Brief Report

Association Between Graft Storage Time and Donor Age With Endothelial Cell Density and Graft Adherence After Descemet Membrane Endothelial Keratoplasty

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IMPORTANCE After retrospectively evaluating the clinical outcome of 500 consecutive cases after Descemet membrane endothelial keratoplasty (DMEK), we extended the analysis in this study by assessing the effect of donor-related parameters on endothelial cell density (ECD) decline and detachment rate in this group.

OBSERVATIONS This retrospective case series included 500 cases who had undergone DMEK from October 2007 to September 2012 at the Netherlands Institute for Innovative Ocular Surgery (NIIOS), Rotterdam, the Netherlands. Logistic regression analysis (n = 332 eyes) showed that donor age might be associated with a 3% increase in the risk for a detachment (odds ratio, 0.97; 95% CI, 0.94-1.00; *P* = .049) (ie, higher donor age seems to be associated with lower chances of a detachment). In addition, linear regression analysis indicated that graft storage time in medium was associated with ECD decrease (ie, the longer the storage time, the larger the decrease at 6 months after DMEK) (*P* = .01).

CONCLUSIONS AND RELEVANCE We showed an association between graft storage time and ECD decline after DMEK and possibly between donor age and graft detachment. Therefore, donor storage times should be kept as short as possible to improve short-term ECDs. More research is needed to draw definite conclusions on the possible effect of donor age on the chance of a detachment after DMEK.

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In the past decade, various techniques for endothelial keratoplasty, including Descemet membrane endothelial keratoplasty (DMEK), have been introduced.[1] To further refine this technique, we assessed the clinical outcome of the first 500 consecutive DMEK eyes at the Netherlands Institute for Innovative Ocular Surgery (NIIOS), Rotterdam, the Netherlands.[1] In that study, various outcome parameters did not seem to correlate with surgical difficulty or donor tissue preparation error. For example, some eyes showed a decrease in endothelial cell density (ECD) that could not be attributed to surgical trauma or a postoperative (partial) graft detachment.[1] If preparation or surgical trauma would not (solely) explain a higher-than-average ECD decrease and/or postoperative graft detachment, donor- and/or recipient-related factors may influence DMEK surgical outcomes or its complications. Therefore, the aim of the current study was to assess whether donor- and tissue-related parameters correlated with short-term ECD reduction and/or detachment rate.

Methods

To test this hypothesis, the clinical outcome of the same cohort of 500 consecutive DMEK eyes was matched to the donor and tissue information available in our eye bank (Amnitrans EyeBank, Rotterdam, the Netherlands) for statistical analysis. All donor tissue was stored under organ culture conditions. The 500 cases evaluated were cases 26 to 525 from a total of 525 consecutive DMEK surgical procedures performed at our institute between October 2007 and September 2012. The first 25 DMEK eyes were excluded to eliminate a potential start-up effect.[1] Retrospective analysis was performed between August 2013 and March 2015. This study was approved by the institutional review board of the Netherlands Institute for Innovative Ocular Surgery. All patients signed an institutional review board–approved informed consent form.

Table 1. Donor and Patient Demographics

Characteristic	Detachment	ECD Decline
No. of donors included	332	299
Patient age, mean (SD), y	67 (13)	68 (12)
Patient sex		
Male	152	132
Female	180	167
Indication for surgery		
FED	290	270
BK	28	21
Other	14	8
Lens status		
Phakic	86	79
Pseudophakic	246	220
Donor age, mean (SD), y	65 (10)	65 (10)
Donor sex		
Male	203	181
Female	129	118
Donor death cause		
Cancer	82	77
Cardiovascular/stroke	174	158
Respiratory	54	44
Trauma	7	6
Other	15	14
Time, mean (SD)		
From death to enucleation, h	8 (3)	8 (3)
From enucleation to first evaluation, h	14 (6)	14 (6)
From death to surgery, d	14.39 (3.99)	14.3 (3.95)
Graft storage time in medium, mean (SD), d	13.65 (4.02)	13.58 (3.98)
DMEK preparation technique[a]		
Unknown	6	5
Traditional[a]	146	127
No touch[a]	150	139
Converted[b]	30	28
Donor preoperative ECD, mean (SD), cells/mm^2	2521 (203)	2525 (208)

Abbreviations: BK, bullous keratopathy, DMEK, Descemet membrane endothelial keratoplasty; ECD, endothelial cell density, FED, Fuchs endothelial dystrophy.

