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The Influence of Rejection Episodes in Recipients of Bilateral Corneal Grafts

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Abstract

We investigated whether a rejection episode in one graft was associated with rejection in the other graft, in recipients with bilateral corneal transplants. In a prospectively-maintained, national register of 14,865 followed corneal grafts, 1,476 patients with bilateral penetrating corneal grafts were identified. Occurrence of rejection was a risk factor for graft failure ($p<0.0001$). Logistic regression was used to calculate the adjusted odds ratio for rejection in one eye following rejection in the other eye. In the subset of 1,118 patients with bilateral grafts but no history of previous grafts or rejections in either eye, the adjusted odds ratio for a rejection episode in the first eye following rejection in the second was 3.27 (95% CI 1.85, 5.79; $p<0.001$). The adjusted odds ratio was 2.04 (95% CI 1.07, 3.91; $p=0.03$) for rejection in the second eye following rejection in the first. The median time between the first rejection episode in one eye and the first rejection episode in the other eye was 15 months. Patients with bilateral corneal grafts who suffer a graft rejection episode in one eye are at significantly greater odds of suffering a rejection episode in the other corneal transplant.

Introduction

Irreversible corneal graft rejection is a common cause of human corneal transplant failure (1, 2). In any cohort of patients with penetrating corneal grafts, a proportion may have bilateral grafts. Over twenty years ago, Meyer reviewed the literature on the likely influence of a second corneal graft on the risk of rejection in either eye in patients with bilateral grafts, and was unable to draw firm conclusions from the available data (3). In a more recent study, Musch and Meyer studied 90 patients with bilateral grafts, with follow-up ranging from 4-108 months for both eyes considered together, and concluded that there was no increased risk of rejection in the first eye after penetrating keratoplasty in the second eye (4).

We were interested in a rather different question: specifically, whether the occurrence of a rejection episode in either grafted eye would affect the risk of rejection in the graft in the other eye. This issue has some implications for clinical management, and is also of interest to investigators exploring the mechanisms involved in corneal graft rejection. Accordingly, we identified 1,476 patients with bilateral corneal grafts in a prospectively-collected clinical database containing records of 14,865 penetrating corneal grafts followed for 1 to 24 years, and investigated the influence of bilateral transplantation on the risk of rejection in either eye.

Subjects and Methods

Australian Corneal Graft Register

The Australian Corneal Graft Register was established in May 1985 to follow the outcomes associated with corneal transplants performed nationally. Records of corneal transplantation and subsequent follow-up have been submitted by 634 ophthalmic

surgeons and other practitioners. Individual surgeons handle the consent process for each patient according to local legislative requirements, to permit information to be lodged with the register. The institutional Clinical Ethics Committee of Flinders University oversees the operations of the register, which are carried out in accordance with the Declaration of Helsinki.

Data collection and verification

Using well-validated proformas, data were collected on the recipient, donor, eye bank, operative procedure, post-operative management and post-operative course, as previously described (5). Each corneal graft was followed at yearly intervals until graft failure or until the death or loss to follow-up of the patient. Missing data were routinely sought by follow-up letter to the contributing ophthalmologist or Eye Bank, as appropriate. Data verification is inherent in the database structure, which contains internal logic checks, but all records were independently verified by a second individual against the record provided by the contributor.

Donor corneas

All donor corneas were assessed within a licensed Eye Bank on the basis of the history, slit-lamp analysis of the enucleated globes, and specular microscopy to confirm a normal corneal endothelium. Serological testing to exclude some communicable diseases was also performed. Advanced donor age was no specific barrier to donation, and tissue matching of donor and recipient was not performed.

Definition of specified events before and after corneal transplantation

Information was collected on specific risk factors for corneal graft failure (5). A history of

past inflammation was recorded if the individual was reported to have had such an episode, if the patient had had one or more previous grafts or any previous intraocular surgery in the same eye, or if there was a history of topical glucocorticosteroids use in the two weeks immediately preceding the graft. Vessel ingrowth into the cornea at the time of graft was scored on a scale of 0-4 (5). No distinction was made between superficial or deep vessels, patent or ghost vessels, or single or multiple vessel leashes. Intraocular pressure (IOP) was considered to be raised if a reading of ≥25 mm mercury was made by applanation tonometry, but the final decision was at the discretion of the ophthalmologist. Indications for transplantation, post-operative complications and reasons for graft failure were coded using the International Classification of Diseases system (ICD.9.CM, US Department of Health and Human Services). To examine the influence of graft diameter, recipient bed size rather than donor button size was used.

Primary non-functions were defined as grafts that never thinned and cleared in the immediate post-operative period. The trial time in survival analysis for such grafts was arbitrarily adjusted to one day. Any existing graft that was replaced by another in the same eye, irrespective of graft clarity and for whatever reason, was classified as a failed graft. In all other cases, graft failure was defined as oedema and irremediable loss of clarity in a previously thin, transparent graft. The day of failure was the first day the patient was seen with an oedematous, opaque graft that subsequently failed to thin and clear. Rejection was defined as the development of inflammation and an epithelial or endothelial rejection line and/or a unilateral anterior chamber reaction with corneal infiltrates and spreading corneal oedema in a previously thin, transparent graft. Graft failure can occur from causes other than rejection, and a rejection episode may be

reversible or irreversible.

Recipients with bilateral grafts

At the census date of October 2009, the Registry held records of 19,387 penetrating corneal grafts in 21,279 patients. Of the 19,387 grafts, 14,865 (77%) had been followed on at least one annual occasion. Records with archival follow-up were available for 2,952 grafts in 1,476 individuals with bilateral corneal grafts. No recipient of bilateral grafts had received two corneas from the same donor. For inclusion in this study, we considered only recipients in whom a graft was performed in the second eye, in the presence of a functioning graft in the other eye (the first eye). Thus, both grafts in both eyes were concurrently transparent immediately after the surgery in the second eye. Follow-up after surgery in the second eye extended for a median of 31 months (range 1-249 months, with short times reflecting graft failure).

