

## SCIENTIFIC CORRESPONDENCE

## Influencing factors on chronic endothelial cell loss characterised in a homogeneous group of patients

Daniel Böhringer, Thomas Reinhard, Helga Spelsberg, Rainer Sundmacher

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**Background/aim:** Advanced donor age, long death to excision time interval, and factors related to organ culture can trigger unfavourable intracellular processes in the graft endothelium and contribute to chronic endothelial cell loss after penetrating keratoplasty. The aim of this study was to investigate factors influencing chronic endothelial cell loss in a homogeneous group of patients.

**Methods:** 177 patients after first normal risk keratoplasties for keratoconus were retrospectively selected from the quality control database of our clinic. For 71 of them at least four central endothelial cell density values were documented in follow up. From these patients, only those 53 without any further intraocular procedures, without glaucoma, and without graft rejection were considered. A scatter plot of logarithmised endothelial cell density values against postoperative time was drawn for each patient. The slope of the regression line then equals the constant of decay in central endothelial cell density. The influence of donor age and storage time in organ culture on this index value of cell loss was investigated by means of linear regression analysis.

**Results:** Mean loss of central endothelial cell density was 16.7% per year. Regression analysis revealed a statistically significant negative linear effect of both postmortem time ( $\beta = -0.324$ ;  $p = 0.014$ ) and donor age ( $\beta = -0.282$ ;  $p = 0.036$ ) and a trend for storage time in organ culture ( $\beta = -0.195$ ;  $p = 0.142$ ) in a combined linear regression model.

**Conclusion:** Increased postmortem time and advanced donor age exert a significant negative effect on chronic endothelial cell loss. Storage time in organ culture seems to be third influencing factor. These negative influences may be reduced by compensating advanced donor age with minimised postmortem and storage time.

Chronic endothelial cell loss is routinely observed after penetrating keratoplasty.<sup>1–6</sup> It is by definition a continuous decrease of endothelial cell density over time in the absence of any clinically evident immune reaction. The annual rate of cell loss is about 7.8% from the third to the fifth year after keratoplasty.<sup>5</sup> When assuming exponential decay of endothelial cell density, this rate is compatible with the finding that after 10 years only 35% of the initial graft endothelial cells are left.<sup>4</sup> Central endothelial cell loss in keratoplasty is at least one order of magnitude above the age related endothelial cell loss in healthy corneas of about 0.5% per year.<sup>7</sup> This finding implies that despite evidence for clear long term graft survival for over 30 years<sup>8</sup> an increased rate of re-keratoplasties due to endothelial failure can not be ruled out in future. Hence, it is mandatory to define and optimise influencing factors on chronic endothelial cell loss in order to improve long term graft prognosis.

The influence of both donor age<sup>9</sup> and storage time in organ culture<sup>10–12</sup> on chronic endothelial cell loss is the subject of controversial discussion. Little is known about the respective influence of death to excision (postmortem) time interval. Patient age as well as the total endothelial age<sup>13</sup> (sum of patient and donor age) may play a part as well. In order to determine the influence of these factors on chronic endothelial cell loss, a homogeneous study population without any confounding additional damaging influences of the graft endothelium (ophthalmological diagnosis, glaucoma, further intraocular surgery, and graft rejection<sup>14</sup>) needs to be followed up using a longitudinal design instead of only a cross sectional one.<sup>15</sup>

## MATERIALS AND METHODS

## Patients

A total of 177 patients after first normal risk keratoplasty for keratoconus were retrospectively selected from the quality control database of our clinic. For 71 of them at least four central endothelial cell density values had been documented on follow up. Only those 53 patients in this group without any further intraocular procedures, without glaucoma, and without immune reactions were included in the analysis. All patient data are given in Table 1.

