Chapter 4.2

Validation of tissue quality parameters for donor corneas, designated for emergency cases: corneal graft survival

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Wilhelmina J Rijneveld, M.D. [1,2]
Rachel Wolff, M.D.[3]
Hennie JM Völker-Dieben, M.D., Ph.D.[4]
Elisabeth Pels, Ph.D.[1]

1 Cornea Bank Amsterdam, department of Euro Tissue Bank, Beverwijk, The Netherlands
2 Westfriesgasthuis, Hoorn, The Netherlands
3 Academisch Medisch Centrum, Amsterdam, The Netherlands
4 Emeritus Professor of Ophthalmology, Vrije Universiteit, Amsterdam, The Netherlands

Abstract

Purpose

To validate tissue quality parameters for donor corneas designated for emergency grafting for corneal graft survival.

Methods

In a longitudinal cohort follow-up study, 131 emergency penetrating grafts were studied. Grafts were performed with a pool of organ cultured donor corneas designated for emergency grafting and prepared for immediate use with all safety tests performed. Assignation criteria were: corneas with a small superficial stromal opacity but meeting all selection criteria for PKP tissue and corneas without stromal opacity, but an endothelial cell density from 1800-2300 cells/mm^2 or mild polymegathism or pleomorphism. Cox multivariate regression analysis, Kaplan Meier survival and log rank test were applied.

Results

Of the 131 keratoplasties, 115 could be followed. One eye was lost during surgery because of an expulsive bleeding. In 15 cases a conjunctival transplantation finished off the penetrating graft. Corneal graft survival was not significantly related to the presence of PKP quality of the donor endothelium, neither with a cloudy graft nor with endothelial decompensation as the cause of failure. Main risk factors for a failed graft were vascularization of the host cornea (p=0.0001), the presence of a systemic auto immune disease in the recipient (p=0.003) and the disease leading to the (imminent) perforation and emergency graft (p=0.021).

Conclusion

A selected pool of donor corneas designated for emergency grafting that does not interfere with the scheduled procedures, allows more efficient and safe use of donor tissue in case of a(n) (imminent) perforation. Corneal graft survival rates justify the criteria for selection.

Key words

Corneal donor selection criteria – corneal graft outcome – corneal preservation- emergency keratoplasty- organ culture.

Introduction

An imminent or complete cornea perforation, as in infectious keratitis or melting disease, is a challenge. It is not always possible to close the gap with tissue glue or amnion (Prabhasawat et al. 2001; Travella & Chang 2001; Sii & Lee 2005). In most eyes, the use of corneal donor tissue on an acute basis is preferred (Franchescetti & Doret 1950; Thiel & Weidle 1985; Kirkness et al. 1991). Preservation of the globe is the first objective and graft transparency is the second objective for requesting a donor cornea (Franchescetti & Doret 1950; Thiel & Weidle 1985; Kirkness et al. 1991; Nobe et al. 2002; Killingsworth et al. 1993). It is also accepted that the requests for emergency corneal tissue should be honoured as soon as possible (Hanada et al. 2008).

To prevent interference with already scheduled surgeries and to meet the demand of availability within 24 hours of fully screened corneal tissue, a separate pool of donor tissue was designated for emergency grafting and prepared for immediate use.

In 1990, the Dutch corneal surgeons and the Cornea Bank Amsterdam started with such a pool of donor corneas. Donor corneas were selected that did not meet all of the criteria for penetrating keratoplasty (Kirkness et al. 1991; Sperling & Sorensen 1981; Pels & Schuchard 1993; Pels et al. 1999). Corneas, with either a small superficial stromal opacity in the optic centre or an endothelial quality judged unsuitable for full thickness keratoplasty, were made available for immediate use with all safety tests performed. The creation of a National Follow-up Registry in 1995 made a long term evaluation possible with a large group of corneas.

In a previous study it was shown that corneal tissue from this pool was able to preserve the globe (Rijneveld et al. in press).The purpose of this study was to validate the use of a selected pool of corneal tissue for corneal graft survival in emergency cases.