Statistical analyses

Data were amalgamated and de-identified prior to analysis using the software packages SPSS v15 (SPSS Inc, Chicago, IL) and Stata v9 (StataCorp, College Station, TX). The Pearson chi-square test was used to compare demographics and indications for corneal transplantation in patients with bilateral grafts compared with all penetrating grafts, with the significance level set at 0.05. Column percentages for each relevant category in the cohort of all grafts were used to generate expected frequencies in the cohort with bilateral grafts. The chi-square test was also used to test for associations between rejection episodes in first and second eyes of patients with bilateral grafts, and to test any association of recipient sex with rejection episodes. Kaplan-Meier survival functions (6-8) were constructed to provide a graphical record of graft survival. For surviving

grafts, trial time was calculated as the time between the date of graft and the date on which the patient was last seen. For failed grafts, trial time was calculated as the time between the date of graft and the date of failure. Kaplan-Meier plots were also used to determine rejection-free survival times. The log-rank statistic was used to examine differences amongst plots. A Cox proportional hazards model was used for multivariate survival analysis to determine risk factors for graft survival in the bilateral cohort. Clustering by patient accounted for the correlation between eyes (9). A backwards selection process was used to find statistically significant covariates ($p \leq 0.05$).

Matched logistic regression was used to determine whether the first or second grafted eye was more at risk for rejection (10). Subsequently, subset analysis was performed using each patient as the unit of analysis, to calculate the adjusted odds ratio for a rejection episode in one grafted eye following a rejection episode in the other grafted eye. Potential confounders considered in multivariate analyses are shown in Table 1; graft size was square root-transformed to ensure linearity. Variables were checked for confounding status using Pearson's χ^2 test for association with rejection episodes in first and second eyes, and univariate logistic regression. Multivariate analysis included all variables with associations at $p \leq 0.1$, in a forward selection process. Variables that were not significant ($p \leq 0.05$) in the multivariate logistic regression were excluded from the final model. A non-parametric K-sample test was used to test the equality of median times to rejection in each eye of patients with bilateral grafts. Further survival analysis was performed with time to rejection (after the second eye was grafted) as the trial time. For patients with one or more rejections in the same eye, time after rejection was analysed separately (10).

Results

Demographics of patients with bilateral grafts

The demographics of recipients of bilateral grafts compared with the cohort of all penetrating grafts are shown in Table 2. The cohorts were comparable, except that patients with bilateral corneal grafts were significantly younger at transplantation ($p<0.001$). The indications for transplantation (Table 3) in patients with bilateral grafts differed significantly from those of the total cohort ($p<0.001$), in that more patients required transplantation for keratoconus or a corneal dystrophy (conditions which are frequently bilateral) in the former. The relative excess of patients with bilateral transplants who were grafted for keratoconus explains the younger age distribution in this cohort, as keratoconus typically manifests itself during adolescence.

Influence of rejection on corneal graft survival in the total cohort of penetrating grafts

We first examined the influence of rejection episodes (whether reversible or irreversible) on graft survival in the cohort of all penetrating grafts (Figure 1). The occurrence of one or more rejection episodes was a significant risk factor for corneal graft failure (log-rank statistic $p<0.0001$). Of the 14,865 penetrating corneal grafts followed, 3,442 had failed and 1,126 (33%) of these had failed from irreversible graft rejection.

Influence of rejection episodes in patients with bilateral grafts

We next examined the cohort of patients with bilateral corneal grafts. Of the 2,952 grafts in 1,476 patients, 376 grafts had failed and of these, 110 (29%) had failed from irreversible rejection. The occurrence of one or more rejection episodes was a

significant risk factor ($p<0.0001$) for graft failure in univariate analysis (Figure 2) and a significant independent risk factor for graft failure in Cox proportional hazards regression (Table 4). Overall, risk factors for graft failure in the cohort with bilateral corneal grafts were similar to those previously reported for the total cohort of penetrating grafts (1, 11).

The occurrence of at least one rejection episode (after the second eye was grafted) in neither, either or both eyes of 1,476 patients with bilateral grafts was then investigated. Irreversible plus reversible rejection episodes, irreversible rejection episodes only, and reversible rejection episodes only, were examined separately (Table 5). Irrespective of whether rejection episodes were reversible or irreversible, the Pearson χ^2 test indicated a significantly different number of rejection episodes between the two eyes. Further one-sided Fisher's exact testing showed that second eyes had a significantly higher number of rejection episodes than first eyes ($p<0.001$). In subsequent analyses, all rejection episodes (irreversible plus reversible) were considered together.

In some instances, a recipient with bilateral grafts had had a history of rejection episodes in a graft in one or both eyes, *prior* to the index graft in the second eye (Table 6). Using the subset of 1,316 patients who had *not* previously suffered a rejection episode in *any* graft in *either* eye, prior to the index graft in the second eye, matched logistic regression was performed to analyse which eye was more likely to undergo graft rejection (Table 7). The adjusted odds ratio for a rejection episode in the second of the two bilateral grafts compared with the first was 2.21 (95% confidence interval (CI) 1.62, 3.02; $p<0.001$). Corneal neovascularization in the graft was a significant covariate in this analysis. Further matched logistic regression was performed to analyse whether the

order of bilateral rejection episodes was significant: it was not, with an odds ratio of 1.21 (95% CI 0.66, 2.22; p=0.54) for second eyes rejecting before first eyes.

Next, logistic regression analysis using the patient (rather than the eye) as the unit of analysis was performed, to examine the influence of a rejection episode in either graft on the likelihood of a subsequent rejection episode in the graft in the other eye. Rejections in the first and second grafted eyes of 1,316 bilateral graft recipients who had no previous history of graft rejection in either eye were analysed (Table 8). With rejection in eye one as the outcome, the adjusted odds ratio was 2.99 (95% CI 1.79, 5.02; p<0.001) for rejection in eye two, compared with *no* rejection in eye two. Similarly, the adjusted odds ratio was 2.15 (95% CI 1.22, 3.78; p=0.008) for rejection in eye two following rejection in eye one, compared with *no* rejection in eye one.