[a] Details on the different preparation techniques are given in the article by Groeneveld-van Beek et al.[7]

[b] Converted = started with no-touch technique but switched to traditional technique because of complications during graft preparation.

Multiple linear regression analysis and logistic regression analysis were used to evaluate how donor- and tissue-related parameters affect ECD decrease and graft detachment at 6 months after surgery. The donor- and tissue-related parameters were selected based on findings in the literature to compare the results.[2-6] Assumptions of the models were checked and anomalies reported, if present. For all statistical tests, a significance level equal to α = .05 was used. The analyses were performed on all unique eyes (ie, first, only 1 operated on eye was included for each patient [n = 393] and of these, only eyes with donor corneas from unique donors were included, leaving 334 eyes for the analysis). For both analyses (ECD and graft detachment), the number of eyes differed owing to missing values on the outcome variable or on the predictor variables (**Table 1**).

At a Glance

- In this study, we retrospectively assessed the effect of donor-related parameters on endothelial cell density decline and detachment rate in Descemet membrane endothelial keratoplasty eyes.
- Longer graft storage times seem to negatively affect endothelial cell density decrease at 6 months after Descemet membrane endothelial keratoplasty.
- Higher donor age may be associated with a lower chance of graft detachment.
- Further research is needed to validate these preliminary findings.

Results

Graft Detachment

At 6 months after DMEK, 79 of 500 eyes (15.8%) showed graft detachment, of which 45 eyes (9.0%) had a detachment of one-third or less of the graft surface area and 34 eyes (6.8%) had a detachment of more than one-third of the graft surface area.[1] Fifteen eyes required air reinjection, and 9 eyes required a secondary keratoplasty because of the detachment within the first 6 months and 2 eyes because of primary and secondary graft failure.[1]

A logistic regression was performed with detachment as the outcome variable with 2 categories: detachment (n = 63) (only 63 eyes [of 79 eyes] with a detachment were included because only unique eyes and donors were used for the analysis) and no detachment (n = 269) and all donor- and tissue-related parameters simultaneously (Table 1). Younger donor age seemed to be associated with a 3% increase in the risk for a detachment (odds ratio, 0.97; 95% CI, 0.94, 1.00; *P* = .049) (**Table 2**; **Figure**, A). This might relate to the observation that older donor DMEK grafts (>45 years) are faster to unfold because of their increased thickness and decreased elasticity,[2] which may result in less surgically induced trauma. However, other studies did not find a correlation between donor age and detachment after DMEK when using organ-cultured grafts,[3,4] possibly because in these studies, only donors older than 50 years were included, whereas in our study, donor age ranged from 38 to 85 years.

Endothelial Cell Density

Endothelial cell density decline was measured as the percentage of change between preoperative eye bank values and those at the 6-month follow-up. The relation between ECD decline and all donor- and tissue-related parameters was analyzed simultaneously with multiple linear regression (n = 299 eyes) (Table 1). For only 1 parameter, storage time in medium, a significant association with ECD decrease was observed (ie, for

Table 2. Results Logistic Regression (Outcome Detachment) and Multiple Regression Analysis (Outcome ECD)

