The Suitability of Corneas Stored by Organ Culture for Penetrating Keratoplasty and Influence of Donor and Recipient Factors on 5-Year Graft Survival

W. John Armitage,[1,2] Mark N. A. Jones,[3] Isaac Zambrano,[4,5] Fiona Carley,[4,5] and Derek M. Tole,[1,6] for the NHSBT Ocular Tissue Advisory Group and Contributing Ophthalmologists (OTAG Audit Study 12)

[1]Corneal Transplant Service Bristol Eye Bank, Bristol, United Kingdom
[2]School of Clinical Sciences, University of Bristol, Bristol, United Kingdom
[3]National Health Service Blood and Transplant, Bristol, United Kingdom
[4]Corneal Transplant Service Manchester Eye Bank, Manchester, United Kingdom
[5]Manchester Royal Eye Hospital, Manchester, United Kingdom
[6]Bristol Eye Hospital, Bristol, United Kingdom

Correspondence: W. John Armitage, CTS Bristol Eye Bank, University of Bristol, Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX, UK; w.j.armitage@bristol.ac.uk.

Submitted: October 4, 2013
Accepted: November 22, 2013

Citation: Armitage WJ, Jones MNA, Zambrano I, Carley F, Tole DM; for the NHSBT Ocular Tissue Advisory Group and Contributing Ophthalmologists. The suitability of corneas stored by organ culture for penetrating keratoplasty and influence of donor and recipient factors on 5-year graft survival. *Invest Ophthalmol Vis Sci*. 2014;55:784–791. DOI:10.1167/iovs.13-13386

Purpose. To determine the impact of donor factors on the suitability of corneas stored by organ culture for penetrating keratoplasty (PK) and the influence of donor and recipient factors on 5-year survival of first PK.

Methods. Logistic regression analyses were carried out to determine the influence of donor factors on, respectively, the risk of microbial contamination during organ culture, the suitability of corneas for PK (endothelial cell density $\geq$ 2200 cells/mm^2), and the quality of corneas (endothelial cell density $\geq$ 2500 cells/mm^2). Only one cornea, randomly selected, from each donor was included in these analyses. A Cox regression analysis was used to determine the influence of donor and recipient factors on 5-year PK survival.

Results. Risk of contamination (n = 8317): Causes of donor death including infection, respiratory disease, and cancer all increased the risk of contamination during organ culture ($P < 0.0001$). Suitability for PK and endothelial quality (n = 7107): Donor age ($P < 0.0001$) and storage time in organ culture ($P < 0.0001$) were the principal factors affecting suitability and quality. Death to enucleation and enucleation to processing times had little influence. Corneas from organ donors were more likely to be suitable for PK ($P = 0.0003$). Five-year graft survival (n = 3014): Graft survival was dominated by the indication for PK ($P < 0.0001$). Allograft rejection was also a major risk factor for failure ($P < 0.0001$). The only donor factor affecting survival was sex ($P = 0.008$).

Conclusions. Donor age and storage time but not postmortem times influenced the suitability of corneas for PK. The indication for PK and other recipient factors were the main predictors of graft failure.

Keywords: corneal organ culture, corneal storage, corneal transplantation

Transplant outcome is ultimately the most relevant measure for assessing criteria for the procurement, processing, storage, and quality assessment of corneas; however, the various recipient factors that are known to influence graft survival and outcome must also be taken into account. Although short-term (1 year) survival for penetrating keratoplasty (PK) across all indications is approximately 90%,[1,2] there follows a steady decline in survival with time that is highly dependent on the original indication for the transplant. By 5 years, data from the Australian Corneal Graft Registry show that while survival for low-risk indications such as keratoconus and Fuchs' endothelial dystrophy remains high, graft survival for other indications such as ulcers and infection (nonherpetic) can be 60% or lower.[2] Data from the Swedish Corneal Transplant Register show that not just survival but visual outcome is similarly influenced by the indication for transplantation.[3] It is important, therefore, to understand the factors influencing the clinical outcome in order to ascertain whether interventions, such as changing donor and cornea selection criteria, could improve graft survival.

In the United Kingdom, during the period of this study there was no upper age limit for eye donors; postmortem retrieval times for eyes up to 24 hours were accepted; the great majority of corneas were stored by organ culture for up to 4 weeks; and the minimum endothelial cell density (ECD) for PK was 2200 cells/mm^2. In order to establish the acceptability of these criteria, it is important to determine how these donor factors influence the suitability of corneas for PK and how donor and recipient factors affect corneal transplant survival. The purpose of this study, therefore, was to evaluate the impact of donor and eye bank variables on the suitability of corneas for PK and, taking into account recipient factors, their influence on 5-year survival of first grafts. It is acknowledged that endothelial keratoplasty (EK) and deep anterior lamellar keratoplasty

(DALK) are increasingly preferred alternatives to PK. These and the introduction of techniques for splitting corneas for use in two patients[4,5] may require eye banks to reassess their selection criteria, but it is important for any changes to be evidence based. Survival analyses of PK not only remain relevant where PK is the procedure of choice but also form a useful baseline for comparison with EK and DALK.

The two principal methods for storing corneas are hypothermia and organ culture; the latter predominates in the United Kingdom.[6,7] We have previously analyzed the influence of donor and storage factors on whether organ-cultured corneas are suitable for PK.[7] Herein, we present an updated analysis of the influence of donor factors on the suitability and quality of corneas for PK and then determine whether any of these donor factors affect first PK survival at 5 years, taking into account recipient factors. Regrafts were excluded from this analysis owing to the heterogeneity of this group, which contains a range of original indications and a variety of causes of failure of the original graft.