The analysis was then repeated in the subset of 1,118 patients with bilateral grafts, but with *no* history of previous grafts *or* rejections in *either* eye (Table 9). For a rejection episode in the first eye following rejection in the second, the adjusted odds ratio was 3.27 (95% CI 1.85, 5.79; p<0.001). For a rejection episode in the second eye following rejection in the first, the adjusted odds ratio was 2.04 (95% CI 1.07, 3.91; p=0.03). Significant covariates were corneal neovascularization and keratoconus. Thus, after an episode of rejection in one graft, the odds of a rejection episode in the other graft were significantly increased, irrespective of whether the episode was reversible or led to graft failure, and irrespective of past history of corneal transplantation or occurrence of rejection episodes.

Time to rejection in patients with bilateral corneal grafts

The median times at which rejection occurred in the 1,476 patients with bilateral grafts were examined (Table 10). Although in some instances an episode of corneal graft rejection in the first eye was followed swiftly by an episode in the other eye, the median time between rejection episodes in bilateral grafts was approximately 15 months. In 21 first-grafted eyes with a previous rejection in the same graft, a further rejection occurred after the second eye was grafted. For the total bilateral cohort and in eyes with no previous rejection, median times from transplantation to the first rejection episode after the second eye was grafted were similar; a test for equality of medians showed no difference for eyes with and without previous rejections (continuity corrected Pearson $\chi2(1)=0.19$, $p=0.66$ for eye one; $\chi2(1)=1.54$, $p=0.21$ for eye two). Thus, a history of previous rejection did not influence the median time to rejection in the same eye.

Since each bilateral graft may have suffered multiple rejection episodes, the effect of a rejection episode *at any time* after graft on subsequent rejection episodes was examined. Time after each rejection episode was analysed separately. There were 2,601 grafts with no rejection episodes, 289 with one, 44 with two, 18 with three, plus 258 rejection-free periods following a rejection episode. Patients with previous rejections in both eyes were excluded, leaving 1,501 at risk for the first eye and 1,659 for the second eye. Kaplan-Meier plots were generated to examine the effect of rejection episodes in either the same or opposite eye on *subsequent* rejection (Figure 3). Grafts with a history of one or more rejections in either eye had significantly worse ($p<0.001$) rejection-free survival compared with grafts with no such history. Grafts with previous rejections in the *opposite* eye had significantly worse rejection-free survival than grafts with no previous rejections, but better rejection-free survival than grafts with previous rejections in the *same* eye ($p<0.0001$).

History of systemic sensitization in patients with bilateral corneal grafts

The data were consistent with the possibility that the occurrence of a rejection episode in the contralateral eye was associated with a history of systemic sensitization of the recipient. A potential confounding factor might thus be recipient systemic sensitization to foreign histocompatibility antigens present on a fetus, resulting from a past pregnancy. Gender was not associated with rejection in the first grafted eye (Pearson's $\chi^2(1)=1.01$, $p=0.31$), therefore gender was not a risk factor in the analysis. A history of pregnancy was thus unlikely to have accounted for the finding that a rejection episode in the graft in one eye predisposes the recipient to rejection in a graft in the other eye.

Discussion

Using Registry data, we report that in a cohort of patients with bilateral corneal grafts, the occurrence of a rejection episode was a significant risk factor for graft failure. Second eyes to be grafted suffered significantly more rejection episodes than first eyes to be grafted. In recipients of bilateral grafts who had no previous history of rejection in either eye, those who suffered a graft rejection episode in one eye were then at a significantly greater risk of suffering a rejection episode in the graft in the other eye. A similar finding was observed when the subset of patients who had never had a previous graft nor a rejection episode in either eye was examined. The median time between rejection episodes in bilateral grafts was 15 months.

Registries, increasingly being used to fill evidence-gaps that may not be amenable to randomised controlled clinical trials (12), have inherent strengths and weaknesses. Strengths include the long-term follow-up of patients who have undergone a surgical intervention “in the real world”. In the context of this study, the approach is accepting of individual surgeon variations in case selection, surgical technique and post-operative management, important because corneal transplantation is performed in a mixture of practice settings. In Australia, all donor corneas must be provided by a licensed Eye Bank and corneal grafts reported to the Registry, so that case ascertainment is high. The major weakness is loss to follow-up, which can occur either because the death of a recipient has not been notified to the contributing ophthalmologist, or because the recipient has chosen not to attend a scheduled appointment. However, all patients with corneal grafts are counselled to seek medical attention, should they notice symptoms of corneal graft rejection such as pain, reddening of the eye, or decreased visual acuity. The non-random selection of cases with rejection episodes is unlikely to have been an

issue, and patients with bilateral grafts were followed for a median of 31 months after corneal transplantation in the second eye. However, a potential source of uncontrolled variation relates to the immunosuppressive regimen provided to corneal graft recipients. There is no gold standard for the prophylaxis or treatment of corneal graft rejection (2). Systemic immunosuppression is seldom used, and although all grafts are treated with topical glucocorticosteroids, the type of steroid, concentration, and regimen of administration vary considerably (13).

All our analyses support the contention that an episode of rejection in one corneal graft significantly increases the odds of rejection of a graft in the other eye. There are at least two possible explanations for our findings, which have not to our knowledge been reported previously in humans. The individual patient who suffers rejection episodes in both grafts may conceivably be immunologically hyper-reactive and therefore prone to rejection. An alternative scenario, which we favour, is that the patient has become systemically sensitized to mismatched histocompatibility antigens present on the graft in one eye, some of which are also present on the graft in the contralateral eye.

Despite being considered as an immune-privileged site, the eye is not sequestered from the immune system (14). It has been known for over 35 years that the combination of corneal graft neovascularization, inflammation, and deliberate systemic sensitization to donor antigens will together ensure penetrating corneal graft rejection in outbred experimental models (15). In a model of orthotopic corneal transplantation in the inbred rat, a second orthotopic corneal graft in either a previously grafted ipsilateral eye or into the normal contralateral eye was rejected at an accelerated tempo compared with the first ipsilateral graft, but the second graft was rejected at the same tempo irrespective of

the eye into which it was placed, suggesting that systemic sensitization had occurred as a result of the first graft (16). The phenomenon of anterior chamber-associated immune deviation, in which introduction of foreign antigen into the anterior chamber of experimental animals results in a systemic depression of the delayed type hypersensitivity response to that antigen (17), is further evidence of an interaction (albeit immunomodulatory) between the eye and the systemic immune response. The relative immune privilege enjoyed by the normal cornea and anterior segment is, however, readily broken by the sequelae of neovascularization and inflammation (2, 18), and human corneal recipients with long-surviving grafts are probably not truly tolerant, in that rejection generally occurs once immune privilege has been perturbed.

