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CLINICAL STUDY

Influencing factors on the suitability of organ-cultured corneas

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Abstract

Purpose **To determine the factors related to donor and tissue retrieval, which influence the suitability of organ-cultured corneas for transplantation.**

Patients and methods **We retrospectively analysed 2596 donor corneas. Polytomic logistic regression analysis was used to assess the influence of various factors (that is, donor age, cause of death, death-to-tissue retrieval time, tissue retrieval-to-reception time, and tissue retrieval method) on the suitability of grafts for transplantation. Positive predictive values (PPVs) were computed.**

Results **Forty-three percent (1118/2596) of corneas were discarded. The leading cause for discarding corneas was poor endothelial quality (21.5%). Corneas from donors older than 80 years were more likely to be discarded because of endothelial insufficiency (OR = 2.37, $P = 0.001$). Longer time between death and tissue retrieval was associated with increased risk of positive serology (OR = 1.43, $P = 0.02$). Increased time between tissue retrieval and reception was associated with increased risk of contamination (OR = 1.57, $P = 0.03$). PPV increased from 38.5% for corneas retrieved from donors older than 80 years featuring a death-to-tissue retrieval time of more than 6 h and a tissue retrieval-to-reception time of more than 24 h to 64.7% for corneas retrieved from donors younger than 80 years featuring a death-to tissue retrieval time shorter than 6 h and a tissue retrieval-to-reception time shorter than 24 h.**

Conclusion **The percentage of discarded corneas can be reduced by including donors aged 80 years or less, using a time from donor's death to tissue retrieval shorter than 6 h, and a tissue retrieval-to-reception time shorter than 24 h.**

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Keywords: corneal assessment; corneal organ culture; corneal storage; corneal suitability for penetrating keratoplasty

Introduction

The initial description of the organ culture technique and evaluation of its effects on the corneal tissue was reported in the seventies by Summerlin *et al*.[1] Following this initial description, repair of the corneal endothelium during organ culture was described by Dougman *et al*.[2] After good clinical results of organ-cultured corneal grafts obtained from old donors were reported by Sperling *et al*,[3] most European eye banks have progressively adopted this method for corneal tissue storage before transplantation. The criteria for tissue selection before transplantation have been described, which include assessment of the corneal endothelium during storage. This led to rejection of corneas with poor endothelial cell density after preservation.[4–7]

For more than 10 years, the number of corneas harvested has increased from 24 763 in 1996 to 39 051 in 2002 in Europe,[8] and from 3073 in 1995 to 6844 in 2002 in France.[9] In 2007, 32 080 donor corneas were processed by European eye banks.[10] However, the percentage of corneas discarded before transplantation has increased during the same period: from 40% in 1996 to 47% in 2007 in Europe,[10] and from 26% in 1995 to 47% in 2002 in France.[9] To improve donor tissue quality, several criteria have been adopted concerning donor medical history, donor serology, and corneal endothelial assessment. Application of these criteria resulted in a larger number of discarded corneas, which represents significant financial cost and wasted energy. The purpose of this study was to assess the factors that influence the suitability of organ-cultured corneas for

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transplantation, and to describe rules that could significantly decrease the number of discarded corneas.

Materials and methods

Corneal procurement

This retrospective study used 2596 corneas with full data from 3024 corneas received at the eye bank of EFS Bourgogne—Franche-Comte (Besançon, France) between 2000 and 2003. Donor tissue material retrieval was performed by *in situ* excision in all cases. Only corneas with full data were included (that is, donor age, cause of death, death-to-tissue retrieval time, tissue retrieval-to-reception time, organ-cultured time, and endothelial cell density). Death-to-tissue retrieval time is the time from donor's death to retrieval of the donor corneas. Tissue retrieval-to-reception time is the time from retrieval of the donor corneas to reception of tissue at the eye bank for processing and storage. No donor age limits were set. Prolonged death-to-tissue retrieval time up to 24 h or more and prolonged tissue retrieval-to-preservation time up to 48 h or more were accepted.

