Cornea Procurement and Processing up to 72 Hours: No Risk for Cornea Transplant Quality

Nicola Hofmann Ilka Wittmershaus Anna-Katharina Salz Martin Börgel

German Society for Tissue Transplantation (DGFG) gGmbH, Hannover, Germany

Keywords

Tissue donation · Death to preservation time · Cornea safety and quality

Abstract

Background: The realization of tissue donations is bound to a tight timeframe. Depending on the type of tissue, time limits are specified within which the donation must be procured and processed. Otherwise, there is a risk of tissue quality loss with increasing time intervals from cardiovascular arrest. According to the European Directorate for the Quality of Medicines and HealthCare (EDQM) guide, cornea must be procured and processed within 72 h after death. The question arises whether this time interval has an influence on the quality of transplanted tissues and how it affects the accomplishment of tissue donations. **Methods:** In order to obtain information on this, the numbers of tissue donations in the network of the German Society for Tissue Transplantation (DGFG) were evaluated as a function of the death to retrieval time (DRT) as well as the death to preservation time (DPT). For this purpose, 21,454 database entries of cornea donations made in the period from 2014 to 2018 were included. **Results:** The results show that nearly 50% of donations realized in the DGFG network could be processed only 48 h or later after cardiovascular death due to the opt-in regulation in Germany. For these donations, there seems to be a higher discard rate compared to donations taken earlier. Nevertheless, there is a transplantation rate for these grafts of more

than 65%, which is comparable to average transplantation rates stated in the literature. **Conclusion:** All corneas finally selected for transplantation must meet the specified quality parameters. Since this naturally also applies to transplants that could only be procured at later time points, it can be concluded that DPT up to 72 h for corneal tissue is adequate and has no influence on the quality of corneas that are ultimately transplanted.

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Introduction

Cornea transplantation is one of the most common and successfully performed kind of transplantations [1]. The reasons are, among other aspects, the diligence of tissue banks that guarantees the quality of donor corneas, and guidelines that lay down the applicable quality and safety framework [2, 3].

The success of corneal transplantation depends to a large extent on the quality of the transplant used [4]. Various factors play a decisive role, such as a flawless stroma without optically relevant, centrally located opacities or alterations (e.g., scars), and an adherent Descemet membrane. One of the most important parameters is the quality of the endothelial cell layer [5, 6]. The most utilized quality features are the morphology and especially the cell density of the corneal endothelium (endothelial cell density; ECD). The corneal endothelium consists of postmi-

Table 1. Evaluation of the data sets from 2014 to 2018

Year	Corneas processed	Evaluable data sets	Allocated grafts (transplantation rate), <i>n</i> (%)	Average donor age, years	Median donor age, years
2014	3,664	3,446	2,313 (67.12)	73.2	76
2015	4,020	3,950	2,605 (65.94)	72.8	75
2016	4,512	4,441	3,000 (67.55)	72.7	75
2017	4,507	4,431	3,051 (68.85)	72.1	75
2018	5,249	5,186	3,653 (70.43)	71.3	74
Total	21,952	21,454	14,622 (68.16)	72.3	75

Corneal donation is feasible until high age. Half of all tissue donors between 2014 and 2018 were 75 years old or older.

totic cells that renew little or not at all by division [7, 8]. It is, therefore, understandable that the question arises how the time interval between cardiac arrest and corneal organ culture (death to preservation time; DPT) influences the quality of the tissue and, ultimately, the success of the transplantation. According to the European Directorate for the Quality of Medicines and HealthCare (EDQM) guide [9], the cornea must be procured and processed within a tight time frame of 72 h after death. At the same time, it is important to understand how these time constraints can affect the realization of tissue donations.

Data on 21,454 procured corneas in the time period from 2014 to 2018 within the German Society for Tissue Transplantation (DGFG) and its network were analysed. First, the transplantation rate as well as the ECD can be used as a measure of the quality of the retrieved and processed corneas. This was examined in relation to different time intervals of procurement and processing of the donated tissue. Another factor is the discard rate, which was also studied in relation to the time intervals. The age of the donor was used as a further parameter, as this can also have an influence on the quality of the retrieved tissue. Additionally, the clinical importance of DPT was evaluated by the need for re-grafting within 1 year.

Material and Methods

During 2014–2018, a total of 3,664–5,249 corneas were processed annually at the tissue banks that are owned by or cooperate with the DGFG (Table 1). In the DGFG network, the cornea is procured under aseptic conditions through trained employees by enucleation of the whole globe. The globes are transported in a humid chamber in a validated transport system at 1–10 °C up to a maximum of 24 h to the processing tissue bank. After disinfection, the corneoscleral disc with a diameter of 16 mm is trephined and stored in a Böhnke corneal holder in organ culture for a maximum of 34 days.

