Descemet membrane endothelial keratoplasty: graft rejection, failure and survival

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PART 2

Prediction of DMEK Rejection and Transplantation of Smaller Endothelial Grafts

CHAPTER 6

Quantitative Assessment of Aqueous Flare after Descemet Membrane Endothelial Keratoplasty for Fuchs Endothelial Dystrophy

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ABSTRACT

Purpose: To assess aqueous flare as a measure of subclinical inflammation after Descemet membrane endothelial keratoplasty (DMEK) for Fuchs endothelial dystrophy.

Methods: In this prospective cross-sectional and longitudinal case series at a tertiary referral center, 173 DMEK eyes of 169 patients and 19 age-matched healthy control eyes were included. Aqueous flare [photon count per millisecond (ph/ms)] was assessed by laser flare photometry at 1 day, 1 week, and 1 month after DMEK in group I (evaluation of postsurgical blood–aqueous barrier recovery; n = 25) and on average 28 (±19) months (range, 3–86 months) after DMEK in group II (evaluation of long-term inflammation; n=148).

Results: In group I, flare levels decreased from 1 day to 1 week [25.1 (±9.1) ph/ms vs. 13.4 (±4.8) ph/ms; P=0.003] and remained stable up to 1 month after DMEK [12.1 (±3.2) ph/ms; P=0.387]. However, average flare at 1 month was higher than that in healthy controls (P<0.001). The long-term flare value after DMEK (group II) was 9.6 (±4.2) ph/ms and was higher in eyes associated with allograft rejection (n=6) versus those without rejection [16.7 (±7.8) ph/ms vs. 9.3 (±3.8) ph/ms, respectively, P<0.001]. All eyes associated with rejection had flare values above 10 ph/ms.

Conclusions: Aqueous flare after DMEK quickly decreased within the first postoperative month, indicating fast recovery of the blood–aqueous barrier. Long-term flare levels were higher in eyes associated with rejection, suggesting persistent subclinical inflammation. A flare level above 10 ph/ms may be used as a threshold for identifying eyes associated with or at risk of allograft rejection after DMEK.

INTRODUCTION

Surgical trauma and associated inflammation cause breakdown of the blood-aqueous barrier, leading to leakage of proteins and inflammatory cells into the anterior chamber, recognized as "flare" with biomicroscopy.[1–5] Laser flare photometry allows for objective and noninvasive assessment of flare levels, so that the device is used in monitoring uveitic eyes for disease progression/remission or recurrence.[4–8] Various studies evaluated aqueous flare levels in ocular diseases (e.g., retinal detachment) or after ocular surgery (phacoemulsification, trabeculectomy, or vitrectomy) to investigate recovery of the blood–aqueous barrier.[9–12] Higher flare levels have also been observed after penetrating keratoplasty, and remarkably higher levels have been associated with allograft reactions.[13–15]

In the past decade, we have introduced several techniques for endothelial keratoplasty including Descemet membrane endothelial keratoplasty (DMEK).[16] DMEK allows for selective replacement of the diseased corneal endothelial cell layer, thus possibly allowing significant reduction in surgical trauma and complications.[16–20] Although the technique is minimally invasive and a much lower antigen load is being transplanted, the DMEK transplant can still induce (subclinical) inflammation.[21] Clinical observation suggests that DMEK eyes hardly show intraocular inflammation after surgery, and even in the event of allograft rejection, flare often seems negligible on slit-lamp examination.[21–23]

However, we recently described signs of endothelial cell activation that may occur up to 18 months before post-DMEK allograft rejection becomes clinically manifest.[24] Therefore, the aim of this study was to evaluate the presence of inflammation in post-DMEK eyes using laser flare photometry and to assess its potential in detecting eyes that may be at risk of developing allograft rejection.

MATERIALS AND METHODS

Aqueous flare was prospectively measured in 173 eyes of 169 patients [mean age: 67 (±10) years] after DMEK for Fuchs endothelial dystrophy during regular follow-up examinations at the Melles Cornea Clinic, Rotterdam, the Netherlands. Exclusion criteria were central corneal edema beyond 3 months after DMEK, scarring, or graft detachment in the central cornea that may influence flare. Because corneal edema,[25] epithelial bullae, and guttae may influence preoperative flare measurements, only postoperative flare values were evaluated.

