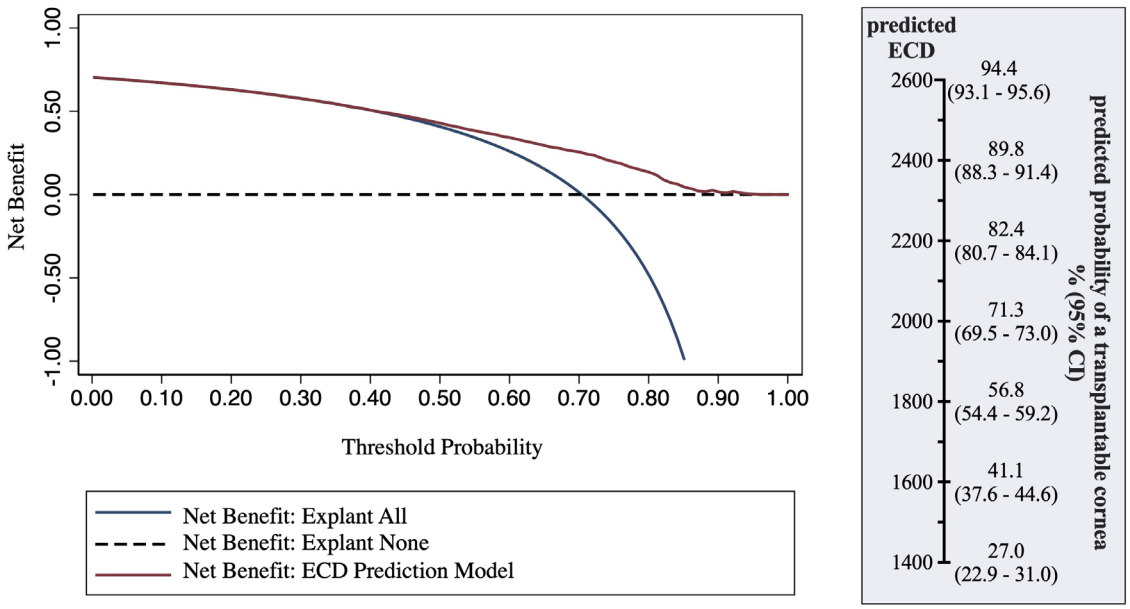

Figure 3. Decision curve analysis. X-axis = threshold probability of a transplantable cornea, at which point the decision for explantation and preparation of the donor cornea would be made. Y-axis = net benefit; proportion of correct positive classifications subtracted from the proportion of false negative results weighted for the risk threshold.

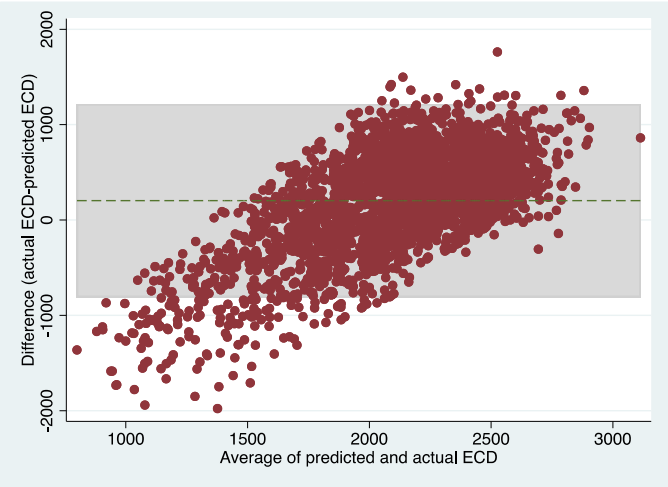

Figure 4. Bland Altman analysis. We performed a Bland Altman analysis. The results of the correlation between the predicted and observed ECD are shown in this figure.

positive classifications subtracted from the proportion of false negatives weighted for the risk threshold. The ECD predictive model yielded a positive net benefit compared to an explant all or explant none approach at a range of threshold probabilities between approximately 0.45 and 0.93 (Fig. 3). Additionally, we performed a Bland Altman analysis to show the results of the correlation between the predicted and observed ECD (Fig. 4).

Discussion

In the present study, we validated our previously developed prognostic model for ECD on an independent cohort of 2999 donor corneas explanted at our institution between 2017 and 2021. Despite the increasing number of tissue donors, the growing demand for corneal grafts not only in Germany, but worldwide still cannot be fully met. Due to the strict guidelines for cornea donation, not every potential donor is eligible.[25] Many potential donors are already out of question before tissue collection because of their medical history, infectious pre-existing conditions, cause of death, DEI, or lack of consent. Furthermore, about one third of the harvested corneas are not suitable for transplantation. The most common reason for the discard is a low ECD.[5–7,26]

The work of an eye bank presents numerous challenges on a daily basis. One important unpredictable factor that can strongly influence the work of eye banks is the daily fluctuating number of suitable tissue donors. This challenge can push eye banks, even those with a well-developed infrastructure, such as the Eye Bank Rhineland-Palatinate, to their limits. Although the aspiration is to harvest every cornea donated so as not to waste valuable tissue, there are unfavorable scenarios where the number of donations exceeds the staff capacity, area to be covered, and time resources of our eye bank. In order not to exceed the DEI, sometimes multiple simultaneous corneal explantations are necessary at widely separated sites with limited staff. In addition, work on weekends and holidays is limited because the number of staff in the eye bank is kept to a minimum. This leads to critical situations where the staff must make a considered decision like, for example, which corneal explantation is "more promising" and worth a trip at the expense of maybe one or two other potential tissue donors. In these tricky situations, a decision must be made between possible donors even before tissue explantation. The fact that the most common reason for corneal exclusion is a low ECD,[5–7] the donor with the highest possible ECD should be chosen to obtain the best condition for its upcoming use.