The relatively long lag time that we observed between rejection episodes in bilateral corneal grafts in some of our patients might argue against the likelihood that systemic sensitization to histoincompatible antigens present on one graft had generated effector cells that were poised to react to the other graft, that by chance carried some of the same incompatible antigens. Should such effector cells have been generated by direct antigen presentation (19) for example, then a more immediate rejection response might have been expected. However, although the median time between rejection episodes in the ipsilateral and contralateral grafts was 15 months, the range was very wide. Furthermore, immune responsiveness was very probably modulated by the administration of topical glucocorticosteroid prophylaxis in all patients. Our data are consistent with the operation of either direct or indirect antigen presentation, or both, occurring to induce systemic sensitization in the individual patient.

Irrespective of the mechanisms involved, our findings have clinical ramifications.

Corneal endothelial cell loss is a major risk factor for late penetrating corneal graft failure (20-22), and thus even reversible rejection episodes may compromise corneal graft survival and function. Patients with bilateral corneal grafts who have suffered a rejection episode in one grafted eye should be counselled to seek prompt ophthalmic care in the event that they notice any untoward symptoms in *either* eye, and ophthalmologists may need to consider carefully, the timeframe over which topical steroids are prescribed. In instances where a graft failure has occurred and a repeat keratoplasty is being contemplated, then consideration might be given to maintenance of topical immunosuppression in the longer term. It is of some concern in this context that although topical glucocorticoid treatment is widely considered to be the gold standard for the prevention and treatment of corneal graft rejection, there is little good evidence to guide selection of the corticosteroid or indeed, any other immunosuppressive drug (23), or the regimen of administration (24) in the post-operative period. Tissue matching, found to be useful in some but not all studies (25, 26) may be another option when contemplating bilateral corneal transplantation in patients with high-risk indications for graft. Further, careful consideration should be given to the need for a corneal graft in the second eye of a patient at high-risk for rejection, who has achieved good vision in a graft in the first eye. Irreversible rejection remains a major cause of corneal graft loss (1, 11, 23, 27, 28), and there is a clear need for an improved evidence-base for the prevention and treatment of rejection, to support clinical practice.

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References

1. Williams KA, Esterman AJ, Bartlett C, Holland H, Hornsby NB, Coster DJ on behalf of all contributors to the Australian Corneal Graft Registry. How effective is penetrating corneal transplantation? Factors influencing long-term outcome in multivariate analysis. Transplantation 2006; 81: 896-901.
2. Coster DJ, Williams KA. The impact of corneal allograft rejection on the long-term outcome of corneal transplantation. Am J Ophthalmol 2005; 140: 1112-1122.
3. Meyer RF. Corneal allograft rejection in bilateral penetrating keratoplasty: clinical and laboratory studies. Trans Am Ophthalmol Soc 1986; 84: 664-742.
4. Musch DC, Meyer RF. Risk of endothelial rejection after bilateral penetrating keratoplasty. Ophthalmology 1989; 96: 1139-1143.
5. Williams KA, Roder D, Esterman A, et al. Factors predictive of corneal graft survival: report from the Australian Corneal Graft Registry. Ophthalmology 1992; 99: 403-414.
6. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 475-481.
7. Peto R, Pike MC, Armitage P, at al. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. I Introduction and design. Br J Cancer 1976; 34: 585-612.
8. Peto R, Pike MC, Armitage P, at al. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II Analysis and examples. Br J Cancer 1977; 5: 1-39.
9. Williams RL. A note on robust variance estimation for cluster-correlated data. Biometrics 2000; 56: 645-646.
10. Prentice, RL, Williams BJ, Peterson AV. On the regression analysis of multivariate

failure time data. Biometrika 1981; 68: 373–379.

11. Williams KA, Lowe M, Bartlett C, Kelly T-L, Coster DJ on behalf of all contributors. Risk factors for human corneal graft failure within the Australian Corneal Graft Registry. Transplantation 2008; 86: 1720–1724.
12. Dreyer NA, Garner S. Registries for robust evidence. JAMA 2009; 302: 790-791.
13. Barker NH, Henderson TRM, Ross CA, Coster DJ, Williams KA. Current Australian practice in the prevention and management of corneal allograft rejection. Clin Experiment Ophthalmol 2000; 28: 357-360.
14. Niederkorn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. Nat Immunol 2006; 7: 354-359.
15. Khodadoust AA, Silverstein AM. Studies on the nature of the privilege enjoyed by corneal allografts. Invest Ophthalmol 1972; 11: 137-148.
16. Banerjee S, Dick AD, Nicholls SM. Factors affecting rejection of second corneal transplants in rats. Transplantation 2004; 77: 492-496.
17. Stein-Streilein J, Streilein JW. Anterior chamber associated immune deviation (ACAID): regulation, biological relevance, and implications for therapy. Int Rev Immunol 2002; 21: 123-152.
18. Williams KA, Coster DJ. The immunobiology of corneal transplantation. Transplantation 2007; 84: 806-13.
19. Boisgerault F, Liu Y, Anosova N, Ehrlich E, Dana MR, Benichou G. Role of CD4+ and CD8+ T cells in allorecognition: lessons from corneal transplantation. J Immunol 2001; 167: 1891-1899.
20. Bourne WM, Hodge DO, Nelson LR. Corneal endothelium five years after transplantation. Am J Ophthalmol 1994; 118: 185-196.
21. Bourne WM. Cellular changes in transplanted human corneas. Cornea 2001; 20:

560-569.