Materials and methods

Donor tissue

Human eyes are donated to the Dutch Transplantation Society (NTS) for transplantation purposes. According to the Dutch law NTS is responsible for the safety screening of the donor. This includes screening of the medical history and the serology testing. NTS has delegated its responsibilities for donor tissues to Bio Implant Services Foundation (BIS). The acceptance of corneal tissue for surgical procedures follows the guidelines of the European Eye Bank Association. Eye retrieval by specifically trained staff is organized by BIS. It generally takes a few days to release the donor tissue for implantation purposes.

Storage of corneal tissue

The Cornea Bank Amsterdam applies Organ Culture as storage method since 1982. Details are described elsewhere (Pels & Schuchard 1993).

In case of corneas designated for emergency grafting the procedure is modified in order to have an organ cultured cornea available for immediate use after reversal of the swelling, with the necessary microbiological tests performed and the tissue released by BIS concerning safety.

After 3 days in Organ Culture the cornea is placed in transport solution consisting of storage medium with 5% Dextran T500. Storage takes place in an incubator at 31°C and transport occurs at room temperature.

Samples are taken for microbiological testing from the storage medium at day three and of the transport solution one day after transfer. These samples are incubated for seven days on blood agar plates (1 ml /plate) at 37° C and room temperature, and in thioglycollate broth medium at 37° C. After minimally 2 days in transport solution (quarantine period for the microbiological tests) the corneas are released for immediate transport and grafting, provided all microbiological tests do not show any growth. The tissue expires after 7 days in transport solution.

Tissue selection

Macroscopic and slit lamp examinations are followed by evaluation of the endothelium after staining with trypan blue (Pels & Schuchard 1993) by light microscopy. Corneas, that meet all selection criteria but one are selected for emergency grafting. They consist of two groups:

Group 1: corneas with a small superficial stromal opacity but meeting all selection criteria for penetrating keratoplasty (PKP) tissue, ie >2300 endothelial cells/mm^2, absence of mild to severe polymegathism and/or pleomorphism (endothelium PKP suitable).

Group 2: corneas with an endothelial cell density between 1800-2300 cells/mm^2 or mild to severe polymegathism and/or pleomorphism but without a stromal opacity (endothelium PKP unsuitable).
To include vital tissue corneas showing endothelium with trypan blue stained nuclei and highly vacuolised cells are excluded (Pels & Schuchard 1993). Corneal tissue was allocated without selection. The availability of cornea tissue determined which type of donor tissue (group 1 or 2) was used for the emergency patient.

Patients
Between 1 January 1995 and 31 December 2002, 2548 scheduled penetrating and 131 emergency penetrating grafts were performed and included in the study. Twenty lamellar emergency keratoplasties were excluded.
The validity of quality criteria for donor cornea endothelium was subject of the study.
The degree of vascularization in the recipient cornea was scored. Vascularization present in more than two quadrants was considered as high risk (Völker-Dieben et al. 1987; Williams et al. 1992; Maguire et al. 1994; Inoue et al. 2001).

Surgery
Although several corneal surgeons performed the emergency keratoplasties, standard surgical techniques were used. Interrupted sutures and oversized buttons were used in all penetrating keratoplasties: 0.25 mm for phakic and 0.5 mm for aphakic eyes. Mean donor graft size was 8.35 mm (4 -13 mm). Conjunctival transplants finished off the emergency grafts when indicated.

National Follow-up Registry
In 1995 a National Follow-up Registry started in which almost all Dutch corneal surgeons participate. Corneas delivered by the Cornea Bank Amsterdam are accompanied by follow-up forms to be completed with clinical information, pre-, per- and postoperatively, including the performance of additional conjunctival auto grafts. Data are collected 0.5, 1, 2, 3, 5 and more than 5 years postoperatively and entered into a computerized data base system. A longitudinal cohort follow-up study was performed from January 1995-December 2002.

Outcome parameters and statistical procedures.
The status of the corneal graft was scored as clear, partly clear or cloudy. Cloudy was considered as a failed graft. The causes of graft failure were recorded, i.e. an ocular surface problem, re-infection, endothelial decompensation, immunologic rejection and surgical interventions responsible for the failure.