Methods

This investigation complied with the tenets of the Declaration of Helsinki.

Eye Donors

Eyes from donors in hospitals throughout the United Kingdom were sent to the Corneal Transplant Service (CTS) Eye Banks in Bristol and Manchester for processing and storage of the corneas by organ culture. While the great majority of eyes were transported to the eye banks in moist chambers, corneoscleral discs in hypothermic storage medium were also received by the eye banks, and these were transferred to organ culture. Consent or, in Scotland, authorization for eye donation was obtained in accordance with, respectively, the Human Tissue Act 2004 and the Human Tissue (Scotland) Act 2006. A detailed medical history and behavioral background of every donor was obtained through interview with the family, a questionnaire sent to the donor's family doctor, and review, where available, of any hospital medical records and autopsy reports. The consent and medical history were recorded on standard National Health Service Blood and Transplant (NHSBT) forms used throughout the United Kingdom for organ and eye donation. Donor blood samples were subjected to mandatory tests for markers of transmissible disease, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-lymphotrophic virus (HTLV), and syphilis.

Storage of Corneas

All corneas in this analysis were stored by organ culture. The organ culture method has been described previously.[7,8] Briefly, sterile, single-use instruments were used throughout the whole process including enucleation, corneoscleral disc excision, and endothelial examination. Before corneoscleral disc excision, eyes were cleaned with sterile 0.9% NaCl and 3% povidone-iodine to reduce the ocular surface microbial load. Corneoscleral discs were suspended in 80 mL medium in sterile glass DIN infusion bottles (Neville and Moore, Southwater, United Kingdom), closed with a silicone rubber stopper. The organ culture medium was Eagle's Minimum Essential Medium (MEM) containing 26 mM sodium bicarbonate, 20 mM HEPES buffer, 2% fetal bovine serum (Australian origin), 2 mM L-glutamine, penicillin (100 U/mL), streptomycin (0.1 mg/mL), and amphotericin B (0.25 μg/mL). (Apart from the fetal bovine serum [Life Technologies Ltd., Paisley, United Kingdom] all chemicals and media used for processing, storage, and assessment of corneas were obtained from Sigma-Aldrich Company Ltd. [Dorset, United Kingdom].) The corneas were stored at 34°C, and after 7 days, a sample of the medium was taken to test for bacteria and fungi using blood agar plates and liquid microbiological media. Microbial contamination was also monitored by daily inspection of the organ culture medium for turbidity. If these tests failed to detect the growth of any microorganisms and there were no known medical contraindications to transplantation, the corneas were released from quarantine.

Before a cornea was issued for PK, the corneal endothelium was examined by transmitted light microscopy after staining with trypan blue to show damaged, dead, or missing cells, and hypoosmotic sucrose to reveal cell borders. If a cornea was considered suitable for PK on the basis of this endothelial assessment (see following section), it was placed in organ culture medium containing 5% Dextran T500 to reverse the stromal edema that occurred during organ culture storage. The cornea was returned to 34°C, and a sample of medium was taken the next day for further microbiological testing. The cornea was dispatched to the recipient's hospital the following day and transplanted on the third day after endothelial examination. For clinically urgent transplants, these timings were typically shorter. During the period of this study, the allocation of corneas took into account donor–recipient age matching to the extent that donors were not more than 30 years older than recipients.

Endothelial Cell Density and Quality Assessment Grade

The minimum ECD for PK was 2200 cells/mm^2. An overall quality assessment was made based principally on ECD but also taking into account qualitative features such as presence and location of dead and/or missing endothelial cells, extent of folds in Descemet's membrane, and stromal opacities (e.g., arcus senilis was acceptable provided there was at least a 9-mm-diameter clear central corneal region). Corneas judged suitable for PK were graded as "Good" (2200–2500 cells/mm^2), "Very good" (2500–3000 cells/mm^2), or "Excellent" ($\geq$3000 cells/mm^2).

Data Analysis

Contamination, Suitability for PK, and Corneal Quality

Three separate analyses were carried out to determine the influence of donor factors on, respectively, the risk of microbial contamination during organ culture, the suitability of corneas for PK (i.e., whether corneas met the minimum endothelial criterion of 2200 cells/mm^2), and the quality of corneas (i.e., whether ECD was $\geq$2500 cells/mm^2). These observational, retrospective, cross-sectional studies included corneas stored by organ culture in the CTS Eye Banks between April 1, 1999 and March 31, 2005. During this period, 20,950 corneas were processed. For the analysis of contamination risk, 2179 corneas withdrawn prematurely from organ culture storage because of medical contraindications were excluded. A further 2337 corneas were excluded owing to missing/incorrect data, resulting in a cohort of 16,434 corneas. To avoid the potential lack of independence between corneas from the same donor, one cornea from each donor was selected at random. With the inclusion of those corneas from donors from whom only one eye was received for processing, an analysis data set of 8317 corneas was compiled. For the analyses of PK

suitability and corneal quality, 5907 corneas discarded because of medical contraindications, contamination, or other non-endothelial reasons were excluded. A further 1601 corneas with missing/incorrect data were excluded to give a cohort of 13,442 corneas. As with the contamination analysis, the analysis data set of 7107 corneas was derived from this cohort by randomly selecting only one cornea from each donor plus the corneas from donors from whom only one eye had been received for processing.