22. Armitage WJ, Dick AD, Bourne WM. Predicting endothelial cell loss and long-term corneal graft survival. Invest Ophthalmol Vis Sci 2003; 44: 3326-3331.
23. Banerjee S, Dick AD. Recent developments in the pharmacological treatment and prevention of corneal graft rejection. Expert Opin Investig Drugs 2003; 12: 29-37.
24. Bertelmann E, Pleyer U. Immunomodulatory therapy in ophthalmology – is there a place for topical application? Ophthalmologica 2004; 218: 359-367.
25. The Collaborative Corneal Transplantation Studies Research Group. The collaborative corneal transplantation studies (CCTS). Effectiveness of histocompatibility matching in high-risk corneal transplantation. Arch Ophthalmol 1992; 110: 1392-1403.
26. Völker-Dieben HJ, Claas FH, Schreuder GM, Schipper RF, Pels E, Persijn GG, Smits J, D'Amaro J. Beneficial effect of HLA-DR matching on the survival of corneal allografts. Transplantation 2000; 70: 640-648.
27. George AJ, Larkin DF. Corneal transplantation: the forgotten graft. Am J Transplant 2004; 4: 678-685.
28. Ing JJ, Ing HH, Nelson LR, Hodge DO, Bourne WM. Ten-year postoperative results of penetrating keratoplasty. Ophthalmology 1998; 105: 1855-1865.

Table 1: Risks factors examined as potential confounders in multivariate logistic regression analysis of 1,476 recipients with bilateral corneal grafts

Risk factor	Category	Number recipients (percent)
Sex of recipient	Female	756 (51%)
	Male	720 (49%)
Recipient age at first graft	<60 years	995 (67%)
	≥60 years	481 (33%)
Graft era (for first eye grafted)	1985-1996 inclusive	968 (65%)
	1997-2009 inclusive	508 (34%)
Corneal vascularization at graft, first eye	0 quadrants	1,235 (84%)
	1-4 quadrants	241 (16%)
Corneal vascularization at graft, second eye	0 quadrants	1,259 (85%)
	1-4 quadrants	217 (15%)
Inflammation in first eye, in past and/or at graft	No	999 (67%)
	Yes	477 (32%)
Inflammation in second eye, in past and/or at graft	No	1,042 (71%)
	Yes	434 (29%)
Keratoconus as bilateral indication for graft	No	705 (48%)
	Yes	771 (52%)
History of previous graft in first eye	No	1,300 (88%)
	Yes	176 (12%)
History of previous graft in second eye	No	1,302 (88%)
	Yes	174 (12%)
Graft size	√ distance from 8 mm diameter	1,476 (100%)
Neovascularization of first graft	No	1,409 (95%)
	Yes	67 (5%)
Neovascularization of second graft	No	1,427 (97%)
	Yes	49 (3%)

Table 2. Demographics of 2,952 bilateral corneal grafts in 1,476 recipients of bilateral grafts, compared with the total cohort of 14,865 penetrating corneal grafts

Demographic		Bilateral grafts	Total cohort
Female		1,510 (51%)	7,337 (49%)
Male		1,442 (49%)	7,528 (51%)
Age*	<30 years at graft	849 (29%)	2,809 (19%)
	30-59 years at graft	1,068 (36%)	4,700 (32%)
	>=60 years at graft	1,035 (35%)	7,349 (49%)
Corneal neovascularisation		116 (4%)	964 (6%)
Graft size	<7 mm diameter	31 (1%)	207 (1%)
	7.0 – 7.4 mm	296 (10%)	1,639 (12%)
	7.5 – 7.9 mm	1,194 (42%)	6,092 (43%)
	8.0 – 8.4 mm	1,152 (41%)	5,265 (37%)
	8.5 – 8.9 mm	123 (4%)	610 (4%)
	9.0 – 9.9 mm	33 (1%)	213 (2%)
	=>10 mm	6 (<1%)	110 (1%)
Era	1985-1988	301 (10%)	1,689 (11%)
	1989-1992	681 (23%)	3,553 (24%)
	1993-1996	621 (21%)	2,894 (20%)
	1997-2000	609 (21%)	2,747 (18%)
	2001-2004	573 (19%)	2,826 (19%)
	2005-2009	167 (6%)	1,156 (8%)

* χ^2 (2)=141.5; p<0.001 for difference between cohort with bilateral grafts and total cohort, in respect (only) of recipient age at graft.

Table 3: Indications for transplantation in 2,952 eyes of 1,476 patients with bilateral corneal grafts, compared with indications in the total cohort of 14, 865 penetrating corneal grafts

Indication	Bilateral grafts	Total cohort
Keratoconus	1,532 (52%)	5,249 (35%)
Bullous keratopathy	429 (15%)	3,908 (26%)
Failed previous graft	335 (11%)	1,817 (12%)
Corneal dystrophy	439 (15%)	1,336 (9%)
Other	217 (7%)	2,555 (17%)
Total	2,952 (100%)	14,865 (100%)

χ^2 (4)=353.3; p<0.001 for difference between cohort with bilateral grafts and total cohort.

Table 4: Significant risk factors for graft failure in 1476 patients with bilateral corneal grafts: Cox proportional hazards regression

Variable		Hazard ratio (95% CI)	p
Rejection episode/s	No	1.0	
	Yes	3.01 (2.13, 4.26)	p<0.001
Keratoconus	No	1.0	
	Yes	0.26 (0.17, 0.40)	p<0.001
Corneal vascularization at graft	0 quadrants	1.0	
	1 or more quadrants	1.56 (1.01, 2.42)	p=0.045
Vascularization post graft	No	1.0	
	Yes	2.04 (1.05, 3.96)	p=0.04
Aphakia	No	1.0	
	Yes	2.16 (1.24, 3.78)	p=0.007
Postoperative microbial keratitis	No	1.0	
	Yes	3.16 (1.70, 5.84)	p<0.001
Raised intraocular pressure at graft	No	1.0	
	Yes	9.45 (2.30, 38.8)	p=0.002
Arrangements for follow-up	By surgeon	1.0	
	Elsewhere	0.52 (0.32, 0.85)	p=0.008
Removal of graft sutures	Every unit increase in ln (year)	0.76 (0.60, 0.95)	p=0.02
Graft size	√ distance from 8 mm diameter	1.85 (1.13, 3.00)	p=0.01

