Risk Factors for Corneal Graft Failure and Rejection in the Collaborative Corneal Transplantation Studies

Maureen G. Maguire, PhD,[1] Walter J. Stark, MD,[1]
John D. Gottsch, MD,[1] R. Doyle Stulting, MD, PhD,[2] Alan Sugar, MD,[3]
Nancy E. Fink, MPH,[1] Ann Schwartz, MD,[4]
The Collaborative Corneal Transplantation Studies Research Group

Purpose: To evaluate comprehensively the magnitude of suspected risk factors for corneal graft failure from any cause, failure from rejection, and immunologic reaction in patients at high risk for graft failure after corneal transplantation.

Methods: The records of the 457 participants in the Collaborative Corneal Transplantation Studies were reviewed. All participants had at least two quadrants of stromal vascularization and/or a history or previous graft rejection. Patients were followed for 2 to 5 years. Characteristics of the patient, study eye, donor, donor–recipient histocompatibility, and surgical procedure were examined for their association with the graft outcomes of failure from any cause, rejection failure, and immunologic reaction. Multivariate survival analysis techniques were used to estimate rates of graft outcome events and to estimate the magnitude of risk factors.

Results: Many apparent risk factors did not maintain their association with graft outcomes after adjustment for other risk factors. Young recipient age, the number of previous grafts, history of previous anterior segment surgery, preoperative glaucoma, quadrants of anterior synechiae, quadrants of stromal vessels, a primary diagnosis of chemical burn, and blood group ABO incompatibility were among the strongest risk factors identified for graft failure. Donor and corneal preservation characteristics had little influence on graft outcome.

Conclusions: Risk of graft failure varies substantially, even within a high-risk population. The number of risk factors present should be considered by the patient and surgeon when contemplating transplantation and planning follow-up.
Ophthalmology 1994;101:1536–1547

Originally received: November 14, 1993.
Revision accepted: April 1, 1994.

[1] The Wilmer Ophthalmologic Institute, Johns Hopkins University, Baltimore.

[2] Emory Eye Center, Emory University, Atlanta.

[3] W. K. Kellogg Eye Center, University of Michigan, Ann Arbor.

[4] University of Wisconsin-Madison, Madison.

For a complete listing of the Collaborative Corneal Transplantation Studies (CCTS) participants, see 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.*

Full-thickness corneal transplantation (penetrating keratoplasty) is one of the most successful forms of trans-

Presented at the Association for Research in Vision and Ophthalmology Annual Meeting, Sarasota, May 1993, and at the American Academy of Ophthalmology Annual Meeting, Chicago, November 1993.

Supported in part through cooperative agreements EY06121, EY07012, EY06158, EY06496, EY06116, EY06155, EY06172, EY06146, and EY06156 with the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

Correspondences to the Collaborative Corneal Transplantation Studies Coordinating Center, 550 N. Broadway, 9th Floor, Baltimore, MD 21205-2010.

plantation. Each year, more than 40,000 patients in the United States undergo corneal transplantation, and clear grafts are maintained in 90% or more of recipients.[1,2] However, for the 10% to 15% of recipients with previous graft failure and/or corneal stromal vascularization, a success rate of 65% or less at 3 years after transplantation has been reported.[3]

The Collaborative Corneal Transplantation Studies (CCTS), a set of two clinical trials sponsored by the National Eye Institute, were initiated in 1985 to investigate whether donor–recipient histocompatibility matching increased the success rate in high-risk patients. Results from the CCTS showed that matching on HLA-A, -B, and -DR and providing corneas from negatively crossmatched donors did not reduce substantially the risk of graft failure from any cause, graft failure from rejection, or episodes of immunologic rejection (reaction).[3] However, high-risk patients receiving a cornea from a blood group ABO-compatible donor were less likely to have graft failure.

Although a number of important questions in corneal transplantation were answered by the CCTS, much remains to be learned about other determinants of graft outcome. The information collected prospectively during the CCTS provides an excellent opportunity to study the influence of known and suspected risk factors in a population known to be at high risk. The objective of this article is to evaluate comprehensively the magnitude of each potential risk factor regarding failure from all causes, failure from rejection, and immunologic reactions.

Patients and Methods

The design and methods of the CCTS have been described in detail in the *Manual of Operations*.[4] The methods also have been summarized in earlier articles.[3,5] Features with direct bearing on the interpretation of the data presented herein are described below.

Patient Selection

Candidates for corneal transplantation were screened at six clinical centers between May 1986 and September 1989. Eyes with two or more quadrants of corneal stromal neovascularization and/or a previous graft rejection were eligible for the CCTS. Patients who were pregnant or who had systemic immunologic disorders, immunosuppressive medications, a need for immediate transplantation, or conditions likely to cause nonrejection graft failure were excluded. Informed consent was obtained, and institutional review boards affiliated with each of the clinical centers approved the study procedures.

Blood samples were obtained during the enrollment visit. Typing for HLA-A, -B, and -DR and blood group ABO was performed at an affiliated histocompatibility laboratory at each of the six centers. The CCTS Central Laboratory (The Johns Hopkins University Immunogenetics Laboratories, Baltimore, Maryland) and the local laboratory each performed lymphocytotoxic antibody screening on two or more occasions. Crossmatch trays of sera from all patients were prepared by the Central Laboratory and distributed to the participating laboratories.

Patients without detectable lymphocytoxic antibodies were assigned to the Antigen Matching Study. The Antigen Matching Study was a prospective, double-masked clinical trial of patients assigned corneas with different levels of HLA matching. All patients in the Antigen Matching Study received corneas from a negatively crossmatched donor. Corneas were allocated without regard to ABO type.

Patients with detectable lymphocytotoxic antibodies were assigned to the Crossmatch Study. The Crossmatch Study was a prospective, randomized, double-masked clinical trial of patients assigned corneas from either positively or negatively crossmatched donors.

Transplantation Procedures

Corneas from hospital, medical examiner, and multiple-organ donors were procured through eye banks and organ-procurement agencies affiliated with the six clinical centers. Blood from donors was typed for HLA and blood group ABO and donor serum was crossmatched with serum from all waiting potential recipients by the local laboratories. The CCTS protocol required corneas to be stored in corneal preservation media within 15 hours of death and transplanted within 120 hours of death. Assignment of corneas to patients in the CCTS was made through use of a computerized algorithm maintained by the United Network for Organ Sharing (Richmond, VA).