Two DMEK groups were evaluated: group I comprised 25 DMEK eyes of 25 patients that were measured prospectively at 1 day, 1 week, and 1 month after DMEK (longitudinal analysis) to evaluate flare levels in the immediate postoperative phase as a measure of recovery of the blood–aqueous barrier (i.e., surgical trauma) (Table 1). Group II comprised 148 primary eyes of 148 patients that were assessed during routine follow-up visits at different time points in a cross-sectional analysis. Only eyes that had reached 3-month follow-up were included in this group to assess

Table 1. Demographics and topical medication regimen of study and control eyes

	Eyes after DMEK (173 eyes, 169 patients)		**Eyes without corneal transplantation**
	Group I (Early flare evaluation)	**Group II (Late flare evaluation)**	**Control eyes**
Number of eyes	25	148	19
Number of patients	25[1]	148[1]	19
Average FU time (±SD), months	--	28 (±19)	--
Evaluated time points	1 day, 1 week, 1 month	--	--
Median FU time (range), months	--	24 (3-86)	--
Patient age, years			
Mean (±SD)	68 (11)[2]	67 (10)[2]	68 (8)[2]
Gender			
Female	16	79	9
Male	9	69	10
Preoperative indication			
Fuchs endothelial dystrophy	25	148	--
Lens status			
Phakic	4	46	18
Pseudophakic	21	102	1
Diabetes	1	13	0
Topical medication			--
None	0	2[3]	
Fluorometholone[4]	0	139[4]	
Dexamethasone	25	5[5]	
Loteprednol/ Rimexolone	0	2	
Antiglaucoma medication	0	5[6]	0

DMEK = Descemet Membrane Endothelial Keratoplasty; FU = Follow-up; SD = Standard Deviation

[1] in 4 patients, contralateral eye in other group

[2] matched (P>0.05)

[3] Medication has been stopped by patient

[4] Frequency according to the standard protocol once every other day and up to 4 times per day

[5] Two of those applied Dexamethasone after an episode of allograft rejection

[6] Two of those were eyes following rejection and 3 without rejection

flare in the later postoperative phase. Four patients had both eyes included: 1 eye in group I and the contralateral eye in group II.

Flare values of group I (at 1-month follow-up) and group II were compared with those of 19 eyes of 19 age-matched healthy subjects with no corneal disease or history of recent ocular surgery (Table 1). None of the included subjects had a history of (non)infectious uveitis or immune disease, and none of the control eyes received topical anti-inflammatory treatment.

All patients signed an institutional review board–approved informed consent form for research participation. The study adhered to the tenets of the Declaration of Helsinki.

Donor Tissue Recovery and DMEK Surgery

DMEK grafts were prepared as has been previously described.[26,27] Corneoscleral buttons were excised from donor globes and stored in organ culture medium (CorneaMax, Eurobio, Courtaboeuf, and France). After evaluation of endothelial cell morphology and viability, corneoscleral buttons were mounted endothelial side up on a custom-made holder to remove a 9- to 10-mm diameter Descemet sheet with its endothelium from the posterior stroma. A "Descemet-roll" formed spontaneously, with the endothelium on the outer side.

All eyes received YAG-laser iridotomy about 2 weeks before DMEK, followed by fluorometholone 3 times daily over 1 week. DMEK surgery was performed as has been previously described.[28] A circular 9.0-mm "descemetorhexis" was performed with a reversed Sinskey hook (DORC International, Zuidland, the Netherlands) under an air-filled anterior chamber. The donor "Descemet-roll" was then stained (0.06% Trypan blue solution, Vision Blue; DORC International), aspirated into a custom-made injector (Melles DMEK injector; DORC International), and inserted through a 3.0-mm limbal tunnel incision into the recipient anterior chamber. The graft, oriented with the endothelium facing the recipient iris and Descemet membrane facing recipient posterior stroma, was then completely unfolded over the iris before an air bubble was injected underneath the graft to position it onto the recipient posterior stroma. The anterior chamber was then completely filled with air for 60 minutes, followed by an air–liquid exchange leaving up to 50% air.

At the end of surgery, subconjunctival dexamethasone and gentamicin were injected. Postoperative medication included antibiotic eye drops for 2 weeks and a steroid regimen of dexamethasone 0.1% drops 4 times daily for 4 weeks,

followed by fluorometholone 0.1% drops 4 times daily, tapered to once daily at 1 year postoperatively, and once daily or once every other day thereafter. In the case of steroid-induced ocular hypertension/glaucoma or ocular discomfort from preservatives, or a previous episode of allograft rejection after DMEK, the patient received an alternative antiinflammatory medication (rimexolone 1%, loteprednol 0.5%, or dexamethasone 0.1% without preservatives) (Table 1). Topical medication was defined as the current medication that was applied for the last 3 to 6 months until the follow-up visit of flare measurement (Table 1).