Five-Year Graft Survival

For consistency with the other analyses, the survival analysis data set comprised those corneas from the 7107 corneas in the PK suitability data set that were transplanted. Corneas were excluded for the following reasons: unsuitable for PK, 1147; suitable for PK but not transplanted, 422; corneas transplanted but not PK, 672; corneas used for PK but not first grafts (i.e., regrafts), 740; and first PKs with missing/incorrect data, 1112. The survival analysis data set thus comprised 3014 first PKs. Data were collected through the NHSBT Ocular Tissue Transplant Audit, which routinely captures 5-year follow-up data on corneal transplants in the United Kingdom. Surgeons submitted a transplant record form at the time of the surgery to confirm the indication for PK and to record any preoperative risk factors and perioperative complications. Follow-up forms were then completed at 1, 2, and 5 years postoperatively. These forms reported whether the graft was still functioning, relevant postoperative events and complications, medication (use of topical steroid, antiviral therapy, and systemic immunosuppression), visual acuity, and keratometry. All donor and recipient data were stored in the UK Transplant Registry maintained by NHSBT.

Statistical Methods

Univariate analyses, Kaplan-Meier survival, multiple logistic regression, and Cox proportional hazards regression were used as appropriate. All statistical analyses were performed with SAS version 9.1 software (SAS Institute, Inc., Cary, NC). Means are quoted with standard deviations (SD). Odds ratios (OR) and hazard ratios (HR) are quoted with 95% confidence intervals (95% CI). The level of significance was set at 5%.

Results

Baseline Eye Bank Data

Outcomes. Of the 20,950 corneas processed during the study period, 68% were assessed as suitable for PK, 2% were issued for other types of transplant, 10% were excluded because of medical contraindications, 6% were excluded because of microbial contamination during organ culture, 13% were excluded for not meeting the minimum endothelial criteria for PK, and <1% were discarded for other reasons.

Postmortem Times and Storage Time. Death to enucleation time was 14.9 hours (SD 7.2). Enucleation to corneoscleral disc excision time, which included transport time to the eye banks from hospitals throughout the United Kingdom, was 16.5 hours (SD 7.5). The mean time from death to corneoscleral disc excision was 31.4 hours (SD 9.6). Corneas were stored on average for 18.3 days (SD 5.2). Twenty-six percent of corneas were stored for 2 weeks or less; 46% were stored for up to 3 weeks, 26% for up to 4 weeks, and 2% for more than 4 weeks.

ECD and Donor Age. The mean donor age and ECD for those corneas assessed as suitable for PK were, respectively, 61 years (SD 18) and 2636 cells/mm^2 (SD 232) (Table 1). Donors of both eyes and organs had a mean age of 48 years (SD 15) compared with 64 years (SD 17) for all other eye donors. The mean ECD and donor age by quality assessment grade and the mean ECD by donor age are shown in Tables 1 and 2.

Table 1. Mean (SD) Endothelial Cell Densities and Mean (SD) Donor Ages for the Endothelial Quality Assessment Categories

Endothelial Assessment	*n*	ECD, cells/mm^2	Donor Age, y
Excellent	789	3044 (177)	41 (21)
Very good	3099	2678 (121)	61 (16)
Good	2169	2426 (107)	68 (14)
Total	6057	2636 (232)	61 (18)

Only corneas that met or exceeded the minimum ECD for PK of 2200 cells/mm^2 are included.

Risk of Contamination

After excluding corneas from donors with medical contraindications and then randomly selecting one cornea from each of the remaining donors, the data set included 8317 corneas. The overall contamination rate was 5.7%. The logistic regression model (Table 3) showed a strong influence of cause of donor death on the risk of contamination, which ranged from less than 4% to more than 17%. A cause of death recorded as infection (i.e., suspected or confirmed bacterial septicemia or other systemic infection not considered to be a contraindication to transplantation) increased the risk of contamination of corneas by more than 4-fold compared with intracranial causes ($P < 0.0001$). Respiratory disease ($P = 0.0007$), cancer ($P = 0.02$), and "other" causes of death ($P < 0.0001$) also increased the risk of contamination. The only other significant factor was transport of corneas to the eye banks in hypothermic storage medium (i.e., corneoscleral disc excision carried out locally in order to retain ocular tissue for research), which reduced the risk of contamination in organ culture ($P = 0.05$). No other donor factors affected the risk of contamination (Table 3).

Suitability for PK

After excluding corneas discarded because of medical contraindications and contamination and then randomly selecting one cornea from each donor, the data set included 7107 corneas. Table 4 shows the influence of donor factors on whether the corneal endothelium failed to meet the minimum suitability criterion for PK (i.e., risk of ECD < 2200 cells/mm^2). Donor age ($P < 0.0001$) and storage time in organ culture ($P < 0.0001$) had the largest influence. Corneas from organ donors were more likely to be suitable for PK ($P = 0.0003$). There was also an unexplained difference between the two eye banks. Times from death to enucleation ($P = 0.9$) and from enucleation to corneoscleral disc excision (i.e., processing) ($P = 0.3$), overall time from death to processing ($P = 0.2$), and

Table 2. Mean (SD) Endothelial Cell Density by Donor Age

Donor Age, y	*n*	ECD, cells/mm^2
0–39	766	2900 (270)
40–59	1795	2666 (201)
60–79	2738	2576 (190)
≥80	758	2511 (174)

Only corneas that met or exceeded the minimum ECD for PK of 2200 cells/mm^2 are included.

