Received: 1 March 2022 | Accepted: 3 September 2022

DOI: 10.1111/aos.15250

Acta Ophthalmologica

ORIGINAL ARTICLE

Posterior stromal ripples increase risk of Descemet's membrane endothelial keratoplasty graft detachment worsening over time

Giulia Coco[1,2] | **Hannah J. Levis**[3] | **Alfredo Borgia**[1] | **Davide Romano**[1] | **Luca Pagano**[1] | **Gianni Virgili**[4] | **Stephen B. Kaye**[1,3] | **Vito Romano**[1,3,5,6]

[1]Ophthalmology, Royal Liverpool University Hospital, Liverpool, UK

[2]Department of Clinical Science and Translational Medicine, University of Rome Tor Vergata, Rome, Italy

[3]Eye and Vision Science, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK

[4]Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), Ophthalmology Clinic, University of Firenze and AOU Careggi, Florence, Italy

[5]Instituto Universitario, Universidad de Oviedo and Fundacion de Investigacion Oftalmologica, Oviedo, Spain

[6]Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Ophthalmology Clinic, University of Brescia, Brescia, Italy

Correspondence
Vito Romano, St Paul's Eye Unit, Department of Ophthalmology, Royal Liverpool University Hospital, Prescot Street, L7 8XP Liverpool, UK.
Email: vito.romano@gmail.com

Abstract

Purpose: To evaluate anterior segment optical coherence tomography (AS-OCT) features of Descemet's membrane endothelial keratoplasty (DMEK) grafts associated with graft attachment worsening over time.

Methods: Retrospective case series on patients who received uncomplicated DMEK surgery and for whom subsequent AS-OCT data were available for analysis. Patients' demographics and surgical details were collected. AS-OCT was analysed for graft detachment axial extension, presence of posterior stromal ripples, quadrant involvement (location and number), degree of detachment extension, peripheral roll, presence and amount of air in the anterior chamber (AC). Features associated with re-bubbling and graft detachment worsening over time were identified.

Results: A total of 147 patients with a mean age of 70.8 ± 9.8 years (63% females) were included. AS-OCT was performed at 2.9 ± 2.4 days after surgery. AS-OCT factors associated with re-bubbling were posterior stromal ripples ($p = 0.004$) and detachment axial extension ($p < 0.001$). At first follow-up, of the 147 DMEK, 67 showed complete attachment and 80 partial detachment. In those cases of initially completely attached grafts, posterior stromal ripples were associated with the risk of subsequent graft detachment ($p = 0.014$) together with recipient age ($p = 0.043$), phaco-combined surgery ($p = 0.018$) and AS-OCT timing ($p = 0.033$); while, in the initially partially detached grafts, detachment worsening was associated with posterior stromal ripples ($p = 0.025$), detachment axial extension ($p = 0.003$), degrees of detachment involvement ($p = 0.029$), peripheral roll-in shape ($p = 0.033$) and presence of air in the AC ($p = 0.032$). Relative risk (RR) of graft detachment worsening in patients with moderate/severe posterior stromal ripples was 1.75 (95% CI = 1.09–2.81).

Conclusion: Posterior stromal ripples and detachment axial extension >1/3 of graft surface area were the main risk factors for detachment worsening over time, and patients showing these features should be monitored closely to identify the need for re-bubbling at an early stage, thus improving surgical outcomes.

KEYWORDS
AS-OCT, Descemet membrane endothelial keratoplasty, DMEK detachment, posterior stromal ripples

1 | INTRODUCTION

Descemet membrane endothelial keratoplasty (DMEK) has become a safe and standardized treatment option for corneal endothelial disorders since it was first introduced by (Melles et al., 2006). However, early postoperative graft detachment and graft failure remain important challenges of the procedure with graft detachment still the predominant reason for repeat DMEK/Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) (Deng et al., 2018); (Heinzelmann et al., 2016); (Romano et al., 2020); (Stuart et al., 2018).