The time of graft failure was defined as the first postoperative examination of which the patient was seen with a failed graft.
Risks factors such as quality criteria of donor tissue, indications for the emergency graft, degree of vascularization of the host cornea, other ocular diseases and accompanying systemic diseases were identified by Cox multivariate logistic regression analysis. Survival curves were calculated using the actuarial life table method by Kaplan-Meier. Differences between classes were assessed with a log rank test. Frequencies were compared using a chi-square test. All analyses were performed using the SPSS software (version 12.0). p Values less than 0.05 were considered statistically significant.

Results

Patients

All emergency patients showed a complete (n=80) or an imminent (n=51) corneal perforation. Differences in ocular and general health between emergency and scheduled patients are listed in Table 1.

Table1 - Recipient data of patients receiving either an emergency or a scheduled penetrating keratoplasty

Status patient eye	*Emergency procedures N=131*	*Scheduled procedures N=2548*	*Difference p value*
Vascularization ≥ 2 quadrants	47.3 %	8.4 %	0.0001
Abnormal eye lid function	11.5 %	1.1 %	0.0001
Tear film abnormal	16.8 %	1.6 %	0.0001
Cells and flare in anterior chamber	26.7 %	2.3 %	0.0001
First graft this eye	61.1 %	82.4 %	0.0001
Male recipients	64.9 %	45.4 %	0.0001
Diabetes	9.2 %	4.8 %	0.0028
Allergies	9.9 %	3.3 %	0.0010
Auto immune diseases *)	9.2 %	*) 1.3 %	
Grafts in other eye	8.4 %	17.0 %	0.0040
Glaucoma	13.7 %	9.0 %	0.0510

*) This was not specifically registered until 2006. For the 131 emergency procedures these data were collected later on. The percentage for scheduled procedures comes from the registrations after 2006; from 840 scheduled procedures, 11 patients suffered from auto immune diseases.

Ocular diseases leading to the (imminent) perforation and emergency graft are shown in Table 2. The largest group (n= 57) of patients suffered from non herpetic ulcers. A subdivision of this group is listed in Table 2a. Herpetic ulcers in 31 patients (30 x Herpes simplex and 1x Herpes zoster) were ranking second. Sixteen patients suffered from a

cornea melting, a subdivision of this group is listed in Table 2b. A complicated trauma was the indication in 13 patients.
Twelve patients showed a systemic auto immune disease. Thirteen patients were diagnosed with diabetes mellitus. Group 1 and 2 grafts were equally distributed amongst the patients with severe vascularization, systemic auto immune disease and diabetes (Table 3).

Surgery

Hundred and thirty one penetrating grafts were performed. One eye was lost during surgery because of an expulsive bleeding. Conjunctival transplantation finished off 15 emergency grafts. Nine conjunctival transplants were performed later,1 month (1x), 2 months (1x), 3 months (2x), 6 months (1x),9 months (1x), 12 months (2x), 36 months (1x) respectively. The proportion of conjunctival grafts in the various indications for an emergency graft was not significantly different. Consequently 115 emergency penetrating keratoplasties were available for long term survival analysis, 61x performed with donor tissue from group 1 (endothelium PKP suitable) and 54 x with tissue from group 2 (endothelium PKP unsuitable). Forty nine percent of the penetrating keratoplasties were combined with other surgical procedures as cataract extraction, lens exchange, vitrectomy, silicone oil addition or removal and glaucoma procedures.

Table 2 - Ocular diseases leading to the (imminent) perforation and emergency penetrating grafting for 131 patients

Indications	*Number*	*Percent*
Non herpetic ulcers	*57*	43
Herpetic ulcers	31	24
Cornea melting	16	12
Trauma	13	10
Others	14	11
Total	131	100

Table 2a - Subdivision for the group of non herpetic ulcers

Non herpetic ulcers	*Number*	*Percent*
Bacterial (culture proven)	22	39
Bacterial (not culture proven)	13	23
Acanthamoeba	3	*5*
Candida	2	4
Other fungi	2	4
Trophic*)	6	10
Not specified	9	15
Total	*57*	100