Aqueous Flare Measurement

All eyes were examined with slit-lamp biomicroscopy before laser flare photometry (KOWA FM-700 laser flare meter; Kowa Company, Chofu, Tokyo, Japan). Laser flare readings were performed by 2 examiners (L.B. and F.C.L.) according to a recommended protocol (Kowa Laser Flare-Cell Photometry Medical Advisory Board 1994).6 Without dilating the pupil, the emitted scanning laser beam (laser diode, 640 nm, 35 μW) was focused at the anterior chamber, and the amount of light scattering (proteins in the anterior chamber) from the beam in a window of 0.3 x 0.5 mm in the anterior chamber was detected by a photomultiplier, where it was converted into electrical signals and analyzed to determine the "flare value" in photon count per millisecond (ph/ms). For each eye, 10 consecutive flare readings with a background scatter of ≤15% were taken, while the highest and lowest measurements were deducted; the remaining 8 measurements were averaged to obtain the flare value.

Endothelial Cell Density Measurement

Endothelial cell density was evaluated with an SP3000p noncontact autofocus specular microscope (Topcon Medical Europe BV, Capelle a/d IJssel, the Netherlands). The automatically delineated cell borders of every image were carefully checked for accuracy and redefined manually if required. Three central images were analyzed and averaged per eye.

Statistics

Paired *t* tests were applied to assess differences in age between all 3 groups and in flare quantity between the 3 follow-up points (1 day, 1 week, and 1 month) of group I and in comparison with the control group. P values of less than 0.05 were considered to be statistically significant.

Multiple linear regression analyses were performed while correcting for covariates (age, sex, lens status, and diabetes) to assess differences in flare between

group II and the control group and to assess whether flare within group II was associated with any of the covariates, the follow-up time or endothelial cell density.[29] Sensitivity analysis evaluated whether eyes with (previous) rejection influenced the results.[29] All multiple tests were controlled for false discovery rate.[30] The relative importance of each predictor (effect sizes) was provided to estimate the relevance of the effect, representing a small (r^2 = 0.01), medium (r^2 = 0.09), or large effect (r^2 = 0.25).[31]

RESULTS

Group I: Flare Levels ≤1 Month After DMEK (Longitudinal Analysis)

In group I (short-term flare measurement within the first month after DMEK), the mean flare value was 25.1 (±9.1), 13.4 (±4.8), and 12.1 (±3.2) ph/ms at 1 day, 1 week, and 1 month, respectively, with a significant decrease in flare levels within the first week (P=0.003), which remained stable up to 1 month (P=0.387) (Figure 1; Table 2). Average flare at 1 month was higher than that in healthy controls (P < 0.001). None of the eyes had subjective or objective signs of allograft rejection within this early postoperative period.

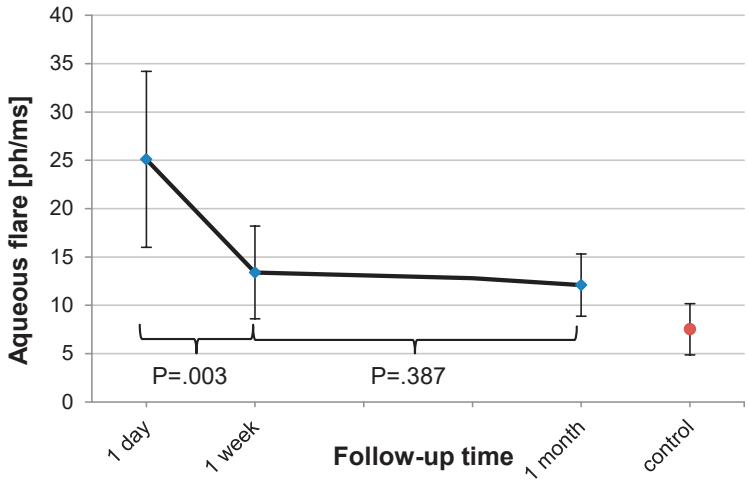

Figure 1. Short-term aqueous flare after Descemet Membrane Endothelial Keratoplasty (DMEK). The figure shows aqueous flare evolution in group I (blue diamonds) at 1 day, 1 week, and 1 month after DMEK. Vertical bars represent standard deviations. The average flare value for the control group of healthy eyes is displayed for comparison (red circle).

