





# Chapter 4.1

Validation of tissue quality parameters for donor corneas, designated for emergency use in preservation of the globe

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## **Abstract**

### **Purpose**

To validate tissue quality parameters for donor corneas designated for emergency grafting to preserve the globe.

### **Methods**

In a longitudinal cohort follow-up study, 151 emergency grafts in the Netherlands 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. Assignment 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<sup>2</sup> or mild polymegathism or pleomorphism. Cox multivariate regression analysis, Kaplan-Meier survival, and log rank test were applied.

### **Results**

All requests for corneal tissue were honoured within 24 hours. Ninety-one patients showed a complete and 60 an imminent perforation. One hundred and thirty one penetrating grafts and 20 lamellar grafts have been used.

The globes were saved in 140 of the 151 patients (92.7%). Globe preservation was not significantly related to the absence of PKP quality of the donor endothelium, the type of grafting, the degree of vascularization in the host cornea, or diabetic disease in the recipient. The main risk factor for globe preservation was the presence of a systemic autoimmune disease in the recipient ( $p=0.0021$ ).

### **Conclusions**

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 an imminent perforation. Globe preservation rates justify the quality criteria for designation of this tissue.

### **Key words**

Corneal donor selection criteria, globe preservation, emergency keratoplasty, therapeutic keratoplasty, endothelial cell density.

## Introduction

An imminent or complete cornea perforation, as in an infectious keratitis or melting disease, is a serious threat for the patient to lose the eye. It is not always possible to postpone a keratoplasty by closing the gap with histoacryl glue, fibrin glue or amnion.<sup>1-3</sup> In most eyes, the use of corneal donor tissue on an acute basis is preferred.<sup>4,6</sup> In the Netherlands, since 1982, corneas for grafting are stored in organ culture (OC) medium and the subsequent swelling is reversed before surgery in transport medium.<sup>7,8</sup>

In the past requests for donor corneas for emergency patients were honored at the cost of the pool of allocated random and HLA-matched tissue. Consequently, patients already scheduled for a penetrating keratoplasty (PKP) had to be canceled to make tissue available with all screening tests for safety of the donor tissue performed.

In 1990, the Dutch corneal surgeons and the Cornea Bank Amsterdam thought to solve this problem by creating a separate pool of donor corneas, not interfering with the normal pool, designated for emergency grafting. Because globe preservation was defined to be the primary goal for this surgery, donor corneas were selected that did not meet all criteria for PKP.<sup>9</sup> Those corneas, with either a small stromal opacity in the optic centre or less than optimal endothelial quality, were made available for immediate use.

After a pilot study in 1992, it was decided to continue with this pool of designated corneas. Results have not yet been published. The creation of a National Follow-up Registry in 1995 made a long-term evaluation possible with a larger group of corneas.

This study was performed to validate the suitability of this selected pool of donor corneas for emergency grafting in order to preserve the globe.

This is the first study with a large group of well-defined donor tissue, from 1 eye bank only, designated for emergency grafts and followed for a long period.

## **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. 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. Screening of the medical history and the organization of the serology testing is also performed by BIS. It generally takes a few days to release the donor tissue for implantation purposes.

### **Storage of corneal tissue**

The Cornea Bank Amsterdam by law is responsible for the excision of the corneoscleral disc, the storage, and selection of the donor corneas. Organ culture is the preferred preservation method since 1982. Details and selection criteria are described elsewhere.<sup>8</sup>

In case of corneas designated for emergency grafting, the procedure is modified in order to have an OC 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 OC, 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 3 and 1 day after transfer to the transport medium. These samples are incubated for 7 days on blood agar plates (0.1 ml/plate) at 37°C and room temperature, and in thioglycollate broth medium at 37°C. After minimally 2 days in transport medium, the corneas are released for immediate transport and grafting provided that all microbiological tests do not show any growth. The tissue expires after 7 days in transport medium.

### **Tissue selection**

Macroscopic and slit-lamp examinations are followed by evaluation of the endothelium by light microscopy. Corneas meeting all selection criteria except one are selected for emergency grafting. In all cases the tissue must be vital, as indicated by the absence of necrotic endothelial cells. They consist of two groups.