Table 5: Occurrence of rejection episode/s in neither, either or both eyes of 1,476 patients with bilateral penetrating corneal grafts

Type of rejection episode			Rejection episode in eye two			χ^2 (1)	p
			No	Yes	Total		
All episodes, (irreversible+ reversible)	Rejection episode in eye one	No	1,176 (80%)	167 (11%)	1,343 (91%)	64.5	p<0.001
		Yes	82 (5%)	51 (4%)	133 (9%)		
		Total	1,258 (85%)	218 (15%)	1,476 (100%)		
Irreversible episodes only	Rejection episode in eye one	No	1,374 (93%)	43 (3%)	1,417 (96%)	18.8	p<0.001
		Yes	51 (4%)	8 (<1%)	59 (4%)		
		Total	1,425 (97%)	51 (3%)	1,476 (100%)		
Reversible episodes only	Rejection episode in eye one	No	1,264 (86%)	138 (9%)	1,402 (95%)	60.3	p<0.001
		Yes	45 (3%)	29 (2%)	74 (5%)		
		Total	1,309 (89%)	167 (11%)	1,476 (100%)		

Table 6: History of previous rejection episodes (reversible or irreversible) in 1,476 recipients with bilateral corneal grafts

Risk factor	Number recipients (percent)
Rejection episode in previous graft in first grafted eye	32* (2%)
Rejection episode in first grafted eye, prior to graft in second eye	121* (8%)
Rejection episode in previous graft in second grafted eye	18* (1%)
No prior rejection episode in any graft in either eye at time of graft in second eye	1316 (89%)

* Note categories are not mutually exclusive.

Table 7: Subset analysis: unadjusted and adjusted odds ratios for rejection in the second eye compared with the first eye in 1,316 recipients* of bilateral grafts who had not previously suffered a rejection episode in *any* graft in *either* eye

Covariate		Unadjusted odds ratio for rejection (95% CI)	p	Adjusted odds ratio for rejection (95% CI)	p
Eye number	One	1.0		1.0	
	Two	2.11 (1.56, 2.86)	p<0.001	2.21 (1.62, 3.02)	p<0.001
Vascularization in grafted eye	No	1.0		1.0	
	Yes	3.60 (1.34, 9.70)	p=0.01	4.45 (1.56, 12.70)	p=0.005

*1,081 patients (82%) had no rejections in either eye, 62 (5%) had rejections in eye one only, 131 (10%) had rejections in eye two only and 42 (3%) had rejections in both eyes.

Table 8: Unadjusted and adjusted odds ratios for rejection, for each eye treated separately, in 1,316 recipients of bilateral corneal grafts* who had not previously suffered a rejection episode in *any* graft in *either* eye

Outcome	Significant covariate		Unadjusted odds ratio for rejection (95% CI)	p	Adjusted odds ratio for rejection (95% CI)	p
Rejection episode in eye one	Rejection episode, eye two	No	1.0		1.0	
		Yes	3.06 (1.84, 5.11)	p<0.001	2.99 (1.79, 5.02)	p<0.001
	Vascularization of graft in eye one	No	1.0		1.0	
		Yes	3.62 (1.75, 7.50)	p=0.001	3.46 (1.65, 7.26)	p=0.001
Rejection episode in eye two	Rejection episode, eye one	No	1.0		1.0	
		Yes	2.53 (1.47, 4.36)	p=0.001	2.15 (1.22, 3.78)	p=0.008
	Keratoconus	No	1.0		1.0	
		Yes	0.59 (0.42, 0.81)	p=0.001	0.54 (0.38, 0.77	p=0.001
	Vascularization of graft in eye two	No	1.0		1.0	
		Yes	5.19 (2.77, 9.74)	p<0.001	4.94 (2.54, 9.62)	p<0.001

*1,081 patients (82%) had no rejections in either eye, 62 (5%) had rejections in eye one only, 131 (10%) had rejections in eye two only, 19 (1%) had a rejection in eye one followed by a rejection in eye two, and 23 (2%) had a rejection in eye two followed by a rejection in eye one.

Table 9: Unadjusted and adjusted odds ratios for rejection, for each eye considered separately, in 1,118 patients* with bilateral grafts but *no* history of previous grafts *or* rejections in *either* eye

Outcome	Covariate		Unadjusted odds ratio for rejection (95% CI)	p	Adjusted odds ratio for rejection (95% CI)	p
Rejection episode in eye one	Rejection episode, eye two	No	1.0		1.0	
		Yes	3.37 (1.92, 5.93)	p<0.001	3.27 (1.85, 5.79)	p<0.001
	Vascularization of graft in eye one	No	1.0		1.0	
		Yes	3.84 (1.71, 8.68)	p=0.001	3.61 (1.57, 8.29)	p=0.002
Rejection episode in eye two	Rejection episode, eye one	No	1.0		1.0	
		Yes	2.48 (1.33, 4.64)	p=0.004	2.04 (1.07, 3.91)	p=0.03
	Keratoconus	No	1.0		1.0	
		Yes	0.58 (0.40, 0.83)	p=0.003	0.50 (0.34, 0.75)	p=0.001
	Vascularization of graft in eye two	No	1.0		1.0	
		Yes	5.77 (2.76, 12.1)	p<0.001	5.32 (2.40, 11.8)	p<0.001

*932 patients (83%) had no rejections in either eye, 52 (5%) had rejections in eye one only, 101 (9%) had rejections in eye two only, 14 (1%) had a rejection in eye one followed by a rejection in eye two, 19 (2%) had a rejection in eye two followed by a rejection in eye one.