Corneal storage

After *in situ* tissue retrieval, corneas were immediately placed in preservation medium at room temperature (CorneaPrep II; Eurobio, Les Ulis, France) and transferred to the eye bank. At the eye bank, corneas were stored by organ culture at 31°C (CorneaMax, Eurobio) for 10–35 days. The endothelium of each cornea was observed twice: at the time of reception in the eye bank and at the end of storage. It was evaluated by light microscopy after staining with Trypan blue (0.4%) and dilatation of intercellular spaces was induced by using normal saline. Endothelial cell density was manually estimated using a grid placed in one ocular. If one of the following was present, cornea was discarded: endothelial cell density less than 2000 cells/mm^2, endothelial cell mortality greater than 2%, irregular cellular mosaic, and endothelial cells loss greater than 20% during storage.[11] The sterility of cornea was evaluated by inspection of the colour of the media three times per week, and by microbiological analysis of the medium samples. Serology of the donor was analysed from a blood sample taken at the time of tissue retrieval. For corneas retrieved from a multi-organ donor, a blood sample was taken before cardio arrest. Non-contributive or unsuitable analysis was considered as positive serology. Exclusion criteria for surgical use were inadequate endothelium, contamination of the medium, positive serology of the donor, and medical contraindication or technical accident.

Statistics

Univariate analysis

The influence of donor and tissue retrieval factors on the rate of donor corneas issued for transplantation was first assessed by χ^2-test and Fisher's exact test for qualitative factors and Student's *t*-test for quantitative factors. The following factors were recorded and analysed as predictive variables: donor age, donor cause of death, multi-organ or single-organ donor, death-to-tissue retrieval time, and tissue retrieval-to-reception time. Spearman's rank correlation was used to study the correlation between quantitative data.

Multivariate analysis

The predictive variables that were significantly ($P<0.10$) associated with the likelihood of acceptance of donor tissue for transplantation were analysed by multivariate polytomic logistic regression. The models were set for each specific risk of unsuitability (that is, inadequate endothelium, positive serology, contamination, and other reasons). A *P*-value below 0.05 in multivariate polytomic regression was considered statistically significant. Odds ratios (ORs) were quoted for significant factors ($P<0.05$). They show for a given factor the risk compared with the baseline. The baseline for age was donor age less than 40 years; for death-to-tissue retrieval time it was less than 6 h; and for tissue retrieval-to-reception time it was less than 24 h. For a given cause of death, the OR was the risk compared with all other causes of death. ORs are shown with their 95% confidence intervals (95% CIs) and means are given with the standard deviations (SD). No adjustment was made for statistical dependence between multiple corneas from the same donor as the analysis was based on corneas rather than on donors. Positive predictive values (PPVs) of suitability of corneas for transplantation were computed according to factors, which were shown to significantly influence suitability in multivariate logistic regression. PPVs were calculated as follows:

PPV = number of suitable corneas/(number of suitable corneas + number of discarded corneas).

Results

Donor cornea characteristics

In this retrospective study, 2596 corneas were included from 1333 donors. The mean donor age was 68.0 ± 16.9 years (SD). Seventy-four percent of donors were older than 60 years (Table 1). The most common causes of death were cardiovascular diseases (30.7%), stroke (22.1%), and cancer (17.6%). Eleven percent of corneas were retrieved from a multi-organ donor. The mean time between death and tissue retrieval was 8.4 ± 6.0 h. The mean time between tissue retrieval and reception at