### Group 1

Corneas with a small superficial stromal opacity but meeting all selection criteria for PKP tissue; for example, >2300 endothelial cells per square millimeter, absence of mild to severe polymegathism and/or pleomorphism.

### Group 2

Corneas with either an endothelial cell density from 1800 to 2300 cells per square millimeter or presence of mild polymegathism and/or pleomorphism but without a stromal opacity.

## **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. 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 were accompanied by follow-up forms to be completed with clinical information, preoperatively, perioperatively, and postoperatively, including the performance of additional conjunctival auto grafts. Data were collected 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 until December 2002. One person (R.W.) visited all centers to collect missing data concerning the corneas used for emergency grafting and entered them in the Registry. The medical records were evaluated by one ophthalmologist (W.J.R.).

## **Outcome parameters and statistical procedures**

Globe preservation was defined as the prevention of enucleation or phthisis bulbi. Risk factors, such as quality criteria of donor tissue, indications for emergency grafting, degree of vascularization of the patient's cornea, other ocular diseases, and systemic diseases, were identified by Cox multivariate logistic regression analysis. Survival curves were calculated using the actuarial life table method by Kaplan and Meier. Differences between classes were assessed with a log rank test. Frequencies were compared using a  $\chi^2$  test. All analyses were performed using the SPSS software (version 12.0). P values less than 0.05 were considered statistically significant.

## Results

### Logistics

In 100% of the cases a request for an emergency graft was honoured within 24 hours, with a cornea from a designated pool of donor corneas. There was no interference with the scheduled keratoplasties.

### Patients

Between January 1, 1995 and December 31, 2002, 2819 regular and 151 emergency grafts were performed. All emergency patients showed a complete (n=91) or an imminent (n=60) corneal perforation.

Differences in ocular and general health between emergency patients and scheduled patients are listed in Table 1. The severity of the ocular and general health problems of the emergency patients is illustrated by 10 significantly different characteristics.

Indications for the emergency grafting are shown in Table 2. The largest group (n=61) of patients suffered from nonherpetic ulcers. A subdivision of this group is shown in Table 3. Herpetic ulcers in 32 patients (31x herpes simplex and 1x herpes zoster) were ranking second. Eighteen patients suffered from a cornea melting, a subdivision of this group is listed in Table 4. Primary Sjögren syndrome was diagnosed in 5 patients. A complicated trauma was the indication for an emergency graft in 16 patients. Sixteen patients showed a systemic auto immune disease. In 11 patients without a systemic autoimmune disease, diabetes mellitus was present. Four patients had a systemic auto-immune disease and diabetes mellitus.

**Table 1 - Recipient data of patients receiving either an emergency or a scheduled keratoplasty**

<i>Status patient eye</i>	<i>Emergency procedures N=151</i>	<i>Scheduled procedures N=2819</i>	<i>Difference p value</i>
Vascularization $\geq$ 2 quadrants	43.7 %	8.5 %	0.0001
Abnormal eye lid function	12.6 %	1.1 %	0.0001
Tear film abnormal	17.2 %	1.7 %	0.0001
Cells and flare in anterior chamber	25.2 %	2.3 %	0.0001
First graft this eye	62.9 %	87.4 %	0.0001
Male recipients	61.6 %	45.9 %	0.0001
Diabetes	9.9 %	4.7 %	0.002
Allergies	8.6 %	3.3 %	0.003
Grafts in other eye	8.0 %	17.0 %	0.005
Glaucoma	13.2 %	8.8 %	0.05

**Table 2 - Indications for emergency grafting for 151 patients**

<i>Indications</i>	<i>Number</i>	<i>Percent</i>
Non herpetic ulcers	61	40.4
Herpetic ulcers	32	21.2
Cornea melting	23	13.2
Trauma	16	10.6
Others	19	12.6
Total	151	100.0