To facilitate and resolve such dilemmas, as described above, we developed a predictive model for the expected ECD from easily accessible predefined potential predictors, such as age, gender, DEI, and lens status.[22] To the best of our knowledge, this is the first investigation to validate such a predictive model on a large cohort of almost 3000 harvested corneas. The results show that the predicted ECD by the model correlates with the actual ECD (correlation coefficient = 0.411; $P < 0.01$). We have shown that this model, in fact, is an acceptable predictor of an actual ECD of over 2000 cells/mm^2 as this number is considered to be the threshold for cornea transplantation involving the endothelium. The median ECD predicted with the formula was 2061 cells/mm^2 (IQR = 1834 to 2221) compared to the median measured ECD of 2377 cells/mm^2 (IQR = 1907 to 2624). A tendency to underestimate the ECD compared to the actual measured ECD is more likely to be beneficial for the upcoming corneal transplant and overall graft survival than the other way around. The results of our ROC curve suggest that our predictive model predicts an ECD > 2000 cells/mm^2 with an AUC of 73% (see Fig. 2). To evaluate the clinical utility of the expected ECD as a screening tool to best manage reprocessing capacities of cornea donor banks when it is limited, we performed a decision curve analysis (see Fig. 3). In clinical settings with a high reprocessing capacity, a low-risk threshold of, for example, 30% for an ECD > 2000 may be sufficient for tissue explantation. In this case, the approach to examine all corneas (see the blue line in the decision curve analysis) has an identical net benefit compared to the ECD predic-

tive model. But in a clinical setting that requires a higher risk threshold for a transplantable donor cornea of, for example, 45% for an ECD > 2000 and above, for example, due to limited processing capacity, our prognostic model shows a higher net benefit (see the red line in the decision curve analysis; see Fig. 3).

The potential implementation of this new prediction model into a cornea bank's donor selection routine brings forth a crucial consideration regarding the rate of false positive results and false negative results depending on the chosen threshold probability. False positive results, where the model incorrectly identifies a cornea as transplantable when it is not, could lead to unnecessary allocation of resources. On the other hand, false negative results, where the model incorrectly identifies a transplantable cornea as non-transplantable, may lead to underutilization of donor tissue. The optimal balance between false positive results and false negative results must be chosen based on the available resources of the eye bank, the availability of donors, and the demand for donor tissue. The rate of false positive results and false negative results for different threshold probabilities in our cohort is shown in Supplementary Table S1. Continued validation of the model through rigorous testing and application in practice are essential steps to balance the occurrence of false positive results and false negative results in different clinical settings and ultimately maximize the utility of the prediction model.

The prognostic model is only used at our Eye Bank Rhineland-Palatinate if circumstances force us to do so. The ultimate goal remains to explant every donated cornea and to examine it for further use. This means that this model does not replace our daily decision making in our eye bank, but is a useful tool in critical situations. It can be used not only for our approach, but also as a preselective decision tool in other countries, for example, emerging or developing countries with a different infrastructure, a larger area to be covered, and a different eye bank organization with its own limited human and logistical resources. In order to apply the model prior to explantation, we would suggest an estimated DEI using the time of death, the distance to the hospital, and the expected time for the recovery. From the economic point of view, preselection may also be more cost-effective by filtering out corneas with an ECD < 2000. A limitation of our study at the time of explantation is the lens status (phakic, aphakic, or pseudophakic) for the application of the model, as it is not always known by each eye bank, but is mandatory for the utility of our predictive model. Therefore, we recommend adding a question about lens status to the standard questionnaire used for donor qualification. Another limitation of our study is that the influence of death to cooling time and its impact on DEI limited to 72 hours in Germany was not assessed in either our model or our study. To meet the growing demand for corneal tissue, it is not sufficient to focus only on an increasing number of tissue donors. Depending on the country, there are differences in the country-specific guidelines for tissue donation. For example, there are no standardized upper or lower age limits worldwide. This might be because unlike other organ transplants, corneal transplants with its immune privilege appear to be less influenced by the age of the donor, as most studies have found no effect of donor age in the first years after transplantation (group 2008). Therefore, different age limits in different countries can affect the model. In scenarios with multiple potential tissue explantations at different locations over a wide area that cannot be covered with limited staff, our preselection model for an ECD > 2000 with easily accessible potential predictors can be a suitable solution to make a solid decision for this challenge. This model has shown to have predictive power for an ECD > 2000 after validation of nearly 3000 donor corneas.

Limitations to the study include that the predictive model was developed based on data from the Eye Bank Rhineland-Palatinate and compared with a new data set from the same Eye Bank. Due to different guidelines for corneal donations worldwide, for example, with regard to DEI or age limit, influences, and restrictions on the utility of our predictive model are possible. To further strengthen the predictive power of the prognostic ECD model and to adapt our model to a wider range, the application of data from other eye banks might be meaningful.

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Data Availability Statements: The data used to support the findings of this study are available from the corresponding author upon request.

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