The CCTS protocol specified guidelines for operative procedures; however, each surgeon chose procedures as warranted by the patient's condition. Protocol recommendations included use of the smallest possible recipient trephine size between 6.5 and 8.0 mm. The trephine size for cutting the donor cornea was to be 0.5 to 0.7 mm larger than the recipient cut for aphakic eyes and 0.2 to 0.5 mm larger for phakic eyes. Suture technique was at the discretion of the surgeon. Use of viscoelastic substances was optional. However, it was recommended that the anterior chamber be reformed with balanced salt solution, rather than viscoelastic substances, at the end of the procedure. Lensectomy, anterior vitrectomy, and intraocular lens insertion or exchange were performed at the discretion of the surgeon.

Patient Follow-up

Patients were scheduled for examination at 1 and 2 weeks and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24 months after surgery and every 6 months thereafter for a minimum of 2 years and a maximum of 5 years. Interim examinations were performed at the patient's request to evaluate new symptoms, or they were scheduled by clinic staff as warranted by each patient's condition. Each examination included an interim history of ocular and immunologic events and a complete evaluation of the study eye.

All patients were treated with topical steroids after surgery. Dexamethasone ointment was used at bedtime for the first month. The 1.0% prednisolone acetate dosage

was tapered from every 2 hours while awake to once daily at 4 months. At 5 months, prednisolone acetate was discontinued and 0.1% fluorometholone was started with dosage tapered from four times daily to once daily at 7 months and thereafter. Alternatively for phakic eyes, prednisolone acetate therapy could be continued at one drop daily after 4 months. Patients in whom signs of immunologic reactions (defined below) developed were treated with a more intensive immunosuppressive regimen beginning with hourly prednisolone acetate while awake with dexamethasone ointment twice nightly and tapering to the level of steroid therapy in effect at the beginning of the reaction. In addition, patients with signs of a severe reaction immediately received 125 mg methylprednisolone sodium succinate intravenously, followed by prednisone (1 mg/kg) orally for 5 days.

Determination of Outcomes

The clarity of the central 3.5 mm of the donor cornea was considered in evaluating graft clarity. Grafts that did not clear within 10 days of surgery were declared primary donor failures. Grafts that cleared after surgery and later became cloudy for a period of 3 months were declared failures.

Reactions were defined by observed clinical signs of immunologic rejection. A mild graft reaction was defined by the presence of one or more of the following: one to five keratic precipitates, any subepithelial infiltrates, an epithelial rejection line, increased corneal thickness on ultrasonic pachymetry (>0.62 mm more than 6 weeks after surgery or a 10% increase in thickness within a 6-week period or between clinic visits) without an increase in aqueous cells, or an increase in aqueous cells without an increase in thickness. A severe graft reaction was defined as the presence of one or more of the following signs: more than five keratic precipitates, cells in the stroma, an endothelial rejection line, or both increased thickness and increased aqueous cells.

Each graft failure was attributed to immunologic rejection or to other causes by the local CCTS ophthalmologist by considering the entire postoperative course. Each of the two CCTS co-chairmen independently classified the failures through a masked review of a brief summary of the clinical findings. Discrepancies among the three evaluations were openly adjudicated and the final, adjudicated classification was used for data analysis of graft failure attributed to rejection.

Statistical Methods

Data from the 419 patients in the Antigen Matching Study, the 37 patients in the Crossmatch Study, and the 1 patient who had lymphocytotoxic antibodies detected on screening but was never randomized in the Crossmatch Study were used in statistical analyses. The CCTS data as of January 31, 1993, were used in the current report. Characteristics of the patient, study eye, degree of histocompatibility between the donor and recipient, donor, and surgical procedure were selected for examination after review of the results of previous investigations of risk factors for graft reactions and graft failure. Characteristics that had been identified in previous investigations as risk factors or that had strong theoretical arguments for their involvement in graft reaction or failure were examined in this article.

Unadjusted survival probabilities were estimated using the Kaplan–Meier method; differences between subgroups were assessed using the log-rank test.[6,7] Relative risks and adjusted survival probabilities were estimated using the Cox proportional hazards model, with ties in event times handled through use of the discrete logistic model.[8] Final models for adjusted survival probabilities were selected by reviewing the models with the "best" subset of explanatory variables and the models generated with a forward selection strategy.[9] Only explanatory variables associated with a two-sided P value of 0.05 or less were included in final models. For the purpose of comparing the strength and consistency of risk factors across the three graft outcomes in Table 5, risk factors included in any one of the final models were examined after adjusting for the risk factors included in the final model for the other graft outcomes.

Results

Follow-up in the CCTS was nearly complete. Of the 7042 clinic visits scheduled for the 457 patients, 260 (4%) were missed. Because of the staggered enrollment of patients, complete information on graft status was available through 2 years on 95% of patients, through 3 years on 75% of patients, through 4 years on 47% of patients, and through 5 years on 20% of patients.

Each characteristic considered to be a potential risk factor for graft rejection or failure was first examined by reviewing Kaplan–Meier survival curves. Three-year rates for graft failure from any cause, graft failure attributed to rejection, and immunologic reaction for subgroups based on characteristics of the recipient are presented in Table 1. Rates for recipients younger than 40 years of age were substantially higher than for older recipients for all three graft outcomes. Young recipients had approximately twice as many failures from all causes and twice as many failures from rejection ($P = 0.0001$). Black recipients had somewhat higher rates of graft reaction ($P = 0.03$) but had failure rates similar to white recipients. Male recipients had a higher rate of failure from any cause than female recipients ($P = 0.04$) but had similar reaction and rejection failure rates. Current smokers had a substantially higher reaction rate ($P = 0.0001$) than nonsmokers and a higher rate of failure from all causes ($P = 0.02$), but had a similar rate of rejection failure. Recipients with the potential for exposure to foreign antigens through pregnancies and transfusions had a lower failure rate than recipients without such exposures ($P = 0.04$) but had similar rates of immunologic reaction and rejection failure. The small percentage of recipients with detectable preformed lymphocytotoxic antibodies did not have an elevated rate of reaction but did have higher rates of failure from any

Table 1. Three-year Rates* of Any Failure, Rejection Failure, and Reaction by Characteristics of the Recipient