	Detachment[a]		ECD Decline[b]	
Characteristic	Odds Ratio (95% CI)	*P* Value	Coeff (Std Coeff) [95% CI]	*P* Value
Patient age	1.00 (0.97 to 1.03)	.96	0.19 (0.02) [−0.04 to 0.43]	.11
Patient sex				
Female	NA	NA	NA	NA
Male	0.78 (0.43 to 1.40)	.41	−0.79 (0.00) [−5.17 to 3.58]	.72
Indication for surgery				
FED	NA	NA	NA	NA
BK	1.78 (0.66 to 4.50)	.24	7.19 (0.01) [−1.55 to 15.95]	.11
Other	0.73 (0.11 to 2.98)	.70	−6.61 (0.00) [−20.21 to 6.99]	.34
Lens status				
Pseudophakic	NA	NA	NA	NA
Phakic	0.44 (0.17 to 1.08)	.08	2.11 (0.00) [−4.12 to 8.35]	.51
Donor age	0.97 (0.94 to 1.00)	.049	−0.18 (0.01) [−0.43 to 0.05]	.12
Donor sex				
Female	NA	NA	NA	NA
Male	0.79 (0.44 to 1.43)	.43	−0.52 (0.00) [−4.95 to 3.90]	.82
Donor death cause				
Cancer	NA	NA	NA	NA
Cardiovascular/stroke	1.36 (0.66 to 2.98)	.42	1.92 (0.00) [−3.32 to 7.17]	.47
Respiratory	1.80 (0.71 to 4.58)	.21	−0.42 (0.00) [−7.54 to 6.69]	.91
Trauma	1.91 (0.22 to 11.65)	.51	3.71 (0.00) [−12.04 to 19.47]	.64
Other	1.15 (0.16 to 5.25)	.87	1.44 (0.00) [−9.37 to 12.26]	.79
Time				
From death to enucleation	0.99 (0.89 to 1.08)	.77	0.19 (0.00) [−0.59 to 0.97]	.63
From enucleation to first evaluation	1.03 (0.98 to 1.08)	.22	0.33 (0.01) [−0.03 to 0.71]	.07
Graft storage time in medium	1.03 (0.96 to 1.11)	.41	0.69 (0.02) [0.15 to 1.24]	.01
DMEK preparation technique				
Unknown	…	…	NA	NA
Traditional	…	…	−16.42 (0.19) [−33.38 to 0.54]	.06
No touch	…	…	−13.14 (0.12) [−30.00 to 3.70]	.13
Converted	…	…	−10.80 (0.03) [−28.67 to 7.05]	.23
Intercept	2.79 (0.02 to 441.40)	.69	32.40 (0.00) [3.58 to 61.23]	.03

Abbreviations: BK, bullous keratopathy, Coeff, regression coefficient; DMEK, Descemet membrane endothelial keratoplasty; ECD, endothelial cell density; ellipses, not available; FED, Fuchs endothelial dystrophy; NA, not applicable (baseline effect, ie, no coefficient estimated); Std Coeff, standardized regression coefficient.

[a] Multiple logistic regression with the outcome of detachment and all predictors included simultaneously in the model (MacFadden R^2 = 0.06). The model with only donor age has an R^2 = 0.01.

[b] Multiple linear regression with outcome of ECD decline (%) and all predictors included simultaneously in the model. The model with all predictors has a multiple R^2 = 0.08 (adjusted R^2 = 0.02) and the model with only storage time in medium as predictor has a multiple R^2 = 0.02 (adjusted R^2 = 0.02).

each extra day in medium, the ECD decline increased on average with 0.7%; estimated coefficient, 0.70; 95% CI, 0.15-1.24; standardized regression coefficient, 0.15; P = .01) (Table 2; Figure, B).