Table 1 General data and data of suitable and discarded corneas

Risk factors	*General data*	*Corneas suitable for surgery*		*Corneas discarded*					
				Contamination		*Positive serology*		*Endothelium*	
	n	n	%	n	%	n	%	n	%
Donor age (years)									
<40	177	113	63.8	15	8.5	14	7.9	24	13.6
40–59	491	311	63.3	41	8.4	60	12.2	58	11.9
60–79	1225	734	59.9	67	5.5	157	12.8	248	20.2
>80	703	320	45.5	34	4.8	107	15.2	228	32.4
Total	**2596**	**1478**	***56.9***	**157**	***6.0***	**338**	***13***	**558**	***21.5***
Cause of death									
Traumatic	249	168	67.5	20	8.0	21	8.4	31	12.4
Cardiac	798	429	53.8	39	4.9	110	13.8	200	22.2
Stroke	573	353	61.6	30	5.2	51	8.9	127	22.1
Respiratory	218	104	47.7	19	8.7	33	15.1	58	26.6
Cancer	458	264	57.7	23	5.0	77	16.8	79	17.2
Intoxication	30	19	63.3	3	10.0	6	20.0	2	6.7
Infection	75	37	49.3	5	6.7	12	16.0	17	22.7
Others	195	104	53.3	18	9.2	28	14.3	44	22.6
Total	**2596**	**1478**	***56.9***	**157**	***6.0***	**338**	***13***	**558**	***21.5***
Harvested corneas									
MOD	303	227	74.9	23	7.6	4	1.3	35	11.6
SOD	2293	1251	54.6	134	5.9	334	14.5	523	22.8
Total	**2596**	**1478**	***56.9***	**157**	***6.0***	**338**	***13***	**558**	***21.5***
DET (hours)									
<6 h	1247	730	58.5	60	4.8	135	10.8	271	21.7
6–12 h	698	396	56.7	55	7.9	83	11.9	148	21.2
>12 h	651	352	54.1	42	6.5	120	18.4	139	21.4
Total	**2596**	**1478**	***56.9***	**157**	***6.0***	**338**	***13***	**558**	***21.5***
ERT (hours)									
<24 h	1017	532	52.3	37	3.6	140	13.8	221	21.7
24–48 h	1181	715	60.5	75	6.4	147	12.4	255	21.6
>48 h	398	231	58	45	11.3	51	12.8	82	20.6
Total	**2596**	**1478**	***56.9***	**157**	***6.1***	**338**	***13***	**558**	***21.5***

Abbreviations: DET, death-to-excision time; ERT, excision-to-reception time; MOD, multi-organ donor; SOD, single-organ donor.
Data of corneas discarded for 'other reasons' are not shown.
% Indicates the percentage corresponding for each group of each risk factors.
Boldfaced values indicate the total for each group.
Italicized values indicate the beginning of each group.

the eye bank was 33.3 ± 20.6 h. The mean endothelial cell density at the time of reception at the eye bank was 2140.7 ± 274.9 cells/mm^2.

The percentage of discarded corneas was 43.1% (1118/2596). The first cause of unsuitability was inadequate endothelium (21.5%, 558 corneas). Thirteen percent (338/2596) of corneas were discarded for positive serology and 6.0% (157/2596) for contamination. Sixty-five corneas (2.5%) were discarded for 'other reasons'.

Influence of donor age on suitability

The percentage of discarded corneas ranged from 36.2% for donors younger than 40 years to 54.5% for donors older than 80. Donor age had a strong influence only for endothelial assessment (Table 2). Thirteen percent of corneas retrieved from donors younger than 40 years (baseline) did not reach the endothelial criteria, compared with 32.4% from donor older than 80 years (Table 1). The specific risk of inadequate endothelium increased only for corneas from donors older than 80 years (OR = 2.37; $P = 0.001$). The mean endothelial cell density significantly decreased with donor age from an average of 2288.5 ± 217.8 cells/mm^2 for corneas retrieved from donors younger than 40 years to 2059.4 ± 313.1 cells/mm^2 for corneas retrieved from donors older than 80 years ($P < 0.001$). This significant decrease in endothelial cell density could also be

Table 2 Influence of factors on specific risk of inadequate endothelium, positive serology, and contamination