Characteristic	No.	Any Failure (%)	Rejection Failure (%)	Reaction (%)
Age (yrs)				
10–39	113	56	41	81
40–59	113	30	20	68
60–69	96	31	21	70
70–89	135	30	19	51
		(0.0001)	(0.0001)	(0.0001)
Race				
White and other	388	37	24	65
Black	69	36	31	72
		(0.53)	(0.08)	(0.03)
Sex				
Male	253	41	26	67
Female	204	32	23	66
		(0.04)	(0.58)	(0.36)
Current smoking				
No	345	34	24	62
Yes	112	46	27	69
		(0.02)	(0.45)	(0.0001)
Pregnancies and transfusions				
0	257	41	27	69
1	92	36	25	66
≥2	107	27	19	61
		(0.04)	(0.65)	(0.25)
Detectable antibodies				
No	419	36	24	66
Yes	38	47	35	68
		(0.04)	(0.05)	(0.73)
Clinical center				
A	136	43	28	78
B	86	16	12	56
C	82	32	22	55
D	66	50	31	73
E	53	48	35	88
F	34	52	34	65
		(0.0001)	(0.02)	(0.0006)
Waiting time (mos)				
≤4	164	37	24	66
5–8	195	33	21	64
9–18	98	45	31	73
		(0.15)	(0.09)	(0.12)

* As estimated by the Kaplan–Meier method. *P* values (in parentheses) are based on the log-rank statistics.

cause and failure from rejection ($P \leq 0.05$). Rates for all three graft outcomes varied markedly across clinical centers with as much as a threefold difference between the clinic with the lowest rates and the clinic with the highest rates. Graft outcome rates were similar for recipients with different amounts of waiting time between CCTS enrollment and transplantation.

Three-year rates for graft outcomes for subgroups based on ocular characteristics of the recipient are presented in Table 2. Patients with herpes keratitis or aphakic or pseu-

Table 2. Three-year Rates* of Any Failure, Rejection Failure, and Reaction by Characteristics of the Eye

Charactistic	No.	Any Failure (%)	Rejection Failure (%)	Reaction (%)
Diagnosis				
Aphakic and pseudophakic corneal edema	117	36	23	65
Herpes keratitis	84	33	27	63
Trauma	43	40	30	70
Keratoconus	40	36	32	75
Chemical burn	39	63	29	75
Corneal ulcer	37	37	22	79
Other	97	30	19	60
		(0.002)	(0.29)	(0.008)
Previous grafts				
0	129	17	8	53
1	171	37	24	70
2	90	53	41	71
≥3	67	53	38	77
		(0.0001)	(0.0001)	(0.0007)
Stromal vessels (quadrants)				
0, 1	81	31	25	71
2, 3	155	32	20	66
4	220	43	28	65
		(0.006)	(0.15)	(0.99)
Anterior synechia (quadrants)				
0	318	31	22	65
1, 2	76	40	29	66
3, 4	62	62	34	79
		(0.0001)	(0.02)	(0.58)
Glaucoma, preoperative				
No	270	29	19	63
Yes	187	48	33	72
		(0.0001)	(0.003)	(0.12)
Natural lens, preoperative				
Absent	267	44	20	67
Present	190	27	28	66
		(0.0003)	(0.05)	(0.87)
Previous surgery, other than graft				
None	122	21	15	61
One or more	335	43	29	69
		(0.0001)	(0.004)	(0.22)

* As estimated by the Kaplan-Meier method. P values (in parentheses) are based on the log-rank statistic.

dophakic keratopathy as a primary diagnosis were among those with the lowest rates of reaction, whereas patients with corneal ulcers, chemical burns, or keratoconus were among those with the highest reaction rates ($P = 0.008$). Eyes with chemical burns had the highest rate of failure from all causes ($P = 0.002$). However, the rate of rejection failure varied relatively little among the primary diagnostic groups. Eyes that had previous grafts were at higher risk for all three outcomes. The rate of failure from any cause increased from 17% with no previous grafts to 53% with two or more previous grafts ($P = 0.0001$) and the rate of rejection failure similarly increased from 8% to approximately 40% ($P = 0.0001$). The rates of reaction and of rejection failure did not increase substantially with in-

Table 3. Three-year Rates* of Any Failure, Rejection Failure, and Reaction by Histocompatibility Characteristics of the Recipient and Donor

Characteristic	No.	Any Failure (%)	Rejection Failure (%)	Reaction (%)
HLA-A, B mismatch				
0, 1 antigens	140	32	20	67
2–4 antigens	317	39	27	66
		(0.22)	(0.15)	(0.61)
HLA-DR mismatch				
0 antigens	201	36	24	65
1, 2 antigens	245	37	25	67
		(0.38)	(0.74)	(0.55)
HLA-B, DR mismatch				
0, 1 antigens	118	38	25	60
2–4 antigens	337	36	25	69
		(0.49)	(0.59)	(0.04)
Blood group ABO				
Compatible	318	34	21	66
Incompatible	137	44	32	69
		(0.07)	(0.01)	(0.31)
Donor and recipient race				
Same	336	39	26	69
Different	121	31	20	66
		(0.44)	(0.29)	(0.16)
HLA-DR6 recipient				
No	363	36	24	66
Yes	94	41	25	69
		(0.16)	(0.40)	(0.28)
HLA-A2 donor				
No	364	36	22	67
Yes	93	41	33	66
		(0.57)	(0.06)	(0.71)

HLA = human leukocyte antigen.

* As estimated by the Kaplan–Meier method. *P* values (in parentheses) are based on the log-rank statistic.

creased quadrants of stromal vascularization. Patients with four quadrants of vascularization did have a higher rate of failure from any cause than patients with less vascularization ($P = 0.006$). The rate of failure from any cause doubled if the eye had three or four quadrants of anterior synechiae from 31% to 62% ($P = 0.0001$) and the rate of rejection failure increased less sharply from 22% to 34% ($P = 0.02$). Eyes with a preoperative designation of glaucoma had higher rates of rejection failure and of failure from any cause. Eyes that had had previous anterior segment surgery, other than a previous graft, were at approximately twice the risk for both failure from any cause and rejection failure.