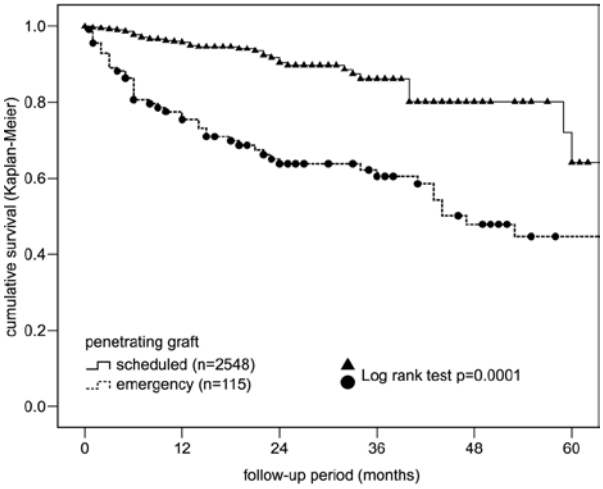
*) stem cell involvement

Table 2b - Subdivision Cornea melting

Cornea melting	*Number*	*Percent*
Rheumatoid arthritis	3	20
Primary Sjögren disease	2	12
Other systemic auto immune diseases	2	12
Other systemic disease	5	31
Not specified	4	25
Total	16	100

Table 3 - Distribution of corneal grafts with group 1 (PKP suitable) or 2 (PKP unsuitable) endothelium in three different risk groups

Risk group		*Donor endothelium PKP quality (numbers)*		*P value*
		group 1	*group 2*	
Vascularization	No	55	58	0.310
	Yes	6	12	
Autoimmune disease	No	54	65	0.546
	Yes	7	5	
Diabetes	No	56	62	0.574
	Yes	5	8	



Population at risk at the different time periods

Penetrating graft	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
Scheduled	2548	426	69	143	10	60	6	27	3	9	1
Emergency	115	73	24	52	11	38	2	21	7	12	1

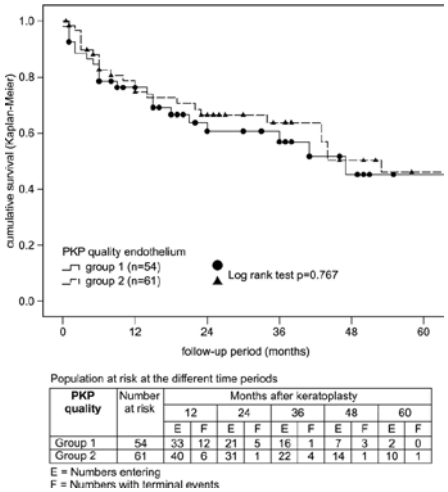
E = Numbers entering
F = Numbers with terminal events

Figure 1
Kaplan Meier survival analysis for emergency compared to scheduled penetrating keratoplasty. On top the Kaplan Meier survival curve and below the number of corneas at risk in each stratum at various intervals after transplantation.

Outcome

A highly significant difference (p =0.0001) in graft survival was identified in the emergency versus the scheduled PKPs (Figure 1). This was explained by the significant differences in ocular and co-morbidity status of the recipient as listed in table 1. Forty eight of the 115 emergency penetrating grafts failed. In all but one the reason of failure is known. Thirty eight failures were accompanied with ocular surface problems (19x), re-infection (14x) (7 x HSV, 7 x bacterial) and 5 x surgical interventions (not specified). Twenty one of these failures occurred during the first 6 months postoperatively, the remaining 17 occurred later in time. Eight grafts failed because of endothelial decompensation. These failures were randomly divided among group 1 and 2 and randomly divided over the follow-up period. In one patient an irreversible immunologic rejection was observed.

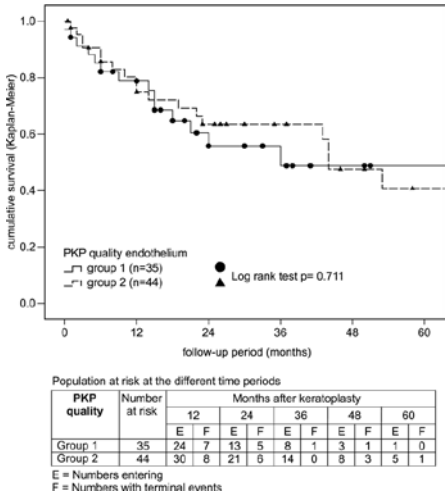
Factors affecting outcome:



Population at risk at the different time periods

PKP quality	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
Group 1	54	33	12	21	5	16	1	7	3	2	0
Group 2	61	40	6	31	1	22	4	14	1	10	1

E = Numbers entering
F = Numbers with terminal events



Population at risk at the different time periods

PKP quality	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
Group 1	35	24	7	13	5	8	1	3	1	1	0
Group 2	44	30	8	21	6	14	0	8	3	5	1

E = Numbers entering
F = Numbers with terminal events

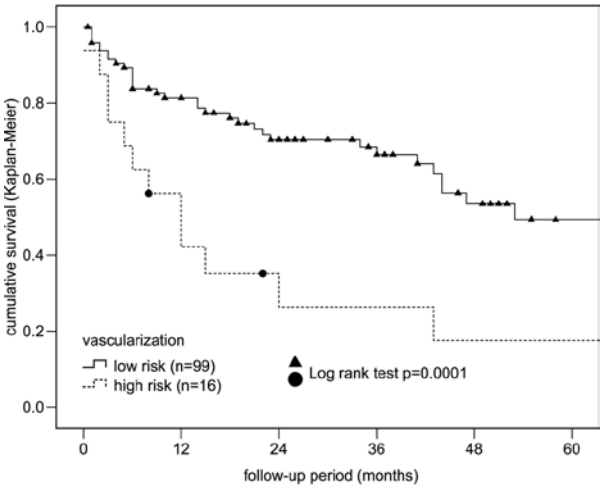
Figure 2a **Figure 2b**

Figure 2

Comparison of effect of endothelium quality by Kaplan Meier survival analysis for group 1 (endothelium PKP suitable) and group 2 (endothelium PKP unsuitable) On top the Kaplan Meier survival curve and below the number of corneas at risk in each stratum at various intervals after transplantation.
Panel a: For all diseases leading to a (n) (imminent) perforation and emergency penetrating graft.
Panel b: For the group ulcers, leading to a (n) imminent perforation and emergency graft.

Donor tissue quality.
Corneal graft survival in penetrating keratoplasties was not related to the selection group of the donor corneas used. In the group of 115 emergency keratoplasties no significant difference in corneal graft survival was observed in the 61 patients whom received grafts from donor tissue group 1 versus the 54 patients ,transplanted with grafts from group 2 (p=0.767) (Figure 2a). In a selected pool of 79 patients treated with a penetrating graft for an ulcer, graft survival was also not significantly different for the two groups of donor tissue (p=0.711) (Figure 2b).

Ocular disease.
Risk factors for graft survival were the degree of vascularization (p= 0.0001) of the recipient and the ocular disease leading to the (imminent) perforation and emergency grafting (p=0.021) (Figure 3a and 3 b); the non herpetic ulcers showed the best prognosis and cornea melting the poorest.

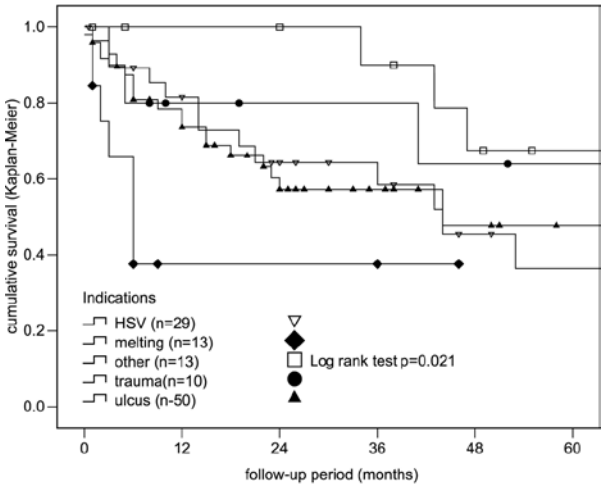


Population at risk at the different time periods

Risk vascularization	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
Low	99	65	17	48	8	35	1	19	6	10	1
High	16	8	7	4	3	3	1	2	1	2	0

E = Numbers entering
F = Numbers with terminal events

Figure 3a
Kaplan Meier survival analysis for clear cornea graft survival after emergency keratoplasty for recipients with a low risk (≤ 2 quadrants) compared to those with a high risk vascularization (> 2 quadrants).
On top the Kaplan Meier survival curve and below the number of corneas at risk in each stratum at various intervals after transplantation.