For each cornea processed, the process data is documented in a pseudonymised form in a central database. This enables the anal-

ysis of individual process steps as well as superordinate correlations.

For this study, cornea donations taken in the period from 2014 to 2018 were considered. 21,454 data sets were examined for various parameters, such as age of the donor, quality of the preparation, ECD, discard rate (including reasons for discarding, such as microbial contamination), and allocation for transplantation. Additionally, 2 separate time periods were considered. The first is the period from cardiac arrest to tissue retrieval (death to retrieval time; DRT), and the second is the total period from cardiac arrest to the actual processing of the cornea in the tissue bank (DPT). For cardiovascular deaths, the time of death corresponds to the moment of cardiac arrest; for multi-organ donors, the time of the start of perfusion is taken.

For the evaluation of the influence of DRT, the data sets were divided into 3 groups in accordance with international practice: the first group represents the collections where the donation could be realized within 24 h ($DRT \leq 24$ h). The second group consists of donations retrieved between over 24 and 48 h after cardiac arrest ($DRT > 24$ and ≤ 48 h), and the third group includes donations retrieved between over 48 and 72 h ($DRT > 48$ and ≤ 72 h). Analogously, the data sets were assigned to 3 groups where the donation could be realized and processed in the tissue bank within the corresponding time periods ($DPT \leq 24$ h, $DPT > 24$ and ≤ 48 h, $DPT > 48$ and ≤ 72 h). It should be noted that donations that could only be collected at 72 h were not included in the evaluation, as the time for possible processing up to a maximum of 72 h after death would have been exceeded.

In order to investigate whether there is a connection between a transplant failure and a time group within the DPT, an indirect method was used: data of all graft allocations in 2018 were examined for registrations for re-transplantation.

The analysis was carried out with Microsoft Excel 365. All complete data sets with a maximum DPT of up to 72 h were included. 498 data records with missing or incorrect time information on the time of death, time of retrieval and/or time of preparation were excluded (Table 1: 21,454 evaluable data sets out of 21,952 processed cornea). For the analysis of the influence of the factors on ECD, only transplanted corneas (14,564 data sets) were included, as there is often no final cell count recorded for corneas that were not released for transplantation.

Statistics

Descriptive statistics comprised medians, means and interquartiles. As statistical test a χ^2 test was used where applicable. For

Fig. 1. a Total donations and donor age related to DRT (DGFG, 2014–2018, $n = 21,454$ corneas). **b** Total donations and donor age related to DPT (DGFG, 2014–2018, $n = 21,454$ corneas).

the evaluation of the influence of DRT, DPT and donor age on ECD, the software *R* was used, and a linear regression model with the dependent variable $y = \text{ECD}$ and the independent variables $x = \text{donor age, DRT and DPT}$ was applied. In order to calculate the strength of influence of the independent variables, a multiple regression analysis was performed. Since DPT and DRT are interdependent, 2 models were created.

Results

Death to Retrieval Time

First, the data were evaluated with regard to the period from cardiac arrest to the retrieval/procurement of the tissue (DRT). Of 21,454 donations during the study period (2014–2018), 8,572 donations could be retrieved

within 24 h after death ($\text{DRT} \leq 24 \text{ h}$). This corresponds to a rate of 39.96%. Another 9,309 (43.39%) donations were collected within 48 h ($\text{DRT} > 24 \text{ h}$ and $\leq 48 \text{ h}$), and 3,573 (16.65%) between 48 and 72 h ($\text{DRT} > 48 \text{ h}$ and $\leq 72 \text{ h}$; Fig. 1a). The mean value of all donations taken during this period was 30.4 h (median 28.3 h, min. 0 h, max. 72 h).