**Table 3 - Subdivision for the group of nonherpetic ulcers**

<i>Non herpetic ulcers</i>	<i>Number</i>	<i>Percent</i>
Bacterial (culture proven)	22	36.0
Bacterial (not culture proven)	13	21.3
Acanthamoeba	4	6.6
Candida	2	3.3
Fungus	2	3.3
Trophic *)	7	11.5
Not defined	11	18.0
Total	61	100.0

\*) stem cell involvement

**Table 4 - Subdivision Cornea melting**

<i>Cornea melting</i>	<i>Number</i>	<i>Percent</i>
Rheumatoid arthritis	4	17.4
Primary Sjögren disease	2	8.7
Other systemic auto immune diseases	4	17.4
Other systemic diseases	8	34.8
Unknown origin	5	21.7
Total	23	100.0

## Surgery

The keratoplasty procedure was 131 x penetrating and 20 x lamellar. Lamellar grafts were significantly ( $p= 0.006$ ) more often applied in patients with melting disease, trauma and miscellaneous indications, 30.4%, 26.3% and 18.8% compared to the nonherpetic ulcers and the herpetic ulcers, 6.6% and 3.1% respectively. Forty nine percent of the penetrating and 45% of the lamellar keratoplasties were combined with other surgical procedures such as cataract extraction, lens exchange, vitrectomy, silicone oil addition or removal and glaucoma procedures. Conjunctival transplantation finished off 18 emergency grafts and 9 conjunctival transplants were performed later on (Table 5); 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.

**Table 5 - Conjunctival grafting (full or partial) during or after the keratoplasty**

<i>Conjunctival Graft</i>		<i>LKP</i>	<i>PKP</i>	<i>Total</i>
Full	During keratoplasty	2	15	17
	After keratoplasty	0	5	5
	Total full	2	20	22
Partial	During keratoplasty	1	0	1
	After keratoplasty	0	4	4
	Total partial	1	4	5
Total conjunctival grafts		3	24	27

LKP = lamellar keratoplasty

PKP = penetrating keratoplasty

## Outcome

The globe was preserved in 140 of the 151 emergency patients (= 97.2 %). Eleven eyes were lost; 9 were enucleated and 2 developed phthisis bulbi. In one patient the phthisis bulbi occurred 10 months after keratoplasty for a herpetic ulcer as consequence of an ocular trauma; the second phthisis occurred 13 months after keratoplasty for a cornea melting, status after vitrectomy and chemotherapy for Hodgkin disease.

One eye was lost during surgery because of an expulsive bleeding and in a second one the severe preoperative inflammation was not controllable post operatively, resulting in an endophthalmitis. The remaining 7 eyes were lost in patients who suffered of cornea melting (3x), chemical burns (2x) or complex penetrating injuries (2x).