Three-year rates for graft outcomes for subgroups based on histocompatibility characteristics of the donor and recipient are presented in Table 3. Overall, there was little variation in the outcome rates in the subgroups of patients with different levels of histocompatibility. Recipients of corneas from blood group ABO-compatible donors had rates of failure from any cause and of rejection failure that were approximately 10% lower than rates of recipients with ABO-incompatible donors.

Three-year rates for graft outcomes for subgroups based on characteristics of the donor and surgical procedure are presented in Table 4. There was little systematic variation in outcome rates by donor age, donor race, or times from donor death to corneal preservation or donor death to surgery. Eyes with donor corneas less than 8.0 mm in diameter had a higher rate for all three graft outcomes than eyes with larger grafts ($P = 0.0001$). Eyes with interrupted sutures had a higher rate for all three graft outcomes than eyes with running sutures or a combination of running and interrupted sutures ($P \leq 0.002$). The rate of failure from any cause was higher in eyes in which

Table 4. Three-year Rates* of Any Failure, Rejection Failure, and Reaction by Characteristics of the Donor and Surgery

Characteristic	No.	Any Failure (%)	Rejection Failure (%)	Reaction (%)
Donor age (yrs)				
≤29	212	36	25	66
30–49	143	35	22	66
50–70	101	41	28	68
		(0.60)	(0.70)	(0.96)
Donor race				
White and other	377	37	24	67
Black	80	36	26	65
		(0.84)	(0.98)	(0.82)
Donor cornea size (mm)				
<8.0	115	51	39	88
8.0	206	35	21	58
>8.0	136	27	19	60
		(0.0001)	(0.0001)	(0.0001)
Time to preservation (hrs)				
≤1	103	41	29	74
2–5	197	31	18	64
6–19	157	41	30	64
		(0.08)	(0.04)	(0.42)
Time to surgery (hrs)				
≤48	119	37	24	65
49–72	151	36	27	69
73–96	112	40	22	60
97–152	75	36	24	73
		(0.85)	(0.81)	(0.28)
Suture technique				
Interrupted only	230	48	31	76
Running only	120	26	16	65
Both	107	24	20	52
		(0.0001)	(0.002)	(0.0002)
Viscoelastic substances				
No	129	45	29	63
Yes	328	34	23	68
		(0.04)	(0.33)	(0.94)

* As estiamted by the Kaplan-Meier method. *P* values (in parentheses) are based on the log-rank statistic.

viscoelastic substances had been used during surgery ($P = 0.04$).

Many of the potential risk factors highlighted above were highly correlated in participants in the CCTS. For example, the eyes with several previous grafts were more likely than other eyes to have four quadrants of stromal vessels. Because of the interdependencies between the many potential risk factors considered, multivariate survival analyses were performed to identify independent risk factors.

Ten different risk factors were included in the final model of time to failure from any cause (Table 5). Recipient age younger than 40 years was the strongest risk factor identified with a risk ratio of 2.5 (Fig 1; $P = 0.0001$). Eyes with chemical burns had a risk ratio for failure of 1.8 (Fig 2; $P = 0.02$); the absence of association with graft reactions and rejection failure implicate nonrejection causes of failure such as epithelial defect. Each additional previous graft was associated with increased risk of failure (Fig 3); an eye with two previous grafts had a risk ratio of 1.44 com-

Table 5. Adjusted Relative Risks and 95% Confidence Intervals for Risk Factors for Graft Outcomes

Risk Factor	Any Failure			Rejection Failure			Reaction		
	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
Recipient age <40 yrs	2.50*	(1.75, 3.58)	0.0001	2.97*	(1.93, 4.57)	0.0001	2.40*	(1.58, 280)	0.0001
Current smoking	1.31	(0.93, 1.85)	0.13	1.05	(0.66, 1.66)	0.83	1.61*	(1.22, 2.11)	0.0006
Chemical burn	1.78	(1.09, 2.88)	0.02	1.04	(0.52, 2.10)	0.91	1.09	(0.69, 1.72)	0.72
Previous grafts, each additional	1.20*	(1.09, 1.32)	0.0002	1.27*	(1.14, 1.42)	0.0001	1.15*	(1.06, 1.26)	0.002
Stromal vessels, quadrants	1.14*	(1.01, 1.28)	0.03	1.08	(0.93, 1.24)	0.34	0.98	(0.90, 1.06)	0.59
Anterior synechia, quadrants	1.19*	(1.07, 1.32)	0.001	1.13	(0.98, 1.30)	0.09	0.98	(0.89, 1.07)	0.61
Glaucoma, preoperative	1.58*	(1.14, 2.21)	0.007	1.46	(0.96, 2.22)	0.08	1.19	(0.92, 1.55)	0.19
Previous surgery	2.16*	(1.37, 3.39)	0.001	2.62*	(1.55, 4.44)	0.0003	1.34*	(1.00, 1.81)	0.05
ABO incompatible	1.37*	(1.00, 1.89)	0.05	1.54*	(1.04, 2.30)	0.03	1.02	(0.79, 1.33)	0.86
HLA-DR6 recipient	1.44*	(1.00, 2.09)	0.05	1.23	(0.76, 1.99)	0.50	1.18	(0.87, 1.59)	0.28
HLA-A2 donor	0.99	(0.68, 1.45)	0.96	1.64*	(1.05, 2.55)	0.03	1.17	(0.87, 1.58)	0.30
Donor cornea <8.0 mm	1.10	(0.76, 1.59)		1.71*	(1.10, 2.67)		1.76*	(1.31, 235)	
Donor cornea >8.0 mm	0.90	(0.59, 1.39)	0.70	0.84*	(0.50, 1.42)	0.01	1.12*	(0.83, 1.51)	0.0006
Interrupted sutures	1.46*	(0.96, 2.22)		0.92	(0.56, 1.52)		1.48	(0.90, 1.88)	
Running and interrupted sutures	0.72*	(0.42, 1.24)	0.004	0.55	(0.28, 1.06)	0.16	1.30	(1.07, 2.04)	0.06

RR = relative risk; CI = confidence interval, HLA = human leukocyte antigen.