## Surgery

Penetrating keratoplasty was performed by only three experienced surgeons according to standardised procedures: a modified Franceschetti's trephine was used for trephination. The graft was cut from the endothelial (7.7 mm) side, the recipient cornea from the epithelial (7.5 mm) side. The graft was temporarily fixed using 10-0 nylon sutures at the 3, 6, 9, and 12 o'clock position. Definitive fixation of the graft was performed with a double running diagonal suture<sup>16</sup> with  $2 \times 8$  cross stitches. The first suture was not removed before the fourth, the second one not before the 12th month after keratoplasty. Suture removal was performed in topical anaesthesia with oxybuprocaine (proxymetacaine) eye drops.

## Grafts

Only donor corneas that met the EEBA criteria<sup>17</sup> were used for surgery. All corneas had been held in organ culture for at least

**Table 1** Relevant data of patients, organ culture, and donors (mean (SD))

Age of patients (years)	35.2 (14.0)
Female/male patients	21/32
Age of donors (years)	46.1 (20.5)
Female/male donors	25/28
Postmortem time (hours)	13.3 (14.0)
Storage time in organ culture (days)	17.2 (20.5)
Endothelial cell density after organ culture (cells/mm <sup>2</sup> )	2395.8 (355.4)
Follow up (years)	2.4 (1.2)

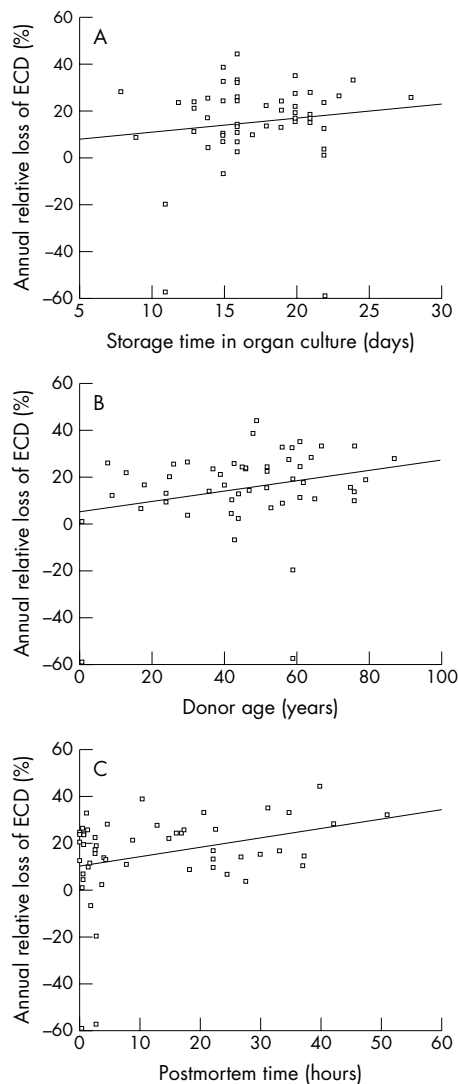
10 days. A postmortem time longer than 72 hours was an exclusion criterion. Data of donors are given in Table 1. Corneal endothelium was assessed using phase contrast microscopy after cell border swelling in hypotonic solution and a digital cell evaluation system<sup>18</sup> in the 36 recent grafts after availability of this analysis tool for routine use at the Lions cornea bank NRW.

### Medical treatment

Directly after keratoplasty 0.5 ml gentamicin 4% and 0.5 ml dexamethasone 0.8% were injected subconjunctivally. Acetazolamide was administered orally in order to prevent a postoperative rise in intraocular pressure. One mg fluocortolone/body mass kg was routinely given orally and tapered off in 2 weeks. Topical gentamicin 0.5% was applied at least five times daily until complete re-epithelialisation. Topical prednisolone 1% eye drops were given five times daily during the first month, four times daily during the second, three times in the third month, twice in the fourth, and once in the fifth month postoperatively. No topical steroids were applied afterwards.

### Follow up

Visits were scheduled 6 weeks, 4, and 12 months postoperatively. Long term follow up was scheduled yearly.