Table 3. Logistic Regression Model Showing Factors Influencing the Risk of Contamination of Corneas Stored in Organ Culture

Factor*	*n*	Contaminated, %	OR	95% CI	*P*
Cause of death, $P < 0.0001$					
Intracranial	1732	4.5	1.0	–	–
Trauma	509	5.7	1.3	0.9, 2.0	0.3
Cardiovascular	2309	3.7	0.8	0.6, 1.1	0.3
Respiratory	700	7.9	1.9	1.3, 2.7	0.0007
Cancer	1729	6.3	1.4	1.1, 1.9	0.02
Infection	173	17.3	4.5	2.8, 7.1	<0.0001
Other	418	11.7	2.8	1.9, 4.1	<0.0001
Missing†	747	5.5	1.2	0.8, 1.8	0.3
Cornea transported to eye bank in hypothermic storage medium, $P = 0.05$‡					
No	7355	5.9	1.0	–	–
Yes	962	4.4	0.7	0.5, 1.0	0.05

The model accounts for 55% of variability of the data.

* Other factors that did not reach the 5% level of significance: time from death to enucleation ($P = 0.6$), time from enucleation to corneoscleral disc excision ($P = 0.5$), interaction between death to enucleation and enucleation to corneoscleral disc excision ($P = 0.5$), overall time from death to corneoscleral disc excision ($P = 0.3$), donor age ($P = 0.14$), organ donor ($P = 0.2$), and eye bank ($P = 0.3$).

† Missing cause of death is a contraindication to transplantation, but these corneas were retrieved and placed into organ culture on the understanding that a cause of death would be forthcoming.

‡ Corneoscleral disc excised before transport to eye bank in hypothermic storage medium.

the interaction between death to enucleation time and enucleation to processing time ($P = 0.8$) had no influence on the suitability of corneas for PK.

Endothelial Quality

The likelihood of ECD < 2500 cells/mm^2 was increased with increasing donor age and longer storage time. This was confirmed by logistic regression (Table 5). Increasing time from enucleation to corneoscleral disc excision also increased the risk of ECD < 2500 cells/mm^2, but the overall effect was small and significant only for times >18 hours (Table 5). None of the other postmortem times reached the 5% level of significance.

Graft Survival at 5 Years

The Kaplan-Meier survival estimate for first PK at 5 years was 73% (95% CI 72%–75%) across all indications (Fig. 1). The Cox model of factors influencing 5-year survival is shown in Table 6. The only donor factor to have an effect on graft survival was donor sex: Transplantation of corneas from male donors increased the risk of failure compared with female donors ($P = 0.008$). None of the other donor factors (see note, Table 6) influenced graft survival. Indication had a major influence on graft survival (Fig. 2). Five-year survival for transplants for bullous keratopathy was only 59% (95% CI 54%–64%) compared with 93% (95% CI 90%–96%) for keratoconus. After taking other factors into account in the Cox regression model (Table 6), this difference represented an almost 4-fold greater risk of failure at 5 years for bullous keratopathy (HR 3.7, 95% CI 2.7–5.1, $P < 0.0001$). Recipient trephine diameter >8 mm ($P < 0.0001$), a difference of >0.25 mm between the donor and recipient trephine sizes ($P < 0.0001$), and several preoperative and postoperative risk factors also increased the risk of failure (Table 6). A major influence on survival was allograft rejection,

Table 4. Logistic Regression Model Showing Factors Influencing the Risk of Corneas Stored in Organ Culture Being Unsuitable for PK (i.e., ECD < 2200 cells/mm^2)

Factor*	*n*	Corneas,† %	OR	95% CI	*P*
Donor age, y, $P < 0.0001$					
0–39	816	9.3	1.0	–	–
40–59	1954	9.9	0.99	0.7, 1.3	0.95
60–79	3256	18.1	1.9	1.5, 2.5	<0.0001
80+	1081	31.3	3.7	2.8, 4.9	<0.0001
Storage time, d, $P < 0.0001$					
7–14	1822	11.9	1.0	–	–
15–21	3248	16.0	1.5	1.3, 1.8	<0.0001
22–28	1874	22.2	2.4	2.0, 2.8	<0.0001
29–35	163	29.5	4.0	2.7, 5.9	<0.0001
Organ donor, $P = 0.0003$					
Yes	995	8.5	1.0	–	–
No	6112	18.2	1.6	1.2, 2.0	0.0003
Eye bank, $P < 0.0001$					
Manchester	2519	12.4	1.0	–	–
Bristol	4588	19.3	1.9	1.6, 2.1	<0.0001

The model accounts for 65% of variability of the data.

* Other factors found not to reach the 5% level of significance: time from death to enucleation ($P = 0.9$), time from enucleation to corneoscleral disc excision ($P = 0.3$), interaction between time from death to enucleation and time from enucleation to corneoscleral disc excision ($P = 0.8$), and overall time from death to corneoscleral disc excision ($P = 0.2$). Cause of death, donor sex, and corneas transported to eye bank in 4°C storage medium were all not significant at the 5% level.

† Percentage of corneas unsuitable for PK (i.e., ECD < 2200 cells/mm^2).

Table 5. Logistic Regression Model Showing Factors Influencing the Likelihood of Corneas Stored in Organ Culture Having an ECD <2500 cells/mm^2

Factor*	*n*	Corneas,† %	OR	95% CI	*P*
Donor age, y, $P < 0.0001$					
0–39	816	17.0	1.0	–	–
40–59	1954	34.8	2.5	2.0, 3.1	<0.0001
60–79	3256	52.8	5.7	4.7, 7.0	<0.0001
80+	1081	69.4	11.4	9.0, 14.3	<0.0001
Storage time, d, $P < 0.0001$					
7–14	1822	35.2	1.0	–	–
15–21	3248	44.0	1.4	1.2, 1.6	<0.0001
22–28	1874	58.5	2.7	2.3, 3.1	<0.0001
29–35	163	76.1	6.3	4.3, 9.3	<0.0001
Time from enucleation to corneoscleral disc excision, h, $P = 0.002$					
≤12	1508	44.4	1.0	–	–
13–18	2111	44.7	1.0	0.9, 1.2	0.8
19–24	3065	47.6	1.2	1.0, 1.4	0.01
25–30	354	50.9	1.4	1.1, 1.8	0.006

The model accounts for 69% of variability of the data.