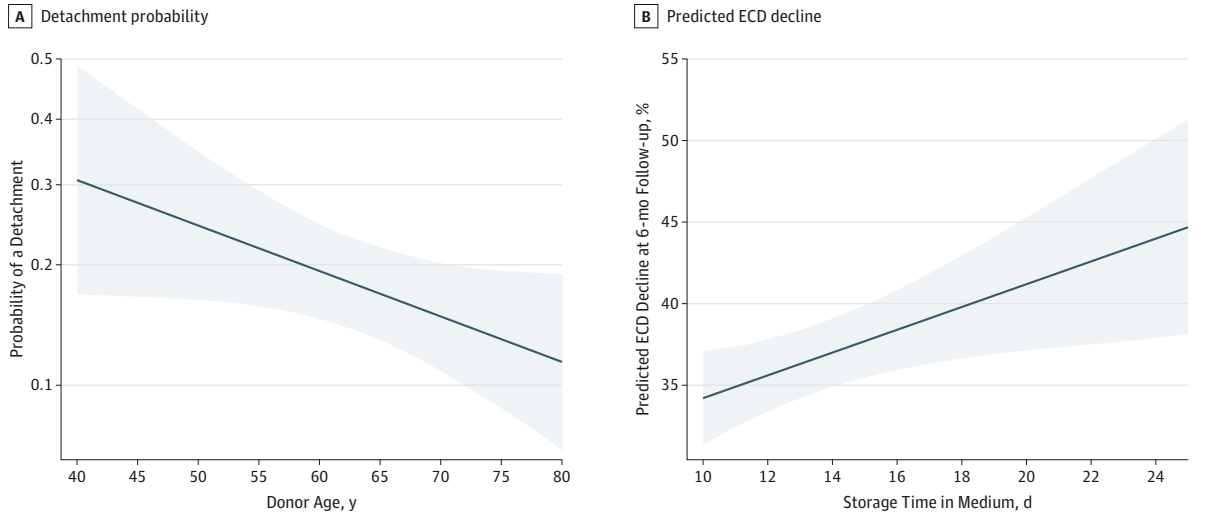
This relation was not found by other studies evaluating DMEK grafts stored in cold-storage medium[5] or when cold storage was compared with organ culture as a preservation method.[6] For the latter study, this difference might be explained by the number of eyes included or the fact that not all parameters were simultaneously tested.

In addition, because complex interactions between donor and recipient characteristics and graft storage conditions might influence graft detachment and rate of ECD decline after DMEK, we also performed classification tree analysis (data not shown). However, conclusive results could not be obtained because of insufficient predictive quality of the trees. To be able to detect complex interactions and to obtain models with sufficient predictive quality, more cases (eyes) are needed.

Conclusions

Based on this preliminary donor parameter analysis, older donors could be better suited for DMEK-graft preparation to reduce the chance of a detachment and donor tissue storage times should be kept as short as possible to improve short-term ECDs. Our eye bank technicians also prefer to prepare DMEK grafts from donors older than 45 years because preparation in older donors seems easier (DM in younger

Figure. Regression Plots for the Outcomes of Detachment and Endothelial Cell Density (ECD) Decline



The effect plots are shown (A) for predictor donor age (years) and outcome probability of a detachment with 95% CIs (shaded region) and (B) for predictor storage time in medium (days) and outcome ECD decline at 6-month follow-up with 95% CIs (shaded region).

donors tends to be more fragile and appears to be more adherent to the stroma). Further investigation is needed to validate the findings of this retrospective study. Future studies with a greater number of eyes may allow for an evaluation of more complex interactions between donor, recipient, and graft parameters to improve the clinical outcome after DMEK by a better selection of donor tissue targeted for various procedures.

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Study concept and design: Rodríguez-Calvo de Mora, Frank, Oellerich, Bruinsma, Melles.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Rodríguez-Calvo de Mora, Bruinsma, Melles.
Critical revision of the manuscript for important intellectual content: Groeneveld-van Beek, Frank, van der Wees, Oellerich, Bruinsma, Melles.
Statistical analysis: Frank.
Administrative, technical, or material support: Groeneveld-van Beek, van der Wees.
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