* Risk factor included in final multivariate Cox model. All relative risk estimates have been adjusted for the risk factors included in the final model.

pared with an eye with no previous grafts (multiplicative risk for each previous graft). Each quadrant of anterior synechiae was associated with approximately the same degree of excess risk as an additional previous graft (Table 5; Fig 3). Risk of graft failure increased with additional quadrants of stromal vascularization, but to a more modest degree (risk ratio of 1.1 per quadrant; Fig 4). Patients designated as having glaucoma before surgery, as determined by review of their medications and ocular history, had a risk ratio of 1.6 (Fig 5; $P = 0.007$). Previous ocular surgery, such as lensectomy or surgery for glaucoma, was associated with a large risk ratio of 2.2 (Fig 6; $P = 0.001$). Both blood group ABO incompatibility and the presence of HLA-DR6 in the recipient were associated with a modest increase in risk of failure from any cause (risk ratio of 1.4; Fig 7), but the risk for rejection failure was elevated only for ABO incompatibility (risk ratio of 1.5). Eyes for which the surgeon chose to use only interrupted sutures were at higher risk of failure than eyes in which running sutures were used; eyes with both running and interrupted sutures had less risk of failure (Fig 8).

Six different risk factors were included in the final model of time to rejection failure (Table 5). Young recipient age, previous grafts, previous anterior segment surgery, and ABO incompatibility were identified as influential risk factors for failure from any cause and were more strongly associated with rejection failure, as evidenced by larger risk ratios and lower *P* values. Patients with HLA-A2 donors had a greater risk of rejection failure, although they were at no excess risk of graft reaction or graft failure from any cause. Surgeons chose to use a 8.0-mm donor graft for almost half of the patients (Table 4). Eyes that received smaller grafts were at increased risk of rejection failure (risk ratio, 1.7), whereas eyes that received larger grafts were at decreased risk (risk ratio, 0.8).

Five different risk factors were included in the final model of time to reaction (Table 5). Young recipient age was again the strongest risk factor (risk ratio, 2.4). Previous grafts and previous anterior segment surgery were identified as influential risk factors for rejection failure and were identified as more modest risk factors for graft reaction. Patients who reported being a regular cigarette smoker at enrollment into the CCTS were more likely to have a graft reaction; however, the excess risk of graft reaction did not translate into an excess risk of rejection failure. Eyes that received donor grafts of less than 8.0 mm were associated with a greater risk of reaction (risk ratio, 1.8) that did translate into an excess risk of rejection failure.

There was no effect of the clinical center of the recipient on the risk of graft failure, graft rejection, or graft reaction after the adjustment procedures described above ($P > 0.30$). The marked variation in event rates among the six clinical centers was attributable to differences in the risk profiles of the patients managed at the centers.

Discussion

The data collected prospectively during the CCTS have provided important information on graft survival during

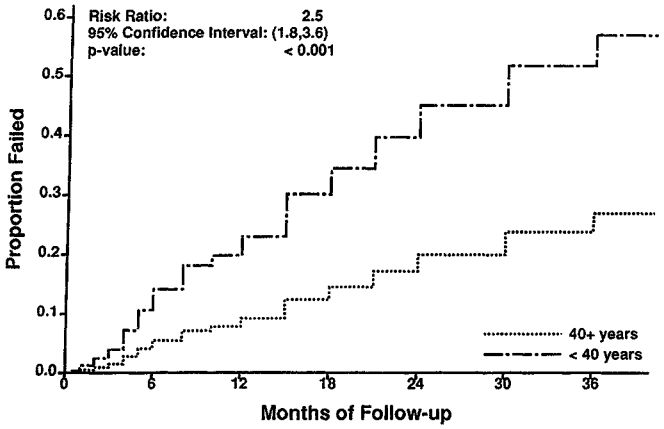

Figure 1. Adjusted failure rates through 3 years by recipient age.

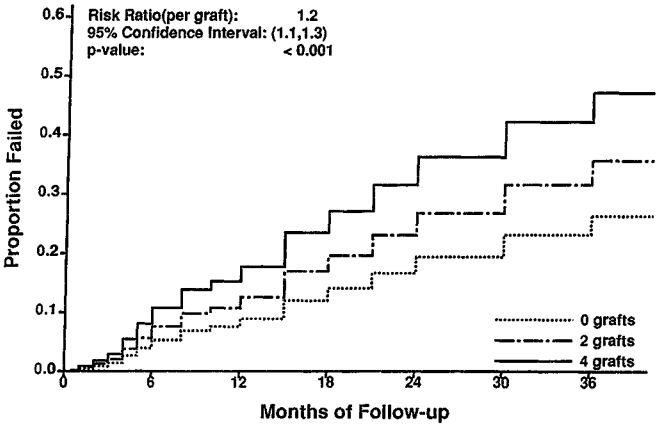

Figure 3. Adjusted failure rates through 3 years by number of previous grafts. Graphs by quadrants of anterior synechiae are nearly identical.

the first 5 postoperative years in high-risk patients. For this report, data from both the Antigen Matching Study and the Crossmatch Study have been pooled and information incorporated from results of the final patient examinations completed after the January 1992 cutoff for the publication of the CCTS main results.[3]

Results of examination of the many potential risk factors for each of the three main graft outcomes under study showed substantial variation in event rates in many of the subgroups (Tables 1–4). Such differences in event rates may be attributable to a true causal relation between the risk factor and the outcome, an apparent association between the risk factor and the outcome induced by correlation of the factor with a true causal factor, or by chance variation. Multivariate analysis techniques, biologic plausibility, consistency of the relation for related outcomes, and replication of findings help distinguish the true causal relations from the artifactual relations. The statistical significance of the association helps rule out chance variation.

Recipients younger than 40 years of age had an elevated risk of graft reaction, rejection failure, and failure from any cause both when considered without regard to other factors (Table 1) and when considered with other factors in the Cox model (risk ratios, ≥2.3; $P = 0.0001$). Risk decreased abruptly between the fourth and fifth decades of life (Table 2). The immune system is known to become impaired with age but there is little basis for an abrupt change at 40 years of age. Musch and Meyer[10] and Boisjoly et al[11] both reported younger age to be a significant risk factor for graft reaction under multivariate models from consecutive series of both high- and low-risk recipients. However, age was not identified as an independent risk factor for graft failure from any cause in other large population series in Australia and The Netherlands[12–14] nor for rejection failure in a small clinical trial involving only high-risk United States patients.[15] Contrary to the CCTS results, *older* age was found to be a risk factor for graft failure under univariate analysis in a large retrospective review of more than 2800 Spanish patients, even when analysis was limited to high-risk patients.[16] However, the large risk ratios and associated high statistical significance in the CCTS population as well as the replication of this finding of excess risk in younger recipients in two other recent prospective studies lead us to the conclusion that younger high-risk patients are at especially high risk for rejection failure.