Population at risk at the different time periods

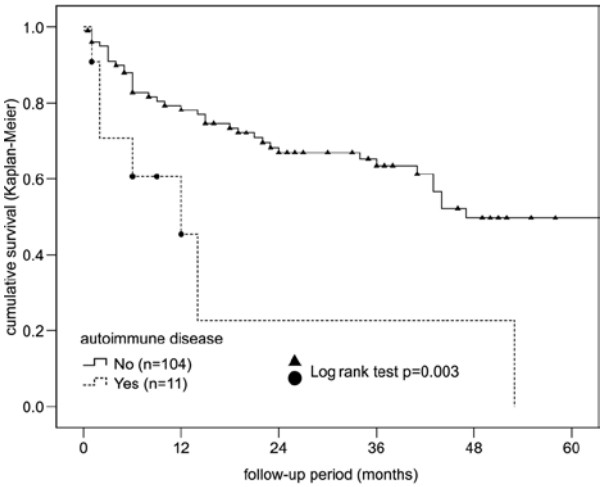
Indications	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
HSV	29	21	3	14	3	11	3	6	2	4	1
Melting	13	2	4	2	0	2	0				
Other	13	11	2	11	1	9	1	6	3	3	1
Trauma	10	6	2	5	1	5	0	4	1	3	0
Ulcus	50	33	7	20	6	11	8	5	3	2	0

E = Numbers entering
F = Numbers with terminal events

Figure 3b
Kaplan Meier survival analysis for clear cornea graft survival the ocular diseases leading to an imminent perforation and emergency graft. On top the Kaplan Meier survival curve and below the number of corneas at risk in each stratum at various intervals after transplantation.

Co-morbidity.

The presence of a systemic auto immune disease represents a serious risk factor for graft survival in the 11 patients (p =0.003) (Figure 4).



Population at risk at the different time periods

Auto immune disease	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
No	104	69	20	59	2	37	7	20	0	12	1
Yes	11	4	4	1	2	1	0	1	0	1	1

E = Numbers entering
F = Numbers with terminal events

Figure 4
Kaplan Meier survival analysis for clear cornea graft survival after emergency penetrating keratoplasty for patients with and without a systemic auto immune disease. On top the Kaplan-Meier survival curves and below the number of corneas at risk in each stratum at various intervals after transplantation.

Discussion

Validating of quality criteria for cornea tissue may lead to the use of donor tissue with various quality parameters for specific surgical procedures resulting in a more efficient use of donor tissue. To our best knowledge studies linking graft outcome with parameters of the cornea endothelium are lacking. Recently a study was published on cornea tissue, judged unsuitable for standard PKP because of anterior corneal pathology, used safely in endothelial keratoplasty (Armour et al. 2007).

In this study, donor tissue, fully screened for safety was always available in 24 hours for emergency grafting. The requests for emergency grafts did not interfere with the scheduled grafts. No differences in corneal graft survival rates were observed whether the endothelium of the donor cornea met the selection criteria or not for the regular scheduled penetrating keratoplasties.

The poor graft survival of emergency grafts when compared to the scheduled grafts is well known and can be ascribed to the specific risk factors (Killingsworth et al. 1993; Stubiger et al. 1995; Nobe et al. 2002; Maier et al. 2007; Hanada et al. 2008).

Globe preservation is the first target in case of a corneal perforation. In a previous study we have demonstrated that this target was reached irrespectively the non PKP quality of the endothelial cell layer of the tissue in a specific pool of donor corneas (Rijneveld et al. in press).

Graft transparency is the second objective. The chance for good graft survival might be jeopardized by the use of corneas with "less than optimal" quality but effective for globe preservation. In particular for the non-herpetic ulcer group the use of excellent quality donor tissue has been advocated by Claerhout (Claerhout et al. 2002).In contrast to Claerhout we found no arguments to endorse her advice. This study demonstrates that the accepted endothelial quality is no significant risk factor for corneal graft survival in those cases. For corneas judged suitable for PKP as well as for corneas with an endothelial quality judged less than optimal, the obtained survival rates were comparable to the published results (Claerhout et al. 2002).