Risk factors	*Inadequate endothelium*		*Positive serology*		*Contamination*	
	OR (95% CI)	P	*OR (95% CI)*	P	*OR (95% CI)*	P
Donor age (years) (baseline: age < 40 (n = 177))						
40–59 ($n = 491$)	0.89 (0.47–1.37)	0.42	1.00 (0.52–1.93)	0.99	0.93 (0.49–1.78)	0.83
60–79 ($n = 1225$)	1.22 (0.74–2.04)	0.43	0.92 (0.44–1.53)	0.53	0.97 (0.51–1.82)	0.23
> 80 ($n = 703$)	2.37 (1.41–3.98)	< 0.001	1.12 (0.60–2.13)	0.71	0.94 (0.49–1.79)	0.41
Harvested (baseline: MOD (n = 303))						
SOD ($n = 2293$)	1.70 (1.11–2.64)	0.01	19.59 (7.1–54.8)	0.0001	0.95 (0.45–2.39)	0.57
DTT (hours) (baseline: DET < 6 (n = 1247))						
6–12 ($n = 698$)	1.02 (0.81–1.31)	0.81	1.43 (1.06–1.94)	0.02	1.04 (0.87–1.59)	0.14
> 12 ($n = 651$)	0.85 (0.62–1.05)	0.11	2.32 (1.74–3.10)	0.0001	1.53 (0.99–2.37)	0.21
TRT (hours) (baseline: ERT < 24 (n = 1017))						
24–48 ($n = 1181$)	0.94 (0.32–1.54)	0.24	0.89 (0.69–1.16)	0.40	1.57 (1.04–2.37)	0.03
> 48 ($n = 398$)	1.09 (0.59–1.65)	0.15	1.08 (0.75–1.57)	0.67	2.82 (1.76–4.54)	0.001

Abbreviations: CI, confidence interval; DTT, death to tissue retrieval time (hours); OR, odds ratio; SOD, cornea harvested from a single-organ donor; TRT, tissue retrieval to reception time (hours).
Odds ratio indicates the risk compared with the baseline. For donor age, baseline is < 40 years. For harvested cornea, baseline is cornea harvested from a multi-organ donor. For DTT, baseline is < 6 h. For TRT, baseline is < 24 h.
Results for corneas discarded for 'other reasons' are not shown.

Table 3 Influence of donor age on the endothelial cell density

	Endothelial cell density (cell/mm²)					
	General data		*Corneas suitable for transplantation*		*Corneas discarded for endothelial non-qualification*	
Donor age						
< 40	2288.5 ± 217.8	*n = 177*	2330.6 ± 94.8	*n = 113*	1934.0 ± 389.2	*n = 24*
40–59	2227.1 ± 246.2	*n = 491*	2269.3 ± 124.9	*n = 311*	1864.6 ± 419.7	*n = 58*
60–79	2139.8 ± 250.8	*n = 1225*	2217.3 ± 126.2	*n = 734*	1770.9 ± 375.9	*n = 248*
> 80	2059.4 ± 313.1	*n = 703*	2199.0 ± 121.3	*n = 32*	1674.4 ± 370.3	*n = 228*
P-value	< 0.001		< 0.001		< 0.001	

observed for corneas suitable for transplantation and those discarded for endothelial non-qualification (Table 3). Donor age had no significant influences on the risk of contamination and positive serology.

Influence of donor status on suitability

For corneas retrieved from a single-organ donor, 45.4% were discarded as compared with 25.1% for corneas retrieved from a multi-organ donor. Among corneas retrieved from a single-organ donor, 14.5% were discarded for positive serology (*vs* 1.3% for corneas retrieved from a multi-organ donor) and 22.8% for inadequate endothelium (*vs* 11.6% for corneas retrieved from a multi-organ donor) (Table 1). As compared with corneas harvested from a multi-organ donor, the specific risk of positive serology (OR = 19.6, $P < 0.0001$) and the risk of inadequate endothelium (OR = 1.7, $P = 0.01$) were significantly higher (Table 2).