Death to Preservation Time

In contrast to DRT, the transport to the tissue bank and handling for organ culture must be included in the calculation for the DPT. Considering this time period, 1,775 corneas were procured, transported to the tissue bank and processed in $< 24 \text{ h}$ after death ($\text{DPT} \leq 24 \text{ h}$). This corresponds to a ratio of 8.27% of all corneal dona-

Fig. 2. a Tx rate and discard rate related to DRT (DGFG, 2014–2018, $n = 21,454$ corneas) (Σ Tx rate: DRT ≤ 24 h: 73.02%; DRT > 24 and ≤ 48 h: 66.39%; DRT > 48 and ≤ 72 h: 61.10%; total: 68.16%). **b** Tx rate and discard rate related to DPT (DGFG, 2014–2018, $n = 21,454$ corneas) (Σ Tx rate: DPT ≤ 24 h: 75.72%; DPT > 24 and ≤ 48 h: 70.77%; DPT > 48 and ≤ 72 h: 64.52%; total: 68.16%). Tx, transplantation.

tions realized (Fig. 1b). A much larger proportion of the donations (9,296; 43.33%) was taken into culture in the tissue bank between 24 and 48 h after cardiac arrest (DPT > 24 and ≤ 48 h). The major part, namely 48.40% (10,383) of corneas had a DPT of 48–72 h. On average, the corneas were processed within 47.1 h (median 47.4 h, min. 1.8 h, max. 72 h).

Transplantation and Discard Rate

The transplantation rate of cornea donations obtained in the DGFG network is about 68% on average. This means that more than 2 thirds of all retrieved and cultivated corneas meet the necessary high-quality criteria and are ultimately used for a patient. This amount includes 63.86% so-called optical corneas which have a cell

Fig. 3. **a** Endothelial cell density (cells/mm²) related to donor age (DGFG, 2014–2018, $n = 14,564$) (grey dots; $F[1.14562] = 869.1$, $p < 2.2e-16$). **b** Endothelial cell density (cells/mm²) related to DRT (DGFG, 2014–2018, $n = 14,564$) (grey dots; $F[1.14562] = 234.5$, $p < 2.2e-16$). **c** Endothelial cell density (cells/mm²) related to DPT (DGFG, 2014–2018, $n = 14,564$) (grey dots; $F[1.14562] = 124.9$, $p < 2.2e-16$).

count of more than 2,000 endothelial cells/mm² at the final evaluation. In addition, 4.29% of corneas are transplanted that have a slightly lower cell count and are, therefore, used as stromal/temporal corneal replacement (Fig. 2a, b, right column each).

If the transplantation rate is considered separately by time of retrieval (DRT), it can be seen that the rate is highest (73.02%; Fig. 2a) within the corneas that could already be procured after a maximum of 24 h (DRT ≤ 24 h). The same is the case when the DPT (<24 h) is selected, where the transplantation rate is 75.72% (Fig. 2b). In the group of DRT >24 and ≤48 h, the transplantation rate is 66.38% (Fig. 2a). If the donation is successfully processed within this period (DPT >24 and ≤48 h), the rate increases to 70.77% (Fig. 2b).

In contrast, the rate is lower if a longer time period elapses before the donation can be procured (DRT >48 and ≤72 h). Here, the transplantation rate is 61.09% (Fig. 2a). It is also lower (64.52%) if the donation can be processed and taken in organ culture within this time frame (DPT >48 and ≤72 h; Fig. 2b).

Accordingly, the discard rate is inversely proportional to the transplantation rate (Fig. 2a, b, upper bars). In general, the main reasons why a donation cannot be selected as a transplant are contamination/lack of sterility, endothelial cell necrosis, low ECD on entry or before delivery from the tissue bank, and scarring or stromal opacity. In the period under investigation, the overall discard rate in the DGFG network averaged 32%.

The longer the period from cardiac arrest, the higher is the probability of tissue damage mainly concerning endothelial cell necrosis or low ECD. This is reflected in higher discard rates, which occur when the tissue can only be collected after a longer period of time (DRT)

or tissue processing and organ culture starts later (DPT).

Within these time periods, it was observed that contamination occurs slightly more frequently the later the donation was realized (DRT ≤ 24 h: 3%; DRT >24 and ≤48 h: 3.7%; DRT >48 and ≤72 h: 5.3%; $p < 0.001$). The contamination rates are similar when the DPT is considered (DPT ≤ 24 h: 3%; DPT >24 and ≤48 h: 3.5%; DPT >48 and ≤72 h: 4%, $p = 0.0156$). The p values of the χ^2 test indicate that the correlation is significant.

Donor Age

Another parameter to be taken into account in terms of transplant quality is the donor's age. The average age of donors in the DGFG network was 72.3 years in the period under investigation (Table 1).

Looking at the average age of the donors in relation to the time of donation, a parallel distribution can be seen. The donors whose tissues were retrieved after the shortest time period were younger, 70.4 years on average, than those whose tissues were procured after 24 h (73.6 years for DRT >24 and ≤48 h and 73.9 years for DRT >48 and ≤72 h; Fig. 1a). An even clearer trend can be seen when looking at the DPT (Fig. 1b). Here, the average age is 67.8 years in the group of donations that were retrieved and processed within 24 h (DPT ≤ 24 h). It is 71.9 years for DPT >24 and ≤48 h and 73.5 years for DPT >48 and ≤72 h.