* Other factors found not to reach the 5% level of significance: time from death to enucleation ($P = 0.2$), interaction between time from death to enucleation and time from enucleation to corneoscleral disc excision ($P = 0.3$), and overall time from death to corneoscleral disc excision ($P = 0.13$). Cause of death, donor sex, corneas transported to eye bank in 4°C storage medium, eye bank, and organ donor were all not significant at the 5% level.

† Percentage of corneas with ECD < 2500 cells/mm^2.

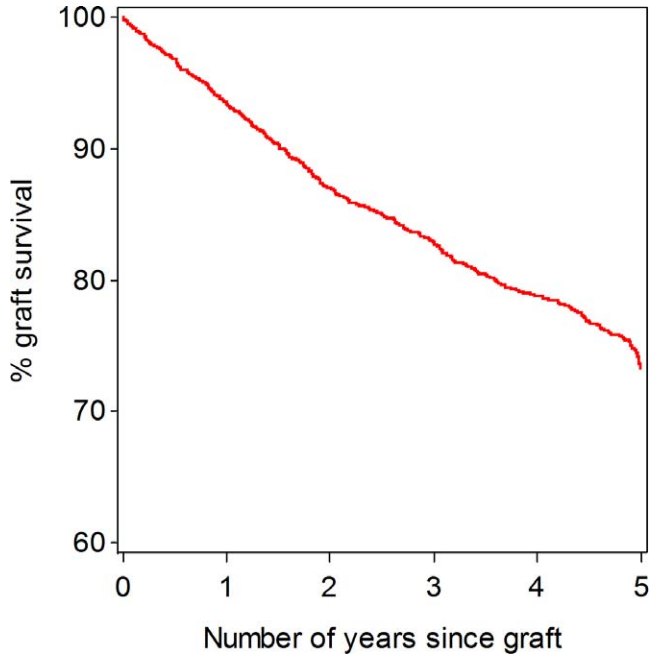


Figure 1. Kaplan-Meier survival plot across all indications. Survival estimate at 5 years: 73% (95% CI 72%–75%). Numbers of grafts at risk: initially, 3014; 1 year, 2601; 2 years, 1864; 5 years, 424.

which increased the risk of failure almost 3-fold (HR 2.6, 95% CI 2.1–3.3, $P < 0.0001$).

Discussion

The introduction of organ culture as a technique for storing corneas offered at the time the prospect of storage for weeks rather than days[8–11]: Even with improvements in hypothermic storage solutions,[12] organ culture still permits longer-term storage. While organ culture preserves the structural integrity of the corneal endothelium,[13] there is a decline in ECD with increasing storage time.[11] Regardless of the mechanism of this cell loss,[13–15] an understanding of the influence of donor factors such as age, postmortem retrieval and processing times, storage time, and ECD both on the suitability of corneas for PK and on graft outcome helps to provide a rational basis for the setting of eye banking standards.[7,16–25]

A perceived advantage of corneal organ culture is the opportunity to detect microbial contamination of the tissue before transplantation. We found cause of death to be the dominating factor influencing risk of contamination, with septicemia posing the greatest risk. Among other causes of death, cancer was associated with increased risk of contamination (Table 3), which may help explain the increased risk of postoperative endophthalmitis associated with cold-stored corneas obtained from donors with malignancies reported by Hassan et al.[26] Donor age and storage time in organ culture, especially the former, were the principal factors affecting endothelial suitability for PK and endothelial quality. We previously found that death to enucleation time and time from enucleation to processing had a slight influence on contamination risk and endothelial suitability when they were treated as continuous variables. Increasing postmortem times have been reported to reduce ECD in donor corneas[18]; however, we found little influence of these factors when they were modeled as categorical variables apart from a slight increase (3%) in the percentage of corneas with ECD < 2500 cells/mm^2 with enucleation to processing times of 19 to 24 hours (Table 5).