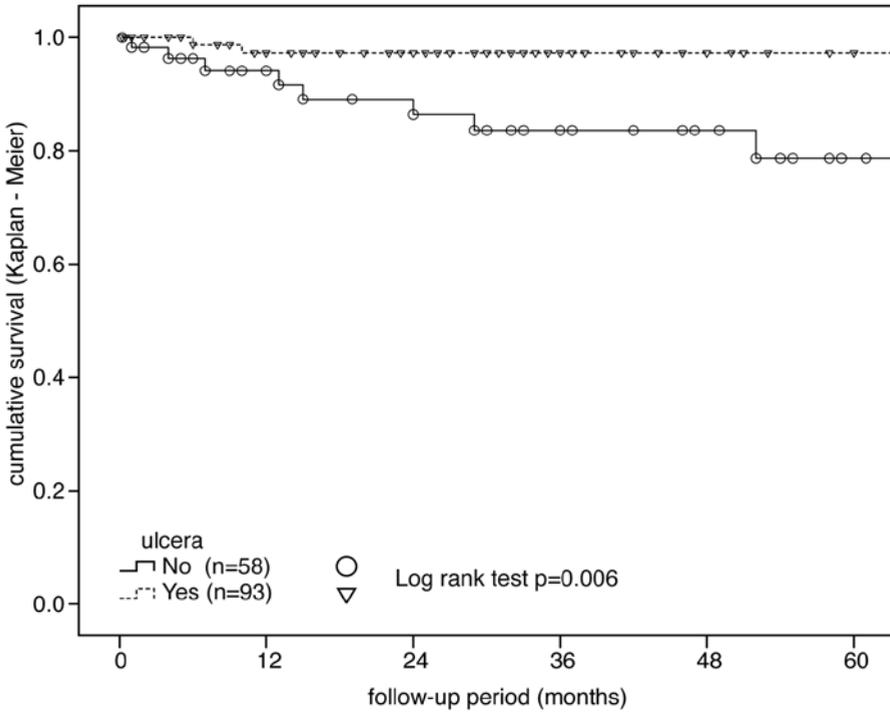
## Factors affecting outcome

### Donor tissue quality

Globe preservation was not related to the quality criteria of the donor corneas used. No significant difference in globe preservation was observed in patients who received emergency grafts from donor tissue group 1 versus group 2. The differences in endothelial cell layer characteristics for donor tissue group 1 and 2 were particularly relevant in penetrating keratoplasty (PKP), but the globe preservation rate in 131 penetrating keratoplasties was also not significantly different between group 1 and 2.

### Ocular disease

For the 93 patients suffering from corneal ulcers, 32 herpetic and 61 non herpetic ulcers, the globe preservation was significantly higher ( $p = 0.006$ ) compared to the nonulcers group (Fig. 1).



Population at risk at the different time periods

ulcera	Number at risk	Months after keratoplasty									
		12		24		36		48		60	
		E	F	E	F	E	F	E	F	E	F
No	58	39	4	32	2	26	0	19	1	11	0
Yes	93	67	1	52	0	31	0	19	0	13	0

E = Numbers entering

F = Numbers with terminal events

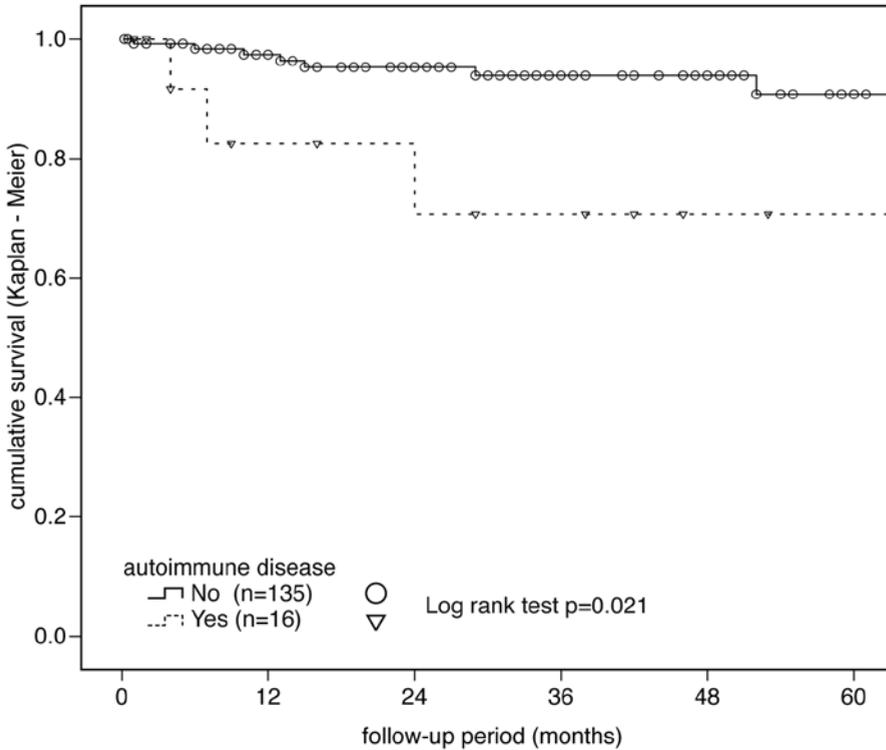
### Figure 1

Globe preservation for the group ulcers, herpetic and non herpetic and the group non ulcers. On top the Kaplan- Meier survival curve, and at the bottom, the number of globes at risk in each stratum at various intervals after transplantation.