Influence of death-to-tissue retrieval time on suitability

Longer time between death and tissue retrieval was associated with increased risk of positive serology: from 10.8% for the time group under 6 h (baseline), to 11.9% for the time group 6–12 h (OR = 1.43; $P = 0.02$), and 18.4% for the time group up to 12 h (OR = 2.32; $P < 0.0001$) (Table 2).

Influence of tissue retrieval-to-reception time on suitability

Increased time between tissue retrieval and reception was associated with increased risk of contamination: from 3.6% for the time group under 24 h (baseline), to 6.4% for the time group 24–48 h (OR = 1.57; $P = 0.03$), and 11.3% for the time group up to 48 h (OR = 2.82; $P < 0.001$) (Table 2).

Table 4 Positive predictive value of suitability of corneas according to influencing factors (donor age, death-to-tissue retrieval time, and tissue retrieval-to-reception time)

	Number of suitable corneas	*Number of discarded corneas*	*Suitability positive predictive value (%)*
DA < 80 years; DTT < 6 h; TRT < 24 h	381	208	64.7
DA < 80 years; DTT < 6 h; TRT > 24 h	197	131	60.1
DA < 80 years; DTT > 6 h; TRT < 24 h	384	253	60.3
DA < 80 years; DTT > 6 h; TRT > 24 h	196	143	57.8
DA > 80 years; DTT < 6 h; TRT < 24 h	81	77	51.3
DA > 80 years; DTT < 6 h; TRT > 24 h	87	95	47.8
DA > 80 years; DTT > 6 h; TRT < 24 h	100	128	43.9
DA > 80 years; DTT > 6 h; TRT > 24 h	52	83	38.5

Abbreviations: DA, donor age (years); DTT, death-to-tissue retrieval time (hours); TRT, tissue retrieval-to-reception time (hours).
It represents the likelihood for a given cornea to be suitable for transplantation according to donor age, death-to- tissue retrieval time, and tissue retrieval-to-reception time.

Influence of donor cause of death on suitability

There were no significant relationships at multivariate level between the donor cause of death and each specific risk of unsuitability.

Positive predictive values

PPVs of suitability of corneas were calculated according to influencing factors (Table 4). The status of the donor (single-organ donor or multi-organ donor) was not included in this analysis as there were no multi-organ donors older than 80 years. The highest PPV (64.7%) was found for corneas retrieved from donors younger than 80 years in the 6 h following the donor's death, with tissue retrieval-to-reception time shorter than 24 h. The lowest PPV (38.5%) was found in the opposite case (donor age >80 years, death-to-tissue retrieval time >6 h, and tissue retrieval-to-reception time >24 h).

Discussion

In the present retrospective study at a single regional eye bank, the leading cause for discarding corneas before transplantation was poor endothelial quality, which was in turn associated with donor age greater than 80 years. Longer time between death and tissue retrieval was associated with increased risk of positive serology. Increased time between tissue retrieval and reception was associated with increased risk of contamination. For corneas obtained from donors older than 80 years, the risk of inadequate endothelium increased by 2.37%. Previous reports have shown that corneas retrieved from old donors were discarded in more than 50% of cases.[5,12,13] For the study by Adán *et al*[14] the highest proportion of corneas classified as 'Excellent' or 'Good' came from young donors. Although a considerable proportion of the donated corneas came from older donors, the majority of corneas used for optical transplants came from younger ones in this study. Our results are in accordance with those of Gain *et al*[13] who established a difference between old and young donors only for endothelial assessment, and with Armitage *et al*[5] who showed that the risk of endothelial non-qualification increased with the age of the donor. The age-related endothelial cell loss was estimated at 0.6% per year for normal patients.[15] However, for corneas retrieved from old donors, the cell loss during storage was shown to be lower than for corneas retrieved from younger donors.[4,5,13] In addition, after transplantation, graft survival for corneal grafts harvested from old donors was not lower than that for corneas from young donors.[4,13] Even if the risk of unsuitability for endothelial non-qualification increases with donor age, the report from the New Zealand National Eye Bank did not find any relationships between donor age and overall suitability.[16]