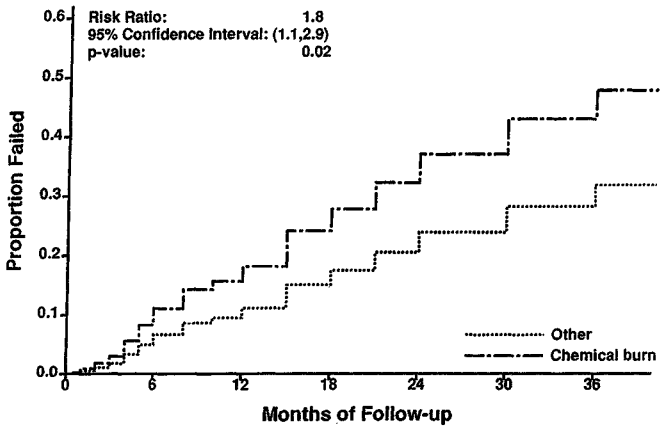

Figure 2. Adjusted failure rates through 3 years by primary diagnosis.

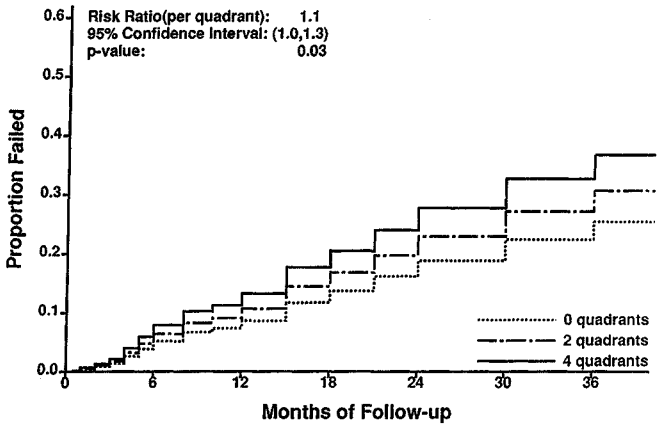

Figure 4. Adjusted failure rates through 3 years by number of quadrants of stromal vessels.

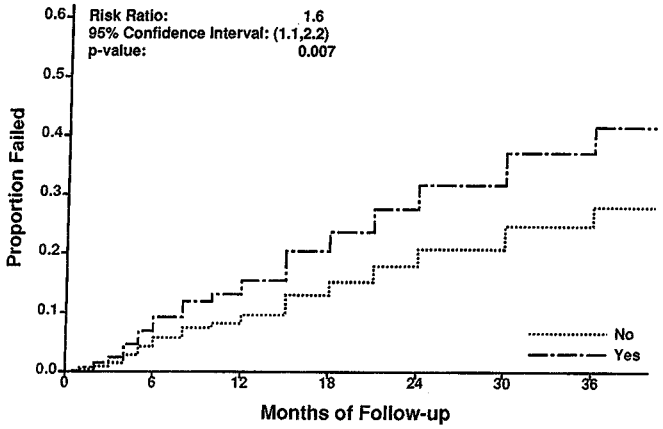

Figure 5. Adjusted failure rates through 3 years by number with history of glaucoma.

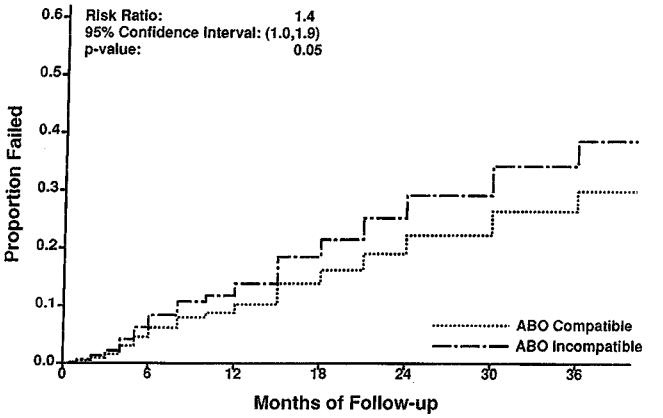

Figure 7. Adjusted failure rates through 3 years by blood group ABO compatibility. Graphs by presence of HLA-DR6 in the recipient are nearly identical.

The eligibility criteria for the CCTS required that all patient eyes have two or more quadrants of stromal vascularization and/or a history of previous graft rejection because these characteristics had been implicated in many early studies of prognostic factors for graft rejection. Approximately half of the CCTS participants had both previous grafts and two or more quadrants of vascularization. Even within the CCTS group composed of high-risk recipients only, the number of previous grafts proved to be a strong risk factor for all three graft outcomes with each additional graft increasing risk by a factor of approximately 1.2 (Tables 2 and 5). Stromal vascularization was a weaker risk factor, achieving statistical significance for only graft failure from any cause. Although the increased risk per quadrant is modest, patients with four quadrants of vessels have an estimated risk ratio of graft failure of 1.7 compared with patients with no vessels.

Previous anterior segment surgery, other than penetrating keratoplasty, was strongly associated with all three graft outcomes (Tables 2 and 5). Approximately one third of the patients in the CCTS with previous ocular surgery had only had their lenses removed; the remainder had had vitrectomy, procedures to control intraocular pressure, or other procedures, usually in addition to lensectomy. Previous anterior segment surgery has not been studied extensively as a risk factor for graft rejection and failure, but was implicated as a risk factor in analysis of the Australian corneal transplant registry.[12,13]

Preoperative glaucoma and anterior synechiae at the time of surgery each were associated with increased risk of graft failure from any cause (Table 5). Both of these conditions, which often lead to problems in controlling postoperative intraocular pressure, have been identified in previous studies as predisposing to graft failure but generally not predisposing to graft reaction.[17–21] In addition, anterior synechiae expose the donor endothelium to blood vessels that may increase the risk of rejection or may cause increased loss of endothelial cells through direct traction or indirectly through inflammation.