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### Comorbidity

A significant inverse correlation was found between globe preservation and the presence of a systemic auto immune disease in the recipient ( $p= 0.021$ ) (Fig. 2). For patients with or without diabetic mellitus, no difference was observed.



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	135	98	3	78	1	52	0	36	1	23	0
Yes	16	8	2	7	0	5	1	2	0	1	0

E = Numbers entering

F = Numbers with terminal events

**Figure 2**

Globe preservation in patients with and without a systemic auto immune disease. On top the Kaplan-Meier survival curve, and at the bottom, the number of globes at risk in each stratum at various intervals after transplantation.

## Surgical procedure

Globe preservation was not related with the type of grafting nor influenced by the presence of a conjunctival transplant postoperatively.

## Discussion

The first target of emergency grafting is the preservation of the globe.<sup>4,6,10,11</sup> It is also accepted that the requests for emergency donors should be satisfied as soon as possible. In this study donor tissue, fully screened for safety, was always available within 24 hours. These emergency requests did not interfere with the scheduled grafts. No difference in globe preservation was observed whether or not the endothelium of the donor corneas met the selection criteria for penetrating keratoplasties.

Various authors reported on anatomical success rates or globe preservation in emergency grafts.<sup>6,12-19</sup> However the effect of quality characteristics of the donor endothelium on globe preservation rates has never been studied.

A few of these authors defined the quality of their donor tissue. Hanada et al<sup>13</sup> reported on twenty corneas stored in Optisol and frozen corneal tissue, but endothelial cell density was not mentioned. Claerhout et al<sup>12</sup> reported on high cell counts in the donor tissue (28 cases), originating from two different banks. Two preservation methods were used, OC and Optisol. Pleyer et al<sup>14</sup> reported on 21 cases of OC donor tissue; however, a minimum cell density was not mentioned. Maier et al, who reported about corneal graft survival and not about globe preservation, described the donor tissue used as cold storage until 1994 and OC since 1995. Only grafts with cell counts of > 2000 cells/mm<sup>2</sup> were used.<sup>20</sup> The limited information on donor tissue quality parameters in these studies does not allow conclusions on a possible influence on globe preservation rates. Globe preservation rates in our study (92.7%) is in line with earlier publications.<sup>12,13</sup> This indicates that the use of preselected corneal tissue not meeting all criteria for PKP does not result in significantly lower globe preservation rates.

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 Keratoplasty (PLKP) than made available for emergency grafting because the demand for PLKP exceeds that for Anterior Lamellar Keratoplasty (ALKP) (2007 Eye Banking Statistical Report EBAA, EEBA Directory 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 affect the globe survival and justifies the assignation procedure including tissue for lamellar grafting. The consequence for the corneal graft survival however has to be carefully investigated. (see chapter 4.2)

The good results of globe preservation for the 93 patients with non herpetic and herpetic ulcers is in line with the observations of Claerhout et al in a group of 15 patients in which

only 10 patients showed an (imminent) perforation suggesting a less severe pathology than in our study.<sup>12</sup>

In the selected group of 16 patients suffering of a systemic auto immune disease, globe preservation was significantly lower than in the group without a systemic auto immune disease. This is in contrast to the better results observed by Pleyer et al in comparable patients.<sup>14</sup> These findings support his recommendation for intensive immune suppressive therapy postoperatively. In the period of our study, 1995-2002 intensive immune suppressive therapy was not yet common practice. Also, others suggested better clinical results with systemic immune suppressive therapy.<sup>18,20</sup>

The majority of the lamellar grafts was performed in the non herpetic ulcer group. Lamellar grafts for impending perforations are described as alternative treatments in case studies. Bessant and Dart published a globe survival of 100% in a group of 10 patients with auto immune diseases, treated with lamellar grafts. However, in contrast to our study, almost all patients were treated with systemic immune suppressive therapy.<sup>21</sup>

In conclusion globe preservation rates in our study are in line with earlier publications.<sup>12,13,15</sup> The following conclusion can be drawn from this study: 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 an imminent perforation. Globe preservation rates justify the quality criteria for designation of this tissue. The fate of the graft will be studied separately as the graft survival is the second objective for requesting a donor cornea.

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