Early identification of DMEK graft detachment is essential to improve graft outcomes, and strategies to predict whether the detachment will worsen over time can aid in the decision to treat with intracameral gas reinjection (re-bubbling) (Parekh et al., 2018).

Anterior segment optical coherence tomography (AS-OCT) is a useful tool to identify early partial graft detachment and to determine whether further intervention is needed (Parekh et al., 2018) (Moutsouris et al., 2011). It provides excellent visualization of the area of graft detachment that cannot be observed at the slit lamp, especially in cases of flat detachment, where the distance between the detached DMEK and the recipient cornea is small (Moutsouris et al., 2011).

Decisions on whether to treat a detachment with re-bubbling are commonly based on visual acuity, slit lamp examination and AS-OCT findings, together with surgeon and patient preference (Deshmukh et al., 2022). When the detachment shows worsening over time, re-bubbling should be performed (Dirisamer et al., 2012); (Gerber-Hollbach et al., 2017) ideally within the first 4–6 weeks after surgery (Yeh et al., 2013). In fact, a delay in the re-bubbling decision may cause scarring and contraction of the Descemet membrane leading to incomplete attachment and unsuccessful outcomes (López et al., 2016). If required, therefore, re-bubbling should be performed at a relatively early stage (Dapena et al., 2009); (Ham et al., 2008); (Yeh et al., 2013).

Although previous studies have extensively evaluated recipient, donor and surgical factors associated with graft detachments (Rodríguez-Calvo De Mora et al., 2016); (Mechels et al., 2017); (Leon et al., 2018); (Tourtas et al., 2014); (Chaurasia et al., 2014); (Röck et al., 2015); (Heinzelmann et al., 2018), only a handful of studies have evaluated the anatomical features of graft detachment as a risk factor for worsening over time (Yeh et al., 2013); (Dirisamer et al., 2012); (Bucher et al., 2015). In the present study, we evaluated which DMEK graft factors seen at AS-OCT scans performed within the first 10 days after surgery were associated with a greater risk of detachment worsening over time, possibly requiring re-bubbling. The ultimate aim was to provide evidence as to which patients are at higher risk of graft detachment worsening and to allow closer monitoring to enable prompt detection of the requirement for re-bubbling.

2 | METHODS

We included all patients who received uncomplicated DMEK surgery between September 2017 and January 2021 at the Royal Liverpool University Hospital and for whom subsequent AS-OCT data were available for analysis. Patients were excluded if no AS-OCT scan was available for analysis or first AS-OCT scan was performed >10 days after surgery. Additionally, we excluded patients who experienced intraoperative and/or early postoperative complications (e.g., prolonged pupillary block, air bubble dislocation, upside-down grafts, complete graft detachment with free-floating graft in the anterior chamber, folded grafts) for whom a different clinical and/or surgical approach was required. Lastly, patients who received hemi-DMEK were excluded due to differences in graft detachment appearance and management.

Descemet's membrane endothelial keratoplasty surgery was performed as previously described.[5] Briefly, descemetorhexis was initially performed at a size of approximately 0.5 mm larger than the DMEK lenticle. Peripheral iridotomy (PI) was performed at this stage according to the surgeon's preference. DMEK tissue was delivered through a glass vial. Manipulation by tapping on the outer corneal surface was used to unfold and centre the graft. Once the graft was correctly positioned, an air bubble was injected into the AC to secure the DMEK graft position. 100% AC fill was achieved at the end of the surgery. Patients were checked 1 h after surgery, and if the AC air fill was still complete, the release of air from the AC was performed to leave its level at approximately 80%–90% air fill.

2.1 | Data collection

Patients' demographics, reason for graft, donor characteristics, type of surgery (DMEK or phaco-DMEK), presence of PI, previous pars plana vitrectomy (ppv) and surgeon level (experience graded as < or >100 DMEK surgeries performed) were collected from an electronic record review. DMEK surgical time was collected from the theatre register. All eyes were clinically examined on day 1, day 3/4, 1 week, 1 and 3 months postoperatively. According to clinical needs, additional follow-up visits were scheduled for each patient.