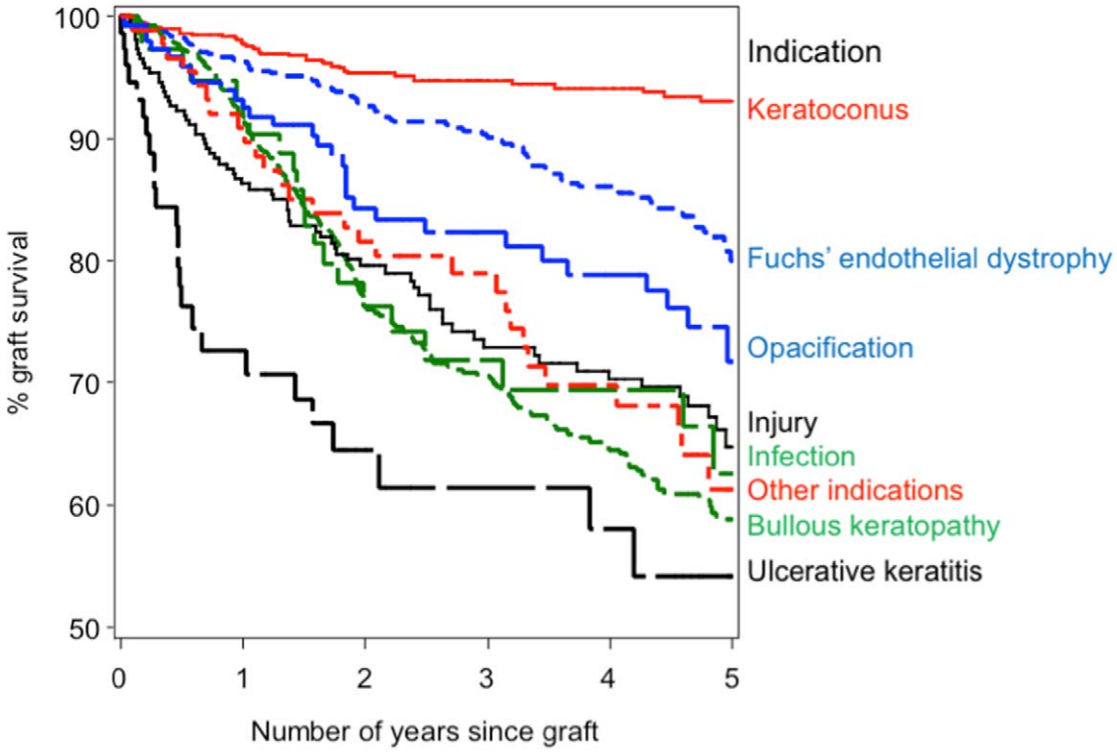
Table 6. Cox Regression Model of Factors Influencing the Risk of Graft Failure at 5 Years, $n = 3014$

Factor*	*n*	HR	95% CI	*P*
Donor sex, $P = 0.008$				
Female	1212	1.0	–	–
Male	1802	1.3	1.1, 1.5	0.008
Diagnosis, $P < 0.0001$				
Ectasias	784	1.0	–	–
Dystrophies	757	1.5	1.1, 2.1	<0.0001
Previous ocular surgery	754	3.7	2.7, 5.1	<0.0001
Infection	307	2.8	1.9, 4.0	<0.0001
Injury	94	4.3	2.7, 6.7	<0.0001
Ulcerative keratitis	89	4.2	2.6, 6.8	<0.0001
Opacification	138	2.3	1.4, 3.7	<0.0001
Other	91	3.4	2.1, 5.4	<0.0001
Infection/inflammation, $P = 0.008$				
No	2580	1.0	–	–
Yes	434	1.4	1.1, 1.7	0.008
Glaucoma, $P = 0.0003$				
No	2725	1.0	–	–
Yes	289	1.5	1.2, 1.9	0.0003
Ocular surface disease, $P < 0.0001$				
No	2704	1.0	–	–
Yes	310	1.7	1.3, 2.1	<0.0001
Vitrectomy at time of transplant, $P = 0.02$				
No	2304	1.0	–	–
Yes	710	1.3	1.0, 1.7	0.02
Recipient trephine diameter, $P = 0.0004$				
<7.5 mm	603	1.0	–	–
7.5–8.0 mm	2211	0.95	0.8, 1.2	0.25
>8.0 mm	200	1.6	1.2, 2.2	0.003
Donor/recipient trephine difference, $P = 0.001$				
0.25 mm	1920	1.0	–	–
0 mm	421	1.3	1.0, 1.7	0.02
>0.25 mm	673	1.4	1.2, 1.7	0.0006
Rejection episodes, $P < 0.0001$				
No	2653	1.0	–	–
Yes	361	2.6	2.1, 3.3	<0.0001
Postoperative surgical procedures, $P = 0.01$†				
No	523	1.0	–	–
Yes	2491	0.7	0.5, 0.9	0.01

* Other factors considered in the model: donor age ($P = 0.8$), endothelial quality assessment ($P = 0.2$), endothelial cell density ($P = 0.3$), time from death to enucleation (not included in Cox model but $P = 0.7$ in univariate analysis), enucleation to corneoscleral disc excision time ($P = 0.94$), death to corneoscleral disc excision ($P = 0.6$), storage time ($P = 0.9$), recipient age ($P = 0.4$), donor–recipient age difference ($P = 0.4$), HLA matched ($P = 0.2$), reason for graft—vision only ($P = 0.3$), suturing method ($P = 0.7$), other risk factors ($P = 0.98$), deep vascularization ($P = 0.1$), superficial vascularization ($P = 0.6$), donor trephine diameter ($P = 0.4$), type of anesthesia ($P = 0.6$), cataract extraction/IOL exchange at time of transplant ($P = 0.6$), other surgical procedures at time of transplant ($P = 0.2$), refractive surgery ($P = 0.4$), postoperative complications ($P = 0.8$).

† Suture adjustment/removal, cataract surgery, refractive surgery.