This finding is of importance for current eye banking practice with an increasing demand for corneal tissue to be used for lamellar grafting procedures. Corneal tissue with endothelium meeting the criteria for PKP will more often be assigned to the pool of posterior lamellar keratoplasties (PLKP) than made available for emergency grafting because the demand for PLKP exceeds that for anterior lamellar keratoplasties (2007 Eye Banking Statistical Report EBAA, Claerhout 2009). As a result the pool for emergency grafting will consist of a larger proportion of tissue with endothelium not meeting PKP criteria. This study shows that such a shift does not effect the corneal survival and justifies this assignation procedure.

Vascularization was observed to be one of the main risk factors for a failed graft.

In many studies the linear correlation between vascularization and immunologic rejections has been described (Maguire et al. 1994; Inoue et al 2001; Price et al 2003; Thompson et al. 2003; Williams et al. 2007).To our surprise the percentage of immunologic failures in the emergency compared to the scheduled procedures was low. The 38 failures of which 21 occurred in the first 6 months suggest that other mechanisms for graft failure than the immunologic reactions prevail in emergency grafting.

The less favourable graft survival in our patients with autoimmune diseases confirms the observations of others (Nobe et al. 2002; Maier et al. 2007). Our results support the conclusion that for those indications alternative treatments should be considered. Lamellar grafts for impending perforations are described as alternatives (Bessant & Dart 1994; Bernauer et al 1995; Shimmura et al. 2003). Maier found a much better graft survival for patients with auto immune disease when compared to our study results (Maier et al. 2007). Compared to our multicentered study, where treatment with immunosuppression was not common practice, he studied patients treated in one clinic with systemic immunosuppression. Also other authors reported on the improved graft survival in combination with systemic immunosuppression or topical cyclosporine A in patients with rheumatoid arthritis (Bernauer et al. 1995; Pleyer et al. 2002).

These results may stimulate the use of a uniform treatment protocol for immunosuppressive treatment in those cases.
Although a system for organ cultured corneas, prepared for emergency cases is described by Bredehorn, details about procedures were not published neither evaluated (Bredehorn et al. 2002a; Bredehorn et al. 2002b). With this study the system has been validated.
These results may not only apply to organ cultured corneas but also to hypothermic storage methods as there is no proof that the storage method itself affect corneal graft survival (Rijneveld et al. 1992, Rijneveld et al. 2008).

Conclusion

A selected pool of organ-cultured donor corneas, designated for emergency grafting that do not interfere with the scheduled procedures, allows more efficient and safe use of donor tissue in case of an (imminent) perforation.
The risk factors for corneal graft survival in emergency cases showed that corneas selected for this type of grafting and not meeting all the criteria for a PKP can be used safely without jeopardizing the chance of a clear graft.

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References

Armour RL, Ousley PJ, Wall J et al. (2007): Endothelial keratoplasty using donor tissue not suitable for full thickness penetrating keratoplasty. Cornea 26:515-19.

Bernauer W, Ficker LA, Watson et al. (1995): The management of corneal perforations associated with rheumatoid arthritis. Ophthalmology 102:1325-1337.

Bessant DA & Dart JK (1994): Lamellar keratoplasty in the management of inflammatory corneal ulceration and perforation. Eye 8:22-28.

Bredehorn T, Langer C, Eichhorst A et al. (2002a): Indications for emergency keratoplasty at the University Eye hospital of Martin-Luther-University Halle-Wittenberg. Transplant Proc 34:2347.

Bredehorn T, Langer C, Duncker G et al. (2002b): Constant availability of emergency cornea transplants in central German Corneabank Halle. Transpl Proc 34:2351-2352.

Claerhout I, Beele H, Van den Abeele K et al. (2002): Therapeutic penetrating keratoplasty. Cornea 21:637- 642.

Claerhout I, Maas H, Pels E. Directory European Eye Bank Association, Amsterdam 2009

Eye Bank Association of America, 2007 Eye Banking Statistical Report, www.restoresight.org

Franchescetti A & Doret M. (1950): Hornhauttransplantation a chaud. Klin Monatsbl Augenheilkd 17:449- 458.

Hanada K, Igarashi S, Muramatsu O et al.(2008): Therapeutic keratoplasty for corneal perforation. Cornea 27:156-160.