Table 2. Aqueous flare in eyes with previous Descemet membrane endothelial keratoplasty and in healthy control eyes

	DMEK eyes (Group I)			DMEK eyes (Group II)			Control group
				Entire Group	Subgroups		
					Eyes without allograft rejection	Eyes associated with allograft rejection	
Follow-up							
Time-point or interval	1 day	1 week	1 month	3 - 86 months	3 - 86 months	7 - 67 months	--
Mean (SD), months				28 (±19)	28 (±19)	25 (±21)	--
Median, months				24	24	19	--
No. of eyes	10	19	12	148	142	6	19
Flare (ph/ms)							
Average (±SD)	25.1 (±9.1)	13.4 (±4.8)	12.1 (3.2)^^	9.6 (±4.2)	9.3 (± 3.8)	16.7 (± 7.8)	7.5 (±2.6)
Median	29.4	12.5	13.2	8.7	8.6	14.1	7.8
Range: Min - Max	7.3 - 35.6	6.5 - 22.7	6.8 - 18.1	3.0 - 31.3	3.0 - 26.6	10.3 - 31.3	2.5 - 14.1
Estimated mean [95%CI]	--	--	--	9.6 [9.0, 10.3]^^	9.2 [8.6, 9.9]^^	16.7 [13.5, 20.0]†	7.3 [5.3, 9.3]
ECD (cells/mm^2)							
Average (±SD)	n.m.	n.m.	n.m.	1360 (±490)	1370 (±490)	1060 (±520)	n.m.
No. of measurements				137	131	6	

SD = Standard deviation; CI = Confidence interval; n.m. = not measured

^^p<0.05 compared to control group

†P<o.o5 compared with DMEK eyes without rejection

Group II: Flare Levels ≥ 3 Months After DMEK (Cross-Sectional Analysis)

In group II (longer-term flare measurements), flare levels were assessed on average 28(±19) months after DMEK (median: 24 months, range: 3–86 months) (Table 1). Except for 2 eyes (flare 7.5 ph/ms and 11.5 ph/ms), all eyes were under low-dose topical anti-inflammatory treatment (Table 1). Three eyes had a history of reversible allograft rejection 5, 13, and 63 months before flare measurement, and 2 eyes were rejection suspects on slit-lamp examination (asymptomatic suspect with suspicious endothelial deposits, flare 31.3 ph/ms, and symptomatic suspect with conjunctival injection/ subjective pain/ keratic precipitates, flare 11.4 ph/ms), and 1 eye was diagnosed with allograft rejection 6 months after flare measurement (flare 12.1 ph/ms). All 6 eyes associated with allograft rejection had flare values above 10 ph/ms, and flare values were higher [estimated mean 16.7 ph/ms, 95% confidence interval (CI) (13.5–20.0)] than eyes without an allograft rejection episode [estimated mean 9.2 ph/ms, CI (8.6–9.9)] ($P < 0.001$, $r^2 = 0.1$). None of the 6 eyes associated with rejection discontinued topical medication before the rejection episode, and none of those eyes developed secondary graft failure.

In total, 102/142 (72%) of DMEK eyes not associated with rejection had a flare value below 10 ph/ms (Table 2; Figure 2).

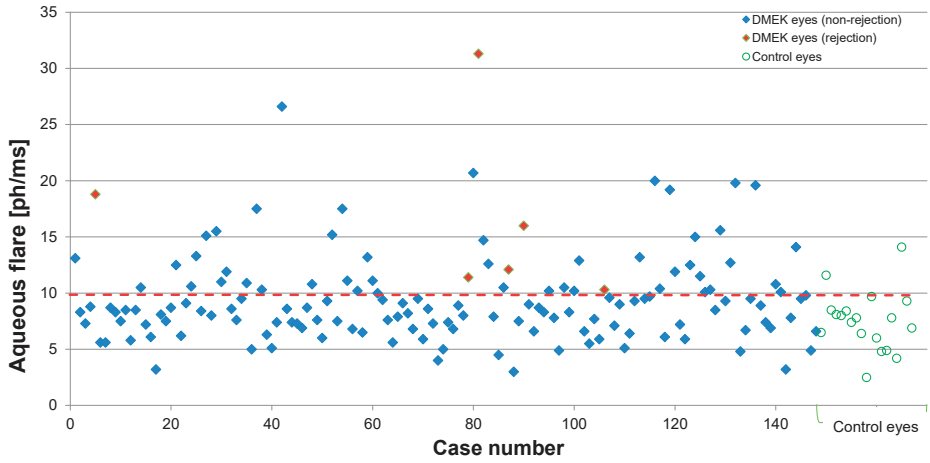

Figure 2. Aqueous flare values after Descemet Membrane Endothelial Keratoplasty (DMEK) of the cross-sectional group. The figure displays all flare values of post-DMEK eyes of group II (blue and red squares) and control eyes (green circles). Note that eyes associated with rejection (red squares) all have flare values above 10 ph/ms (red dashed line) and most post-DMEK eyes without rejection (blue squares) had flare values below 10 ph/ms.