Anterior segment optical coherence tomography data were extracted from the instrument (CASIA AS-OCT SS-1000, Tomey). AS-OCT scans were performed at each outpatient clinical visit; however, when the patient was admitted after surgery and examined on day 1 postoperatively as an inpatient in the ward, AS-OCT scans were frequently not performed on that day. First and subsequent available AS-OCT for each patient were analysed. Each AS-OCT was analysed over 360 degrees (radial scans) and any type of detachment was described, regardless of its size. The maximal axial extension of detachment in the 360-degree scans was defined as detachment involving: (1) lateral 1/4 if less than/equal to 1/4 of the surface area, (2) lateral 1/3, if less than/equal to 1/3, (3) the paracentral area if involving more than 1/3 but not the central area, (4) the central area, defined as the central 2 mm of the entire cornea. The number of quadrants involved in detachment were reported, as well as the involvement of (1) superior quadrant +/− others,

(2) lateral (nasal and/or temporal) quadrant +/− inferior and (3) inferior quadrant. The number of quadrants involved was not necessarily related to the extension of the detachment, so degrees of extension of detachment were calculated separately and categorized as follows: (1) ≤45, (2) 46–90, (3) 91–135, (4) 136–180, (5) 181–270, (6) 271–360. To calculate degrees of extension of detachment, the radial scan line was manually moved throughout the 360 degrees. Grades corresponding to graft detachment were recorded and, in case of multiple areas of detachment, summed up together.

Peripheral graft shape at the area of detachment was assessed for the presence of a peripheral roll (absent, mild if only a tendency of roll-in shape was found and moderate/severe if a clear peripheral roll was noted). Posterior corneal profile was assessed for the presence of a feature we have termed 'stromal ripples'. Posterior stromal ripple was defined as an irregularity in the posterior corneal profile that assumed the shape of a ripple, as shown in Figure 1. Degree of posterior stromal ripples was graded as none, mild or moderate/severe. Two independent observers (cornea specialists) graded the level of posterior stromal ripples as mild or moderate/severe based on their number (</=4 or >4) and their appearance (less evident or more evident). In case of disagreement between observers, the number of posterior stromal ripples was used to categorize them into the mild (</=4) or moderate/severe (>4) grade (Figure 1).

Presence of air in the anterior chamber (AC) and amount of air visible from the AS-OCT scan were recorded. Grafts were defined as attached if full DMEK graft attachment was noted and partially detached in all other cases, even if a minimal area of DMEK graft was detached in any of the 360-degree AS-OCT scan. Cases of grafts completely detached from the recipient stroma were defined as 'free floating' and excluded from the analysis as explained in the exclusion criteria. At follow-up, the detached grafts were assessed as (1) stable, if no change in the parameters analysed was noted, (2) improved, if a decrease in the extent of the detached area was noted or (3) worsened, if an increase in the area of detachment was noted.

Descemet's membrane endothelial keratoplasty grafts were re-bubbled at first follow-up if the detachment was involving 1/3 of the axial extension for more than four quadrants and if the detachment was involving the central 5 mm of the cornea (pupillary area). Re-bubbling was performed at a later stage in cases of worsening DMEK detachments meeting the above-described criteria.

2.2 | Grouping and analysis

Graft features assessed with AS-OCT within 10 days of surgery were used to create two cohorts: attached grafts and partially detached grafts, as previously defined. Based on natural history, attached grafts were divided into (1) grafts that remained attached (stable) and (2) grafts that partially detached during follow-up (worsened), while partially detached grafts were divided into (1) detached grafts that remained stable or improved over time and (2) detached grafts that worsened, as defined previously.