These findings are in the main consistent with other studies. Most show an influence of donor age either on the percentage of corneas suitable for PK or on ECD. For example, Gavrilov et al.[16] reported that 13% of organ-cultured corneas from donors 40 years were unsuitable for PK because of



Indication	N	Survival	95% CI	p
Keratoconus	784	93	90-96	
Fuchs' endothelial dystrophy	757	80	76-84	
Opacification	138	72	60-80	
Injury	94	63	46-74	
Infection	307	65	56-72	
Other indications	91	61	48-72	
Bullous keratopathy	754	59	54-64	
Ulcerative keratitis	89	54	38-68	< 0.0001

FIGURE 2. Kaplan-Meier plot showing effect of indication on 5-year estimated graft survival of first corneal transplants (see Table 6 for Cox regression model).

insufficient endothelium, and this rose to 32% for corneas from donors 80 years (cf. Table 4). The Cornea Donor Study, which was undertaken in the United States to determine the suitability of cold-stored corneas from older donors for PK, showed a negative association between increasing donor age and ECD.[27]

Given that corneal transplants lose endothelial cells at an accelerated rate,[28] a higher initial ECD in the donor cornea would imply a better prognosis for long-term graft survival; however, there is currently little direct supporting evidence for the setting of an acceptable minimum donor ECD for PK. Corneas from older donors and corneas stored for longer periods are likely to have lower ECDs; but, provided the ECD is above a given minimum at the time of transplantation, it is assumed that these donor variables will have little impact on long-term graft survival. Our results lend support to this contention. The Cornea Donor Study failed to find an association between donor ECD and graft failure caused by endothelial decompensation, although the 6-month postoperative ECD was predictive of failure.[22] Since postmortem times in our study had no effect on overall graft survival, exploratory analyses were carried out to further investigate the influence of postmortem times on graft failure caused by endothelial decompensation in Fuchs' and bullous keratopathy recipients. No effects of postmortem times were seen in the Fuchs' group. In the bullous keratopathy group, death to enucleation times of 19 to 24 hours did appear to increase the risk of endothelial decompensation; but the overall trend was not consistent when longer death to enucleation times were considered and, taken as a global variable, death to enucleation time did not reach the 5% level of significance.

It is noteworthy in the present study that no donor factors except sex affected 5-year graft survival. The Cornea Donor Study found no influence of sex on risk of PK failure at 5 years, but corneas from female donors did have higher postoperative ECD.[24,29] Whether the sex effect we observed is related to expression of the H-Y antigen, which could be a target for allograft rejection where corneas from male donors are transplanted into female recipients, is a moot point.[30] Although further analysis did not show any of the specific donor–recipient sex combinations to be significant, this observation does warrant further investigation. A lack of effect of donor age and donor ECD on 5-year graft survival was also found in the Cornea Donor Study.[20–22,24] In the

present study, corneas were stored by organ culture; there was no upper donor age limit, and corneas from older donors tended to be allocated to older recipients. By contrast, corneas in the Cornea Donor Study were stored by hypothermia; there was a maximum upper age limit for donors of 75 years; there was no donor–recipient age matching; and only moderate risk grafts were included in the study. However, the results of the Cornea Donor Study do provide strong support for a lack of influence of donor age, at least up to 75 years in moderate-risk grafts. There are reports of effects of donor factors on postoperative ECD,[18,31] but other studies do not consistently support such findings.[21,25] The Cornea Donor Study did show a slight association between postoperative endothelial cell loss and donor age but, as acknowledged by the authors, the clinical significance of this for graft survival in the longer term is unknown.[20] Longer-term follow-up will be needed to establish the ultimate impact of donor factors such as age and ECD; however, loss to follow-up becomes an important consideration in long-term studies.[32]

In summary, donor age and storage time in organ culture were the main factors determining the suitability of corneas for PK. Postmortem times to enucleation and to processing had little influence on tissue suitability or quality. Although postmortem times up to 30 hours were included in the analyses, current practice in the CTS Eye Banks is not to accept death to enucleation times greater than 24 hours. Similarly, a few corneas (2%) included in the analyses were organ cultured for more than 4 weeks, whereas the standard practice is to outdate corneas after 4 weeks. Based on the outcome of the present analyses, these time limits appear to be acceptable in terms of their influence on the suitability of corneas for PK stored by organ culture. All of the transplanted corneas met or exceeded the minimum endothelial quality assessment based principally on ECD $\geq$ 2200 cells/mm^2. The indication for PK was a major factor influencing graft survival. The 5-year survival estimates shown in Figure 2 and the HR in Table 6 for the main indications are similar to those reported by the Australian Corneal Graft Registry.[2,19] Since the Australian registry is primarily based on cold-stored corneas, these similarities in outcomes with our results suggest that there is little influence of method of corneal storage on graft outcome. As with the Australian data, graft survival was dominated by the indication for PK, allograft rejection, and other recipient-related factors.[19] Whether this conclusion will be modified by longer-term follow-up studies or whether it applies to endothelial keratoplasty remains to be determined; however, within the limits of this study, donor and cornea storage criteria as applied in the United Kingdom appear to be acceptable based on 5-year graft survival for first PK.

Acknowledgments

Supported by institutional funding from NHSBT and the University of Bristol.

Disclosure: **W.J. Armitage**, None; **M.N.A. Jones**, None; **I. Zambrano**, None; **F. Carley**, None; **D.M. Tole**, None