The mean flare value was higher in group II than in healthy controls {9.6 ph/ms [95% CI (9.0–10.3)] vs. 7.3 ph/ms [CI (5.3–9.3)]}, respectively (P=0.03), estimated mean difference: -2.35 ph/ms [Ci (-4.5-0.19)], r^2 = 0.03] (Table 2). Sensitivity analysis showed that this result did not change after discarding the rejection/suspect eyes (n = 6).

Within group II, age was associated with flare (r^2 = 0.04, P = 0.009), while flare quantity was not associated with the follow-up time or endothelial cell density, sex, lens status, or presence of diabetes (P > 0.800 for all parameters).

DISCUSSION

Laser flare photometry has become a routine diagnostic tool in monitoring patients with uveitis and intraocular inflammation.[6,8,25,32,33] There have been various studies assessing aqueous flare also after penetrating keratoplasty;[13–15] however, to date, there is no study measuring flare values after endothelial keratoplasty, and in particular, after DMEK, probably because eyes after DMEK look so intriguingly "quiet" and "safe," even during a rejection episode. Hence, although inflammation may be less and a lower rejection incidence with milder forms of rejection has been reported, a clinically "invisible" immune reaction may lead to a faster endothelial cell density decrease, a main risk factor for secondary graft failure.[34,35] It may, therefore, be important to monitor the "inflammatory status" in post-DMEK eyes, especially while patients tend to be "asymptomatic."[21,22]

Interestingly, even some post-DMEK eyes that were not associated with rejection still showed on average higher flare values than virgin control eyes, although all but 2 eyes were still on a topical steroid medication (mostly low dose fluorometholone) (Table 1). This may suggest that DMEK eyes suffer from persistent inflammation, in other words, seemingly uncomplicated DMEK eyes may subclinically still be subject to long-term activation. If so, it may be hypothesized that such persistent upregulation results from a chronic immune response to the allograft or incomplete restoration of the blood-aqueous barrier after DMEK surgery. Still, the chronic immune response seems to be "controlled" by low-dose topical steroids, which is supported by the higher incidence of rejection in uncomplicated DMEK eyes after steroid discontinuation.[21] This would underline the importance of a long-term or even indefinite steroid regimen after DMEK.

Notably, the 3 post-rejection eyes showed an elevated flare level, despite the "clinically" reversed rejection episode. Hence, these eyes may have persistent breakdown in their blood-aqueous barrier because of long-term subclinical inflammation, which could explain enhanced endothelial cell density decrease and higher risk of graft failure in post-rejection eyes.

To further evaluate repair of the blood-aqueous barrier after surgery, we studied the flare curves of post-DMEK eyes within the first postoperative month. Measurements were difficult to perform on day 1 (because of residual corneal edema). At 1 week, more reliable measurements could be obtained, showing a quick decrease in flare levels stabilizing at 1 month postoperatively. However, flare levels at 1 month were on average still higher than those in non-DMEK control eyes. This observation could explain the presence of cystoid macular edema observed by some authors after DMEK or triple-DMEK that could be prevented by intensified steroid medication in the immediate postoperative phase after DMEK.[36,37] Earlier studies also reported that recovery of the blood-aqueous barrier can be related to the type of surgery, incision size, and intraoperative trauma to uveal tissues.[1–3,10–12,38,39] Although with DMEK, endothelial disease is nowadays treated in a minimally invasive manner, the presence of an allograft may still have a (persistent) effect on flare levels after DMEK.

We have recently described specular microscopy and Scheimpflug imaging as possible ophthalmic tools to identify eyes at risk of developing allograft rejection.[22,24] In the current study, we used laser flare photometry as a tool for screening post-DMEK eyes. Remarkably, all DMEK eyes associated with allograft rejection at some point (either suspects or post-rejection) showed a flare level above 10 ph/ms, whereas about 1/3 of DMEK eyes not associated with rejection showed flare values above 10 ph/ms. The 2 rejection-suspect eyes had flare levels of 11.4 and 31.3 ph/ms. Hence, flare assessment may aid in deciding on treatment (yes or no) in specific cases. Also, the eye that was 6 months later diagnosed with allograft rejection had a flare level of 12.4 ph/ms. Although it may be difficult to draw conclusions from these limited cases, if considered "at risk," eyes with flare levels above 10 ph/ms may be monitored more closely, whereas those with lower flare values approximating virgin control eyes may be managed with routine follow-up examinations (Figure 2). Further long-term studies need to evaluate the potential of laser flare photometry as an additional device or as a stand-alone diagnostic in this important field.

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