**Figure 1** Relative annual loss of endothelial cell density (ECD) with respect to (A) storage time in organ culture, (B) donor age, and (C) postmortem time.

### Measurement of chronic endothelial cell loss and statistical analysis

Photographs of graft endothelium were taken with the non-contact specular microscope Robo Noncon (Konan, Japan). This system enables the investigator to calculate semi-automatically the central endothelial cell density in a reliable and correct way.<sup>19</sup> At least 15 cells were entered in the semi-automatic cell density algorithm of the Robo Noncon for each photograph.

Reduction of central endothelial cell density was assumed to follow the dynamics of first order decay over follow up. For each patient at least four individual central endothelial cell density values had been recorded. A scatter plot of the logarithmically transformed cell density values against postoperative time was drawn for each patient. The slope of the regression line then equals the constant of decay of individual loss of central endothelial cell density. This highly derived index is suitable to assess individual loss of cell density.<sup>15, 20</sup>

The influence of total endothelial age, patient and donor age, postmortem time, and time in organ culture on this derived index value was investigated by means of linear regression analysis.

Patient age, donor age, and total endothelial age were highly significantly correlated ( $p < 0.00001$ , coefficients  $> 0.6$ ) due to strict donor and patient age matching policy. Hence, the influence on central endothelial cell density of donor age, patient age and total endothelial age were analysed separately, each in combination with postmortem time and storage time in organ culture, in three linear regression models.

Statistical analysis was done using SPSS 10 on Windows NT 4.

### RESULTS

The overall constant of decay of loss of central endothelial cell density was found to be  $-5.03 \times 10^{-4}$  (SD  $5.18 \times 10^{-4}$ ) per day. This value can be interpreted as mean annual loss of 16.7% of the respective endothelial cell density left. The annual rates of loss of endothelial cell density are plotted against the respective factors (Fig 1) in order to vividly demonstrate the clinical significance of the factors considered.

The results of the three linear regression models are summarised in Table 2. The factors postmortem time, donor age, and storage time in combination (first model in Table 2) show the strongest influence on loss of endothelial cell density. This suggests that from the highly correlated factors of donor age, patient age, and total endothelial age, donor age is most important. A statistically significant negative influence on loss of endothelial cell density could only be demonstrated for postmortem time and donor age. Storage time in organ

**Table 2** Results from three linear regression models for the negative influences of total endothelial age, donor age, patient age, storage time in organ culture, and postmortem time on loss of endothelial cell density

	Standardised regression coefficient	p Value
Storage time in organ culture	-0.195	0.142
Postmortem time	-0.324	0.014
Age of donor	-0.282	0.036
Storage time in organ culture	-0.147	0.264
Postmortem time	-0.356	0.009
Age of patient	-0.214	0.109
Storage time in organ culture	-0.181	0.169
Postmortem time	-0.341	0.010
Total endothelial age	-0.278	0.037

culture seems to exert a negative influence on cell loss in this model as well, but misses statistical significance (Table 2). The standardised regression coefficients ( $\beta$  values) can be compared quantitatively with respect to degree of influence on loss of endothelial cell density.

## DISCUSSION

The aim of this study has been to evaluate influences of post-mortem time, storage time in organ culture, patient age, and donor age on chronic endothelial cell loss. This was done by correlating loss of central endothelial cell density to the above putative influencing factors. This approach assumes equivalence of loss of central endothelial cell density and chronic endothelial cell loss.

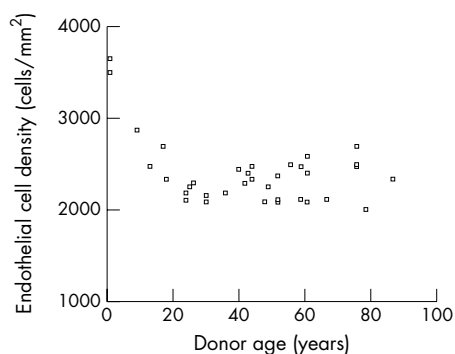
A homogeneous study group with only one ophthalmological diagnosis has the advantage of few potential confounding factors such as variable endothelial cell migration from graft to host.<sup>21</sup> Absence of confounding factors and longitudinal analysis of endothelial cell density are optimal prerequisites for statistically demonstrating existing influences from patient, organ culture, and donor variables and further underlines the assumption of equivalence of loss of central endothelial cell density and loss of endothelial cells.