Although the prognosis of first grafts into avascular corneal beds is known to vary by the primary diagnosis,[2,22–24] among the CCTS high-risk patients, the only primary diagnosis identified with a worse prognosis was chemical burn (Tables 2 and 5).[21] Patients with chemical burns did not have a greater risk of graft reaction and

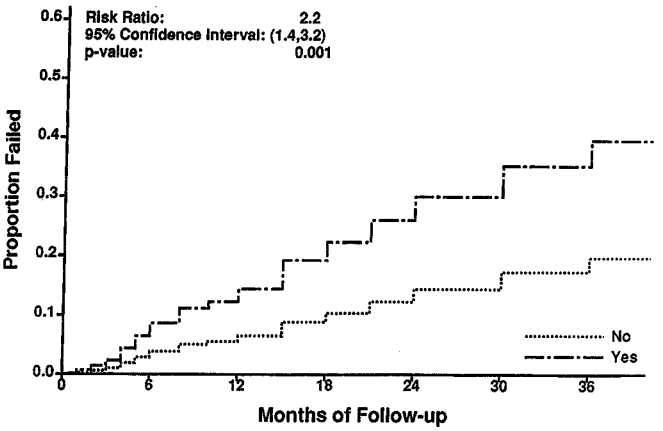

Figure 6. Adjusted failure rates through 3 years by previous anterior segment surgery.

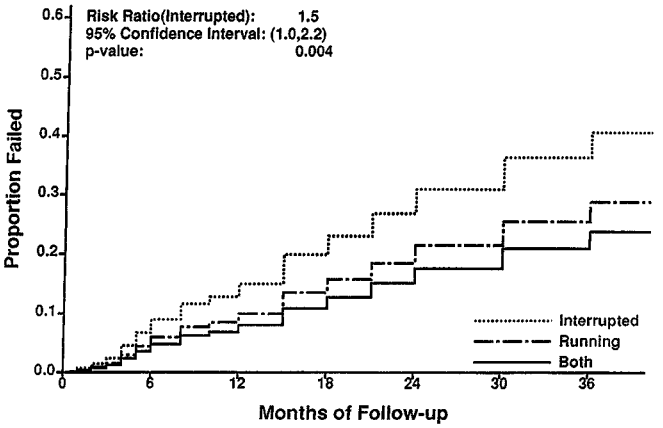

Figure 8. Adjusted failure rates through 3 years by suture technique.

rejection failure, but were at increased risk of failure from epithelial defects.

As reported previously,[3] blood group ABO incompatibility was associated with an increased risk of rejection failure as well as failure from any cause. The possible biologic mechanisms and lack of agreement with other large studies of graft failure have been discussed at length in the previous publication.[3] Two particular histocompatibility antigens, HLA-DR6 in the recipient and HLA-A2 in the donor, have been cited as risk factors for rejection in renal transplantation.[25] The risk ratio for graft failure from any cause for recipients in the CCTS with HLA-DR6 was elevated (1.4; $P = 0.05$) but the association was weaker for rejection failure (risk ratio, 1.2; $P = 0.50$). The risk ratio of rejection failure was elevated for recipients of corneas from HLA-A2 donors (1.6; $P = 0.03$), but the excess risk of irreversible rejection did not translate into an overall risk of graft failure (Table 2). Other studies in corneal transplantation have not been able to examine these two factors because of small population size and/or low event rates.

Interpretation of risk factors that are subject to manipulation by the surgeon is particularly difficult. Both donor size and suture technique were selected by the surgeon in the CCTS. Theoretically, larger grafts might increase the risk of rejection by presenting a greater antigen load closer to the recipient limbal vasculature, whereas one might predict that suture technique would have relatively little effect on graft outcome.

Interestingly, smaller grafts were at greater risk of rejection in the CCTS, and interrupted sutures were associated with graft failure. Some previous studies have found that larger grafts are more likely to be rejected,[11,14] consistent with theoretical predictions. Others have found that smaller grafts are more likely to fail[15] or that grafts that are *either* smaller or larger than average are more likely to fail.[12] We suspect that CCTS surgeons were more likely to use small grafts and interrupted sutures in patients with risk factors that were recognized by the surgeon but not accounted for by the factors included in the multivariate Cox regression model.

Most characteristics of the patient, clinical center, donor, and histocompatibility match had little influence on graft outcome. In addition to the strong effect of young age, the most influential factors for graft failure in the CCTS were previous grafts, previous anterior segment surgery, preoperative glaucoma, anterior synechiae, stromal vessels, and a primary diagnosis of chemical burn. Costly histocompatibility matching did not substantially improve graft survival, although selection of corneas from ABO-compatible donors appears promising. The data from participants in the CCTS should be of great interest to patients and surgeons who want to estimate the likelihood of successful corneal transplantation in high-risk eyes.

References

1. Activity Report, 1992. Washington, DC: Eye Bank Association of America. 1992. Available from: Eye Bank Assoc. of America, 1725 Eye St., N.W., Suite 308, Washington, DC 20006-2403.
2. Price FW Jr, Whitson WE, Marks RG. Graft survival in four common groups of patients undergoing penetrating keratoplasty. Ophthalmology 1991; 98:322–8.
3. 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; 110:1392–403.
4. Coordinating Center for the Collaborative Corneal Transplantation Studies (CCTS). Manual of Operations, 1987. Available from: National Technical Information Service. (#PB 90-134925).
5. The Collaborative Corneal Transplantation Studies Research Group. Design and methods of the Collaborative Corneal Transplantation Studies. Cornea 1993; 12:93–103.
6. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457–81.
7. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966; 50:163–70.
8. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York: John Wiley & Sons, 1980; 37.
9. SAS/STAT Software: The PHREG Procedure. Version 6. Cary, NC: SAS Institute, 1991. (SAS Tech Rep; P-217)
10. Musch DC, Meyer RF. Risk of endothelial rejection after bilateral penetrating keratoplasty. Ophthalmology 1989; 96: 1139–43.
11. Boisjoly HM, Bernard P-M, Dubé I, et al. Effect of factors unrelated to tissue matching on corneal transplant endothelial rejection. Am J Ophthalmol 1989; 107:647–54.
12. Williams KA, Roder D, Esterman A, et al. Factors predictive of corneal graft survival. Report from the Australian Corneal Graft Registry. Ophthalmology 1992; 99:403–14.
13. Williams KA, Muehlberg SM, Wing SJ, Coster DJ. The Australian Corneal Graft Registry. 1990 to 1992 report. Aust N Z J Ophthalmol 1993; 21: Suppl 2.
14. Volker-Dieben HJ, D'Amaro J, Kok-Van Alphen CC. Hierarchy of prognostic factors for corneal allograft survival. Aust N Z J Ophthalmol 1987; 15:11–18.
15. Sanfilippo F, MacQueen JM, Vaughn WK, Foulks GN. Reduced graft rejection with good HLA-A and B matching in high-risk corneal transplantation. N Engl J Med 1986; 315: 29–35.
16. Barraquer RI, Kargacin M. Prognostic factors in penetrating keratoplasty. In: Draeger J, Winter R, eds. New Microsurgical Concepts II: Cornea, Posterior Segment, External Microsurgery. Basel: Karger, 1989; 165–71. (Dev Ophthalmol; 18).
17. Bourne WM. Reduction of endothelial cell loss during phakic penetrating keratoplasty. Am J Ophthalmol 1980; 89:787–90.
18. Tragakis MP, Brown SI. The significance of anterior synechiae after corneal transplantation. Am J Ophthalmol 1972; 74:532–3.
19. Owens WC. Symposium: corneal transplantation. V. Results. VI. Discussion. Trans Am Acad Ophthalmol Otolaryngol 1948; 52:341–53.
20. Foulks GN. Glaucoma associated with penetrating keratoplasty. Ophthalmology 1987; 94:871–4.
21. Robinson CH, Jr. Indications, complications, and prognosis for repeat penetrating keratoplasty. Ophthalmic Surg 1979; 10(5):27–34.