The whole study population was described, and AS-OCT factors associated with re-bubbling at any stage were determined. AS-OCT parameters associated with stability/improvement and worsening were analysed in both groups of attached and partially detached grafts. Clinical parameters known as risk factors for graft detachment were controlled for. Among partially detached grafts, group-by-group comparison between stable/improved detachments and worsened detachments was performed to detect differences among them. Differences in central corneal thickness (CCT) and corneal volume (CV) were evaluated at this stage.

Risk of worsening was calculated for attached grafts showing stromal ripples and for partially detached grafts showing detachments of ≤ or >1/3 of the surface area and in the presence of stromal ripples (moderate/severe, all, none).

2.3 | Statistical analysis

Data are presented as the mean ± standard deviation (SD), or as percentages (%) for categorical variables. A generalized linear model (GLM) (logit function) was fit with predictors through backward elimination (until $p \leq 0.1$) controlling for confounders to identify risk factors associated with (1) factors at first follow-up

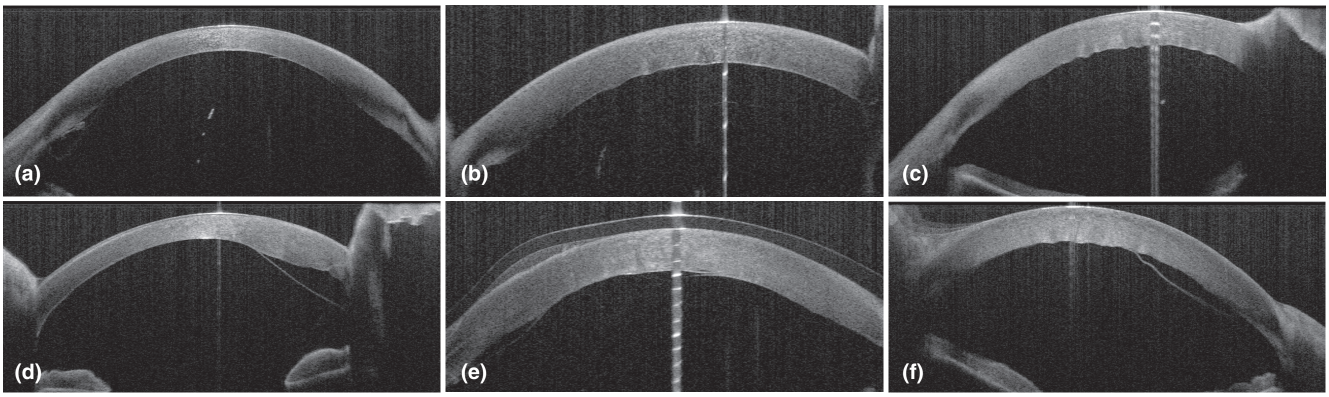


FIGURE 1 Posterior stromal ripples and classification. (a and b)show AS-OCT scan with no posterior stromal ripples in attached and partially detached DMEK grafts, respectively. (c and d) show mild posterior stromal ripples in attached and partially detached DMEK grafts, respectively. (e and f) show moderate/severe posterior stromal ripples in attached and partially detached DMEK grafts, respectively.

associated with graft detachment requiring re-bubbling at any time, (2) AS-OCT factors associated with partial graft detachment after initial attachment, (3) AS-OCT factors associated with graft stability/improvement versus worsening in partially detached grafts. Parameters analysed in the model included AS-OCT DMEK graft detachment characteristics (axial extension, stromal ripples, number of quadrants involved, type of quadrant involved, degrees of detached area, peripheral roll-in shape). We also included known clinical risk factors for graft detachment like recipient age, reason for graft, type of tissue used (surgeon stripped, prestripped or preloaded), surgeon level and phaco-combined procedures.

Fisher's exact test and Mann–Whitney U test were used to test differences between groups of grafts showing stability/improvement and worsening. The statistical analyses were performed using STATA 13.0 (StataCorp.) and a p-value of less than 0.05 was considered statistically significant. The study was approved by the Institutional Review Board (IRB) (A0002786) and followed the tenets of the Declaration of Helsinki.