Postmortem time was not found to influence long term endothelial cell loss in cross sectional studies with heterogeneous ophthalmological diagnoses,<sup>10–22</sup> but had the strongest negative effect on long term chronic endothelial cell loss in the homogeneous group of this study. The trigger for this dependence might be the postmortem change in intraocular solute concentrations.<sup>23</sup>

The influence of storage time in organ culture on long term chronic endothelial cell loss is subject to controversial discussion.<sup>10–12</sup> The same is true for the acceptance of donor age as an influencing factor on chronic endothelial cell loss. Only two of five studies reviewed recently<sup>9</sup> were able to demonstrate a statistically significant negative influence of donor age on long term chronic endothelial cell loss. Controversy, however, might again be due to the poor statistical power of cross sectional approaches and to the heterogeneity of respective study populations.

In the presented study donor age was found to have a significant negative effect on the long term endothelial prognosis. The influence of storage time in organ culture on the rate of endothelial cell loss missed statistical significance ( $p = 0.14$ ), but might play a part as well.

Metabolic changes<sup>24</sup> and changes in genetic expression profile<sup>25</sup> are known to occur during organ culture. These may cause the reported decline in the percentage of corneas suitable for keratoplasty with increasing storage time<sup>26</sup> as well as an increase in chronic endothelial cell loss.<sup>10–27</sup>



**Figure 2** Central endothelial cell density from phase contrast microscopy directly after culture and before keratoplasty plotted against donor age. Only the 36 most recent donors after availability of the digital automatic endothelial cell assessment system at the Lions cornea bank NRW are shown.

Since loss of endothelial cells in healthy corneas for life is exponential,<sup>28</sup> the rate of cellular loss only depends on current cell density, a finding well reflected by the donors of this study (Fig 2). This means that the rate of cellular loss should be the same in old and young donors. In theory, the percentage of endothelial cell density that is lost per year should thus be independent of donor age. The mechanisms responsible for the observed dependence of chronic endothelial cell loss on donor age might be triggered by death (toxic metabolites), organ culture (metabolism), or keratoplasty (denervation, trauma). In this context, the finding that the endothelium of old donors is more prone to endothelial damage in organ culture deserves some interest.<sup>26</sup> These robust cells in organ culture might be those that disappear early after keratoplasty and thus contribute to chronic endothelial cell loss.

Old donors can not generally be excluded from cornea donation due to shortage of grafts available for keratoplasty in Germany.<sup>29</sup> Furthermore, from a practical point of view, the difference between donors of, for example, 60 and 80 years on annual loss of cell density is quite moderate (Fig 1B). Hence, when grafts from old donors are given to old patients, late endothelial failure due to chronic endothelial cell loss should generally not be a problem within the patient's lifetime.

Since all influencing factors are shown to be additive by means of linear regression analysis, the adverse negative influence of advanced donor age on chronic endothelial cell loss can be alleviated by minimising postmortem time and possibly by limiting storage time in organ culture as well. This policy might help to reduce the problem of late endothelial failure due to chronic endothelial cell loss in future but still needs confirmation by means of a prospective randomised long term study.

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## Authors' affiliations

**D Böhlinger, T Reinhard, H Spelsberg, R Sundmacher**, Eye Hospital of Heinrich-Heine-University and LIONS Cornea Bank North Rhine Westphalia, Düsseldorf, Germany

Correspondence to: Dr med Daniel Böhlinger, Eye Hospital, Heinrich-Heine-University, D-40225 Düsseldorf, Germany; daniel.boehlinger@uni-duesseldorf.de

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