Endothelial Cell Density

Since ECD is considered one of the main quality criteria for the grade of a corneal graft, this parameter was also considered in relation to the different time intervals for DRT and DPT or donor age. Figure 3 shows that the cor-

Table 2. Registration for re-transplantation in relation to DPT (2018)

DPT (death to preservation time)	Allocated transplants in 2018	Re-registrations	% registration for re-transplantation	Ø donor age in years
DPT ≤24 h	331	10	3.02	64.5
DPT >24 and ≤48 h	1,720	60	3.49	68.3
DPT >48 and ≤72 h	1,602	62	3.87	74.5
Total	3,653	132	3.61	70.9

relation between donor age and ECD is significant. From the slope of the straight line (R^2), the strength of the influence of the considered parameters on ECD can be concluded. The steeper the straight line, the stronger the effect. Accordingly, the impact of donor age ($R^2 = 0.05632$; Fig. 3a) on ECD is stronger than DRT ($R^2 = 0.01587$; Fig. 3b) and DPT ($R^2 = 0.0085$; Fig. 3c). This result was also confirmed by the multiple regression analysis, where the standardized regression coefficient of donor age of -0.22 was larger than that of -0.09 for DRT, or of -0.23 for donor age compared to -0.06 for DPT.

Registration for Re-Transplantation

In 2018, a total of 3,653 corneas from the DGFG network were delivered for transplantation. Of these, 132 patients underwent a second operation on the same eye within the same year. If these registrations for a re-transplantation are considered separately according to the groups of DPT, an even distribution of the graft failure rate can be observed independent of the time of retrieval and processing. In all 3 time groups, a re-transplantation rate of about 3% was detected (Table 2). χ^2 test resulted in a $p = 0.6998$, showing that there was no significant difference between the 3 groups.

Discussion

The present study seems to confirm that the period of time between cardiac arrest and the realization of a donation has an influence on the procured and processed cornea tissue. After evaluating the data of 21,454 cornea donations, it can be seen that an up to 12% higher discard rate must be accepted if a donation can only be taken at a later time. This result could be related to the negative influence of longer time intervals on the ECD. At the same time, the transplantation rate is naturally also lower in these cases, a finding that is also described by Röck et al. [10]. A distinction must still be made between the time when the donation is retrieved and the time when the tissue is finally processed in the tissue bank and transferred to the organ culture.

While in the DGFG network almost 40% of all donations are procured within 24 h and a total of more than 80% are still realised within 48 h, the processing of the majority of donations takes place after a longer period of time, with almost 50% only between 48 and 72 h. Hence, this survey makes it clear that a substantial proportion of all donations could no longer be realised if the permitted processing period would be shortened.

The extended time periods are partly due to the opt-in solution in Germany. Before any consent to donation, the relatives are given the opportunity for several interviews to make an informed decision. In addition, the network structure of DGFG accounts for the DPT, as donations are collected nationwide and then transported to one of the network's tissue banks. While this concept ensures a high supply rate of corneas in Germany, it must accept more extensive logistics.

The analysis points out that all tissues released for transplantation have the same high quality, regardless of the time at which they were retrieved and processed, because only a very weak effect of higher DRT and DPT on ECD of corneas that were allocated for transplantation was found. This is also supported by the findings of analysing the registrations for a re-transplantation. Among the patients re-registered in 2018, no accumulation of transplant failure was found in any of the DPT time groups studied. The high standards on the quality parameters must be met in any case, otherwise the graft will not be approved for transplantation into patients.

Since tissue quality is of particular importance for the success of the transplantation, other authors have already dealt with the question of what influence the time of retrieval and processing might have on corneal integrity. However, most of these investigations refer to short periods of time of maximum 12 h DPT. Especially in those countries where cold storage is predominant, all processes are very much shortened. Within this short period of time, the authors could not detect any negative effects on graft quality or clinical outcome [11–15]. The epithelium seems to be slightly affected with increasing time [16]. However, this occurs anyway during organ culture that is mainly used in Europe and has no influence on the trans-

plantation result due to the regeneration potency of the epithelial cells [17, 18].

The results of the present study are in agreement with other investigations. Andersen and Ehlers [19] found no influence with a mean post-mortem time of 40.5 h on graft survival after 5 years.