Table 10: Time between transplantation and rejection episodes in 1,476 patients with bilateral corneal grafts

Time (months) between:	Median	Range
Transplant (date of graft, eye one) and first rejection episode in eye one	54	5-258
Transplant (date of graft, eye two) and first rejection episode in eye two	12	<1-190
First rejection in either eye and first rejection in other eye	15	<1-197
First rejection in eye one and second rejection in eye one	3	1-35
First rejection in eye two and second rejection in eye two	8	<1-124

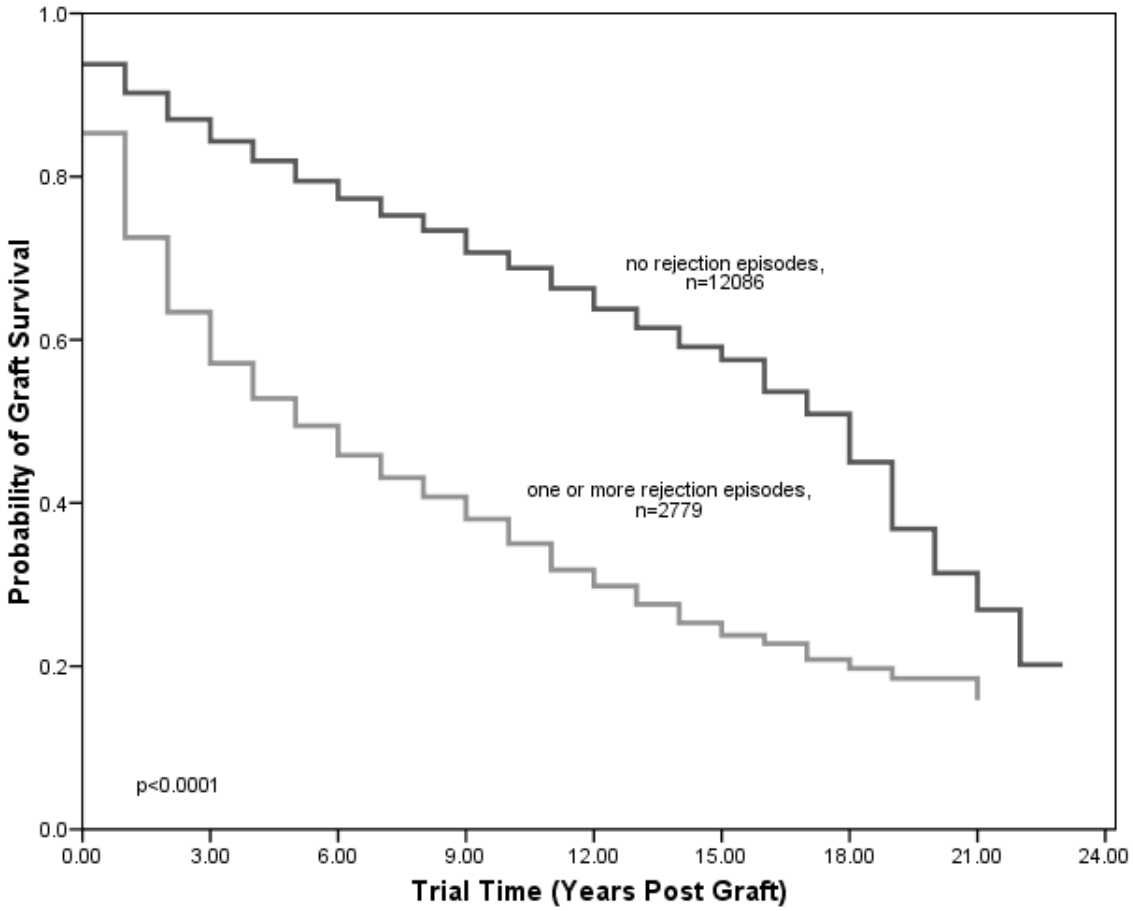
Figure Legends

Figure 1. Kaplan-Meier survival plot of all penetrating corneal grafts with follow-up, stratified according to the occurrence or otherwise of episodes of corneal graft rejection. This univariate analysis was performed on the complete dataset (n=14,865) without patient clustering. The numbers at risk at intervals of 3 years are shown below the plot. The difference between the curves is significant at $p<0.0001$ (log-rank statistic).

Figure 2. Kaplan-Meier survival plot of penetrating corneal grafts in recipients of followed bilateral grafts, stratified according to the occurrence or otherwise of episodes of corneal graft rejection. This univariate analysis was performed on the complete data set (n=2,952) without patient clustering. The numbers at risk at intervals of 3 years are shown below the plot. The difference between the curves is significant at $p<0.0001$ (log-rank statistic).

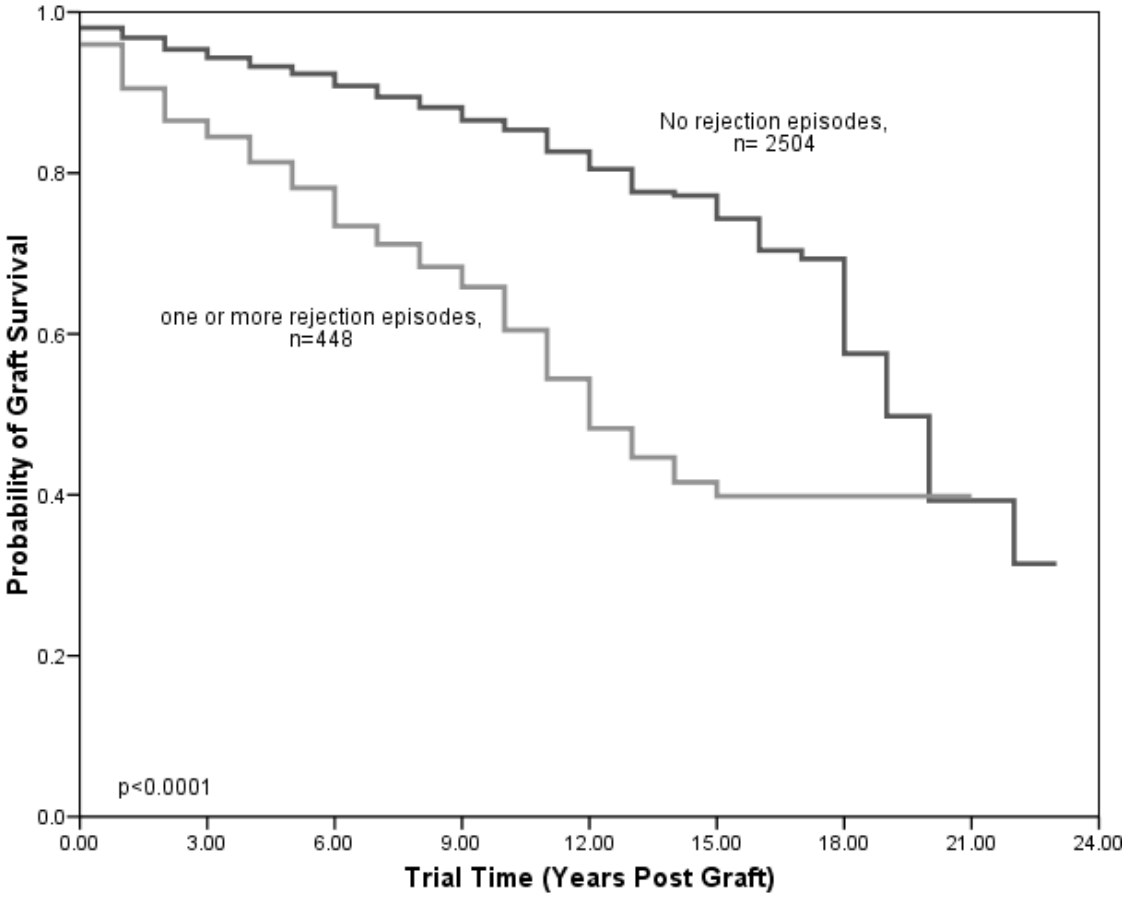
Figure 3. Kaplan-Meier plot of rejection-free survival stratified by first and second eyes separately, with and without previous rejection episodes in the same eye or the opposite eye, for the cohort of recipients with bilateral grafts. After a rejection episode, time to a subsequent rejection in the same eye was analysed as separate event, giving 1,501 at risk for the first eye (Eye 1) and 1,659 for the second eye (Eye 2) to be grafted. The numbers at risk at intervals of three years are shown below the plot (n/a = not applicable). The differences between the curves in each set of plots (Eye 1, Eye 2) are significant at $p< 0.0001$ (log-rank statistic).