References

1. Vail A, Gore SM, Bradley BA, Easty DL, Rogers CA, Armitage WJ. Conclusions of the corneal transplant follow up study. *Br J Ophthalmol*. 1997;81:631–636.
2. Williams KA, Lowe MT, Keane MC, Jones VJ, Loh RS, Coster DJ, eds. *The Australian Corneal Graft Registry 2012 Report*. Adelaide: Snap Printing; 2012. Available at: http://hdl.handle.net/2328/25859. Accessed January 10, 2014.
3. Claesson M, Armitage WJ, Fagerholm P, Stenevi U. Visual outcome in corneal grafts: a preliminary analysis of the Swedish Corneal Transplant Register. *Br J Ophthalmol*. 2002;86:174–180.
4. Heindl LM, Riss S, Adler W, Bucher F, Hos D, Cursiefen C. Split cornea transplantation: relationship between storage time of split donor tissue and outcome. *Ophthalmology*. 2013;120:899–907.
5. Heindl LM, Hofmann-Rummelt C, Schlötzer-Schrehardt U, Kruse FE, Cursiefen C. Histologic analysis of descemet stripping in posterior lamellar keratoplasty. *Arch Ophthalmol*. 2008;126:461–464.
6. Armitage WJ. Developments in corneal preservation. In: Reinhard T, Larkin F, eds. *Cornea and External Eye Disease*. Berlin: Springer; 2008:101–109.
7. Armitage WJ, Easty DL. Factors influencing the suitability of organ-cultured corneas for transplantation. *Invest Ophthalmol Vis Sci*. 1997;38:16–24.
8. Pels E, Rijneveld WJ. Organ culture preservation for corneal tissue. In: Bredehorn-Meyr T, Duncker GIW, Armitage WJ. eds. *Eye Banking*. Basel: Karger; 2009:31–46.
9. Summerlin WT, Miller GE, Harris JE, Good RA. The organ-cultured cornea: an in vitro study. *Invest Ophthalmol*. 1973;12:176–180.
10. Doughman DJ, Harris JE, Mindrup E, Lindstrom RL. Prolonged donor cornea preservation in organ culture: long-term clinical evaluation. *Cornea*. 1982;1:7–20.
11. Pels E, Schuchard Y. Organ-culture preservation of human corneas. *Doc Ophthalmologica*. 1983;56:147–153.
12. Lindstrom RL, Kaufman HE, Skelnik DL, et al. Optisol corneal storage medium. *Am J Ophthalmol*. 1992;114:345–356.
13. Crewe JM, Armitage WJ. Integrity of epithelium and endothelium in organ-cultured human corneas. *Invest Ophthalmol Vis Sci*. 2001;42:1757–1761.
14. Albon J, Tullo AB, Aktar S, Boulton ME. Apoptosis in the endothelium of human corneas for transplantation. *Invest Ophthalmol Vis Sci*. 2000;41:2887–2893.
15. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun*. 2005;73:1907–1916.
16. Gavrilov JC, Borderie VM, Laroche L, Delbosc B. Influencing factors on the suitability of organ-cultured corneas. *Eye (Lond)*. 2010;24:1227–1233.
17. Patel HY, Brookes NH, Moffatt L, et al. The New Zealand National Eye Bank study 1991-2003: a review of the source and management of corneal tissue. *Cornea*. 2005;24:576–582.
18. Borderie VM, Scheer S, Touzeau O, Vedie F, Carvajal-Gonzalez S, Laroche L. Donor organ cultured corneal tissue selection before penetrating keratoplasty. *Br J Ophthalmol*. 1998;82:382–388.
19. Williams KA, Lowe M, Bartlett C, Kelly TL, Coster DJ. Risk factors for human corneal graft failure within the Australian corneal graft registry. *Transplantation*. 2008;86:1720–1724.
20. Gal RL, Dontchev M, Beck RW, et al. The effect of donor age on corneal transplantation outcome results of the cornea donor study. *Ophthalmology*. 2008;115:620–626, e626.
21. Lass JH, Gal RL, Dontchev M, et al. Donor age and corneal endothelial cell loss 5 years after successful corneal transplantation. Specular microscopy ancillary study results. *Ophthalmology*. 2008;115:627–632.
22. Lass JH, Sugar A, Benetz BA, et al. Endothelial cell density to predict endothelial graft failure after penetrating keratoplasty. *Arch Ophthalmol*. 2010;128:63–69.
23. Sugar A, Tanner JP, Dontchev M, et al. Recipient risk factors for graft failure in the cornea donor study. *Ophthalmology*. 2009;116:1023–1028.

24. Sugar J, Montoya M, Dontchev M, et al. Donor risk factors for graft failure in the cornea donor study. *Cornea*. 2009;28:981–985.
25. Gain P, Thuret G, Chiquet C, et al. Cornea procurement from very old donors: post organ culture cornea outcome and recipient graft outcome. *Br J Ophthalmol*. 2002;86:404–411.
26. Hassan SS, Wilhelmus KR, Dahl P, et al. Infectious disease risk factors of corneal graft donors. *Arch Ophthalmol*. 2008;126:235–239.
27. Sugar A, Gal RL, Beck W, et al. Baseline donor characteristics in the Cornea Donor Study. *Cornea*. 2005;24:389–396.
28. Armitage WJ, Dick AD, Bourne WM. Predicting endothelial cell loss and long-term corneal graft survival. *Invest Ophthalmol Vis Sci*. 2003;44:3326–3331.
29. Lass JH, Beck RW, Benetz B, et al. Baseline factors related to endothelial cell loss following penetrating keratoplasty. *Arch Ophthalmol*. 2011;129:1149–1154.
30. Böhringer D, Spierings E, Enczmann J, et al. Matching of the minor histocompatibility antigen HLA-A1/H-Y may improve prognosis in corneal transplantation. *Transplantation*. 2006;82:1037–1041.
31. Böhringer D, Reinhard T, Spelsberg H, Sundmacher R. Influencing factors on chronic endothelial cell loss characterised in a homogeneous group of patients. *Br J Ophthalmol*. 2002;86:35–38.
32. Claesson M, Armitage WJ. Ten-year follow-up of graft survival and visual outcome after penetrating keratoplasty in Sweden. *Cornea*. 2009;28:1124–1129.