22. Epstein RJ, Seedor JA, Dreizen NG, et al. Penetrating keratoplasty for herpes simplex keratitis and keratoconus. Allograft rejection and survival. Ophthalmology 1987; 94:935–44.
23. Wilson SE, Kaufman HE. Graft failure after penetrating keratoplasty. Surv Ophthalmol 1990; 34:325–56.
24. Mader TH, Stulting RD. The high-risk penetrating keratoplasty. Ophthalmol Clin North Am 1991; 4:411–26.
25. Hendricks GF, Schreuder GM, D'Amaro J, van Rood JJ. The regulatory role of the HLA-DRw6 in renal transplantation. Tissue Antigens 1986; 27:121–30.

Discussion
by
Bartly J. Mondino, MD

The authors reviewed the records of 457 participants in the Collaborative Corneal Transplantation Studies (CCTS). All participants had at least two quadrants of stromal vascularization and/or a history of previous graft rejection. Patients with corneal grafts were followed between 2 and 5 years for three outcomes: immunologic reaction, rejection failure, and failure from any cause. Multivariate survival analysis techniques were used to estimate rates of graft outcome events and to estimate the magnitude of suspected risk factors. The strongest risk factors identified for graft failure were the following: young recipient age, number of previous grafts, history of previous anterior segment surgery, preoperative glaucoma, quadrants of anterior synechiae, quadrants of stromal vessels, a primary diagnosis of chemical burn, and blood group ABO incompatibility. There are other risk factors such as socioeconomic status that may be important and were not analyzed.

One potential problem of this type of study is "confounding by indication."[1] Although the CCTS protocol specified guidelines for surgery, each surgeon could deviate from the protocol recommendations if the patient's condition warranted such action. This approach may result in "confounding by indication." Because the surgeon may choose donor corneal size, suture technique, and use of viscoelastic substances, eyes with the worst prognosis may receive a smaller donor cornea, interrupted sutures, and viscoelastic substances.

The authors found that the rate of failure from any cause was higher in eyes in which viscoelastic substances had been used during surgery. However, the relation of viscoelastic substances to graft failure is not clear. Perhaps, the patients with the more complicated conditions, who may be more likely to have problems later, require viscoelastic substances during surgery. The use of viscoelastic substances did not make it as a risk factor in the final model.

Another curious result was that eyes with interrupted sutures had a higher rate for graft failure from any cause than eyes with running sutures or with a combination of running and interrupted sutures. Could the interrupted suture technique have been preferred by surgeons for more difficult cases? If so, this could help explain the higher failure rate. It is not clear why eyes with interrupted sutures were at higher risk of failure than eyes with both running and interrupted sutures. The protective effect of a running suture when used with interrupted sutures is not clear to me.

The finding that donor grafts that were less than 8.0 mm were subject to more immunologic reactions and rejection failures than donor grafts greater than 8.0 mm is curious. One would suspect that larger donor grafts may increase the chance of graft rejection by providing a greater number of donor antigens closer to the limbus than smaller grafts. Perhaps, there was a surgical bias toward using smaller grafts in eyes thought to have a greater chance of rejection. The authors analyzed the influence of donor grafts less than 8.0 mm and greater than 8.0 mm. What were the results for donor corneas that were equal to 8.0 mm?

Donor cornea size, suture technique, and use of viscoelastic substances were at the discretion of the surgeon so that "confounding by indication" may be a problem. To determine conclusively whether these are risk factors, randomized clinical trials would be necessary.

In summary, the authors have presented a well-designed study of great interest and importance to corneal transplantation surgeons operating on high-risk eyes. Many suspected and reported high-risk factors[2–6] were confirmed. The authors should analyze the clinical data at 4 and 5 years just as they did for 3 years.

From the Department of Ophthalmology, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles.

References

1. Rothman KJ. Modern Epidemiology. Boston: Little, Brown, 1986.
2. Boisjoly HM, Bernard P-M, Dubé I, et al. Effect of factors unrelated to tissue matching on corneal transplant endothelial rejection. Am J Ophthalmol 1989; 107:647–54.
3. Price FW Jr, Whitson WE, Marks RG. Graft survival in four common groups of patients undergoing penetrating keratoplasty. Ophthalmology 1991; 98:322–8.
4. Robinson CH Jr. Indications, complications, and prognosis for repeat penetrating keratoplasty. Ophthalmic Surg 1979; 10(5):27–34.
5. Tragakis MP, Brown SI. The significance of anterior synechiae after corneal transplantation. Am J Ophthalmol 1972; 74:532–3.
6. Williams KA, Roder D, Esterman A, et al. Factors predictive of corneal graft survival. Report from the Australian Corneal Graft Registry. Ophthalmology 1992; 99:403–14.