Figure 1



Identity	Number at risk, years post graft							
	Initially	3	6	9	12	15	18	21
No rejection episodes	12,086	4,534	2,292	1,169	629	296	95	21
≥1 rejection episodes	2,279	1,232	657	329	161	66	19	7

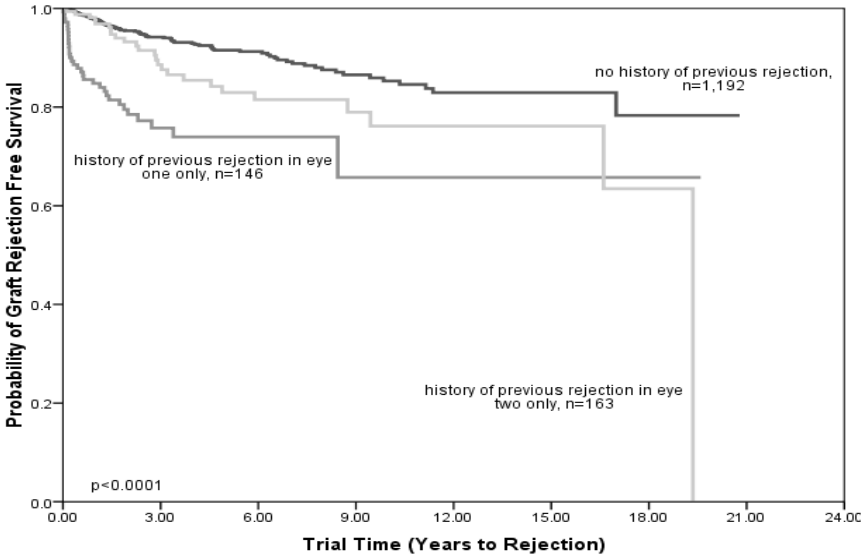
Figure 2



Identity	Number at risk, years post graft							
	Initially	**3**	**6**	**9**	**12**	**15**	**18**	**21**
No rejection episodes	2,504	1,368	793	442	265	135	53	12
≥1 rejection episodes	448	304	198	109	53	24	12	6

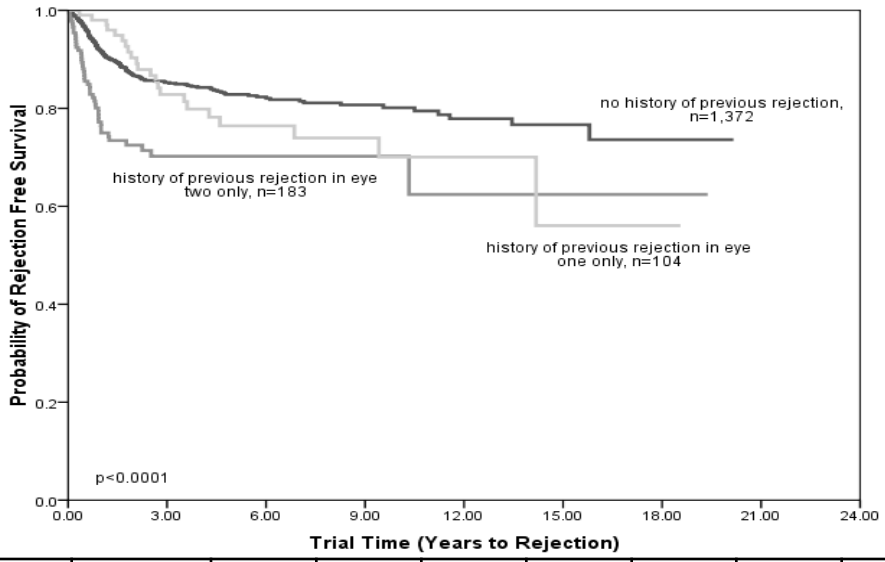
Figure 3

Eye 1



Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
No history of previous rejection	1192	560	290	154	90	38	9	0
History of previous rejection in eye one	146	48	18	7	6	2	1	n/a
History of previous rejection in eye two	163	90	56	31	16	8	2	n/a

Eye 2



Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
No history of previous rejection	1372	572	300	163	90	36	8	1
History of previous rejection in eye two	183	55	29	15	4	2	1	n/a
History of previous rejection in eye one	104	61	38	21	8	4	2	n/a