Also, according to Armitage et al. [20], post-mortem times have no influence on 5-year graft survival, although it is not clear which overall time span was considered. In their work, the time from death to retrieval and from retrieval to preparation (RPT) was listed separately with DRT of a maximum of 24 h and time from retrieval to preparation of a maximum of 30 h, so time intervals of up to 54 h from death to preparation might have been included. The authors conclude that, for graft failure, the indication for penetrating keratoplasty and other recipient factors were the main predictors.

Various other groups also conclude that longer post-mortem periods (up to 72 h DPT) have no influence on the clinical outcome [21–25] or the postoperative ECD loss [5, 26]. This also applies when lamellar keratoplasty techniques (e.g., Descemet membrane endothelial keratoplasty or deep anterior lamellar keratoplasty) are used. Post-mortem intervals of up to 63 h did not influence the clinical outcome of these grafts [27, 28].

Only one report [29] showed a clearly negative influence of longer DPT on chronic endothelial cell loss after transplantation in a small group of 53 keratoconus patients. It was calculated according to a linear regression model, but this approach assumes equivalence of loss of central ECD and chronic endothelial cell loss. In parallel, there was also a significant correlation with donor age.

In a study in France [30], as in the present study, an influence of different time intervals on a higher discard rate was detected. However, a direct comparison is difficult, as a distinction was made between the time from death to retrieval and from retrieval to preparation. It remains unclear whether time intervals of up to 72 h from death to preparation were included. Nevertheless, the authors also found a correlation between the time interval between donation and death and the occurrence of contamination in organ culture.

An increased contamination rate during longer transport (i.e., DRT times) was also observed by Gruenert et al. [31]. Interestingly, they found a link between younger donor age and higher risk of contamination.

This connection could not be identified in the present study. On the contrary, the lowest contamination rates were found in the group of younger donors. Rather, the correlation of donor age with the discard rate or inversely proportional to the transplantation rate is remarkable. The older the donors were on average, the fewer corneas ultimately met the necessary quality criteria for transplantation. This result is not surprising since the endothe-

lial cell count, which is one of the main quality criteria, decreases with increasing age [30]. This observation was again confirmed in the present study where the donor age was found to have a higher impact on ECD than longer DRT or DPT. In addition, a larger number of donors have already undergone previous interventions, such as cataract surgery, which also has a negative effect on cell density [32]. Besides this, these surgeries cause scars, which also can lead to the fact that the cornea cannot be transplanted if the optical zone is affected.

In a review of data of more than 30 years from a German tissue bank, Linke et al. [33] also found a correlation between the discard rate and donor age. In addition, they also examined the influence of DRT. Their results are consistent with the present study, as the same distributions of discard rate, transplantation rate, donor age and DRT were found. They conclude that the higher discard rates with longer DRT are probably more likely attributable to the higher donor age than to the longer time span until procurement. The average DRT in their study was approximately 38 h and, thus, higher than the average in the network of the DGFG (~30 h). Like in the present investigation, times of 72 h DRT are considered also by Linke et al. [33].

For the patient's safety and the transplantation result, the quality of the cornea that is donated is most important. Although other authors have described that the discard rate of donations increases with the age of the donor, for example, due to insufficient endothelial cell count, the clinical outcome after cornea transplantation does not depend on the donor age [34, 35].

Summary and Conclusion

There is no clear correlation between clinical outcome of transplantation and DPT. Increasing duration of DPT can be accompanied by a decrease in ECD. However, duration of DPT also correlates with donor age, and lower ECD is more likely to be attributed to the age of the donors, leading to a reduced transplantation rate of corneas with longer DPT intervals. Additionally, the contamination rate of corneas with longer DPT might be slightly higher.

Nearly 50% of the donations realized in the DGFG network can only be retrieved and preserved more than 48 h after death. Therefore, a reduction of the permitted retrieval and preservation period would have a significantly negative impact on the supply of high-quality transplants in Germany.

Consistent with the literature data, our report shows that a time span of up to 72 h for retrieval and preparation of donor corneas has no influence on the tissue quality nor the transplantation success.

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Statement of Ethics

The reported data are results from an analysis of data that are collected as part of standard procedures in relation to cornea donation, thus not requiring approval by the Ethics Committee. All data were documented after receiving informed consent of the relatives of the cornea donors according to German legislation. The authors confirm that the study and data accumulation conform with all federal or state laws and that the study was in adherence to the tenets of the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

N.H.: study design, data collection and analysis, writing the manuscript, administrative necessities. I.W.: data collection and analysis, writing the manuscript. A.-K.S.: writing the manuscript. M.B.: supervision, writing the manuscript.

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