Inoue K, Amano S, Oshika T et al. (2001): Risk factors for corneal graft failure and rejection in penetrating keratoplasty. Acta Ophthalmol 79:251-255.

Killingsworth DW, Stern GA, Driebe WT et al. (1993): Results of therapeutic penetrating keratoplasty. Ophthalmology 100:534-541.

Kirkness CM, Ficker LA, Steele AD et al. (1991): The role of penetrating keratoplasty in the management of microbial keratitis. Eye 5:425-431.

Maguire MG, Stark WJ, Gottsch JD et al. (1994): Risk factors for corneal graft failure and rejection in the Collaborative Corneal Transplantation Studies. Ophthalmology 101:1536-1547.

Maier P, Bohringer D. & Reinhard T (2007): Clear graft survival and immune reactions following keratoplasty. Graefe's Arch Clin Exp Ophthalmol 245:351-359.

Nobe JR, Sharma N, Tiyal JS et al. (2002): Tectonic grafts for corneal thinning and perforations. Cornea 21:792-797.

Pels E & Schuchard Y (1993): Organ culture in the Netherlands. In: Brightbill FS, ed Corneal Surgery. Theory, Technique and Tissue. St. Louis: Mosby 622- 632.

Pels E, Beekhuis WH & Völker- Dieben HJ. (1999): Long term storage for keratoplasty. In: Brightbill FS, ed. Corneal Surgery. Theory, Technique and Tissue. St. Louis: Mosby 897-906.

Pleyer U, Bertelmann E, Rieck P et al. (2002): Outcome of penetrating keratoplasty in rheumatoid arthritis. Ophthalmologica 216:249-255.

Prabhasawat P, Tesavibuk N & Komolsuradej W (2001): Single and multilayer amniotic membrane transplantation for persistent corneal epithelial defect with and without stromal thinning and perforation. Br J Ophthalmol 85:1455-1463.

Price MO, Thompson RW & Price FW (2003): Risk factors for various causes of failure in initial corneal grafts. Arch Ophthalmol 121:1087-1092.

Rijneveld WJ, Beekhuis WH, van Rij G et al. (1992): Clinical comparison of grafts in McCarey Kaufman medium at 4°C and in corneal organ culture at 31°C. Arch Ophthalmol 110:203-205.

Rijneveld WJ, Remeijer L, van Rij G et al. (2008): Prospective evaluation of McCarey-Kaufman and organ culture cornea preservation media: 14 year follow-up. Cornea 27: 996-1000.

Rijneveld WJ, Wolff R, Völker-Dieben et al: Validation of tissue quality parameters for donor corneas, designated for emergency cases: preservation of the globe. Accepted Cornea 30 march 2009.

Shimmura S, Shimazaki J & Tsubota K (2003): Therapeutic deep lamellar keratoplasty for corneal perforation. Am J Ophthalmol 135:896- 897.

Sii F & Lee GA. (2005): Fibrin glue in the management of corneal melt. Ophthalmology 33:532-534.

Sperling S & Sorensen IG (1981): Contamination of cadaver corneas. Acta Ophthalmol 59:126-133.

Stubiger N, Pleyer U, Erb C et al. (1995): Keratoplasty a chaud. Ophthalmologe 92:427-432.

Thiel HJ & Weidle EG (1985): Keratoplasty a chaud: results and complications. Dev Ophthalmol 1:68.

Travella M & Chang C (2001): 2-Octyl cyanoacrylate medical adhesive in treatment of a corneal perforation. Cornea 20:220-221.

Thompson RW, Price MO, Bowers PJ et al. (2003): Long term graft survival after penetrating keratoplasty. Ophthalmology 110:1396-1402.

Völker-Dieben HJ, D' Amaro J & Kok-van Alphen CC (1987): Hierarchy of prognostic factors for corneal allograft survival. Aust NZ J Ophthalmol 15:11-18.

Williams KA, Roder D, Esterman A et al. (1992): Factors predictive of corneal graft survival. Ophthalmology 99:403-14.

Williams KA, Lowe MT, Barlett CM et al. The Australian Corneal Graft Registry 2007 Report. Flinders University Press 2007, Aidelaide, Australia.