In the present study, none of the causes of death showed any significant association with the different causes of unsuitability. Robert *et al*[17] who conducted a specific study of corneas from septic donors did not find the risk of contamination for septic donors to be greater than that for aseptic donors. This finding was confirmed by Spelsberg *et al*.[18] Conversely, Armitage *et al*[5] reported from a large series of more than 9000 corneas an increased risk of contamination for corneas obtained from septic donors (OR = 1.9). In the report from the New Zealand National Eye Bank,[16] corneal tissue from donors with a cardiovascular or cerebrovascular cause of death was more likely to be suitable for transplantation. Nevertheless, as was discussed by the authors, the high proportion of donors from these groups (67.2%) who died suddenly with less morbidity may account for higher suitability. Another short series suggested a possible link between post-keratoplasty endophthalmitis and donor malignancy.[19] This was confirmed by a study by the Eye Bank Association of America,[20] which reported that hospitalization or cancer at the time of

death more than doubled the OR of post-keratoplasty endophthalmitis. This is possibly due to exposition of donors to nosocomial pathogens. In addition, Van Meter[21] reported five cases of dense central sub-epithelial scarring after penetrating keratoplasty using corneal grafts obtained from donors with a history of cancer and systemic chemotherapy with antimetabolite drugs during the last 8 weeks before death. In all five patients, corneal epithelial damage was attributed in part to the effect of alkylating agents present in the corneal tear film.

We found a strong correlation between death-to-tissue retrieval time and the risk of positive serology. Cahane *et al*[22] suggested that delay in harvesting corneas may give rise to false-positive serology. In contrast with previous reports, we did not find any relationships between death-to-tissue retrieval time and the risk of inadequate endothelium.[5,23] Nevertheless, for Armitage *et al*[5] this relationship was weak (OR = 0.988). In the present study, the mean death-to-tissue retrieval time (8.4 h) was quite short as compared with that in previous reports (10–26 h).[5,13,18,24,25]

We did not find any relationships between death-to-preservation time and the risk of contamination, whereas previous reports had suggested that a death-to-preservation time of more than 15 h was associated with increased risk of positive microbial rim culture.[26] However, this result was significant only in univariate analysis and not in multivariate analysis.[26] Interestingly, the authors showed that slit-lamp quality rating of corneal tissue was associated with the results of donor corneal rim cultures. It has been suggested that stromal oedema may allow bacteria to enter the donor cornea.[27] Finally, in the New Zealand National Eye Bank report,[16] the mean death-to-preservation time was 15.2 h and it did not differ between corneas that were suitable for transplantation and those that were unsuitable.

In the present study, the risk of contamination increased with tissue retrieval-to-reception time. Armitage *et al*[5] showed weak influence of this factor on the likelihood of contamination (OR = 1.004, 95% CI = 0.999–1.009). For the study by Hassan *et al*,[20] corneas obtained and transplanted after 5 days of donation were more likely to be associated with post-keratoplasty endophthalmitis. In addition, the risk of endophthalmitis rose by 17% for every 10 years of recipient age in this study. As opposed to our results, two studies reported an influence of the tissue retrieval-to-reception time on the risk of endothelial non-qualification.[12,23]

Conclusion

After analysis of 2596 donor corneas processed in a unique regional eye bank and stored by organ culture, the following criteria may be used to reduce the percentage of discarded corneas: donor age 80 years or less, time from donor's death to tissue and blood sample retrieval shorter than 6 h, and time from tissue retrieval to reception at the eye bank shorter than 24 h.

Summary

What was known before

- To determine the factors related to donor and tissue retrieval, which influence the suitability of organ-cultured corneas for transplantation.

What this study adds

- Donor age, death-to-tissue retrieval time, and tissue retrieval-to-reception time are important predictive factors for corneal suitability

Conflict of interest

The authors declare no conflict of interest.

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