3 | RESULTS

One hundred forty-seven eyes of 147 patients were included in the study. The mean age was 70.8 ± 9.8 years (range 45.4–96.6 years) and 63% were females (92/147). The most common indication for DMEK was Fuchs endothelial dystrophy (FED; 94 eyes, 64%). Other patients had pseudophakic bullous keratopathy (PBK) (53 eyes, 36%). Only one patient had previous ppv for retinal detachment. No difference was present between included and excluded patients for age (70.8 ± 9.8 vs. 72.5 ± 10.8; $p = 0.237$, Mann–Whitney U test), sex (females 63% vs. 60%; $p = 0.861$, Fisher's exact test) and reason for grafting (FED 64% in both groups; $p = 1.00$). In most eyes, phacoemulsification cataract surgery was performed at the same time as the DMEK (triple procedure; 89 eyes, 60.5%), while in 38% of cases (56/147) patients were pseudophakic at the time of surgery, and 2 eyes were phakic (1.4%). PI was performed in 46% of patients. Donor age was 66.3 ± 9.3 years, and 62% were males. Mean ECD of the graft was 2548 ± 187 cells/mm^2, and mean graft diameter was 8.4 ± 0.4 (range 7.75–9.5 mm). After surgery, all patients received a subconjunctival injection of gentamicin and dexamethasone. Postoperatively, all patients were prescribed topical chloramphenicol 0.5% 4xday for 14 days and 2-hourly prednisolone 1%, which was tapered to 6xday after a week and 4xday at 1 month. In 12 patients (8%), air was reduced after surgery at approximately 80%–90% air fill; of these, in three cases the need for air release presented regardless of the PI.

3.1 | All patients—AS-OCT factors associated with re-bubbling at any stage

First AS-OCT scan was acquired at a mean of 2.9 ± 2.4 days after surgery. At the time of first scan, 46% (67/147) of patients showed completely attached grafts, while the remaining 54% (80/147) showed partial detachments; 17% showed detachment of ≤1/4, 15% of ≤1/3, 10% showed paracentral detachment and 12% detachment involving the central area. The mean number (n) of quadrants involved was 1.1 ± 1.4. The superior quadrant was involved in 22% of cases. Nineteen grafts showed a peripheral roll at the edge (13%). Posterior stromal ripples were noted as mild in 21% of cases and moderate/severe in 19%. Overall, air was still present in 104 eyes (71%) with an average of 40.1% ± 20.7% of AC fill when present. A total of 52 (35.4%) eyes were re-bubbled at some stage, of which 15 eyes (10.2%) were re-bubbled at this visit and 29.3% at a later follow-up visit (also accounting for 2nd re-bubbling).

Overall, significant AS-OCT factors at first follow-up associated with graft detachment requiring re-bubbling at any stage were: stromal ripples ($p = 0.004$, GLM) and detachment axial extension ($p < 0.001$). Not significant were the presence of air in the AC ($p = 0.090$), type of tissue used for surgery ($p = 0.076$) and phaco-combined procedures ($p = 0.100$). All the other parameters analysed (e.g., number of quadrants involved, type of quadrant involved, degrees of detached area, peripheral roll-in shape, recipient age, reason for graft and surgeon level) were largely not significant, therefore not kept in the model during backward elimination. Neither the presence of the PI nor the need for air release did influence the rate of re-bubbling (both $p = 1.00$, Fisher's exact test). Detailed AS-OCT characteristics of attached and partially detached DMEK grafts at first scan are reported in Figure S1.

3.2 | Attached grafts—AS-OCT factors associated with subsequent partial detachment

Of the grafts that showed complete attachment at first follow-up (mean 2.4 ± 2.2 days), air was still present in the anterior chamber in 56/67 (84%) with an average of 44.9% ± 21.6% anterior chamber fill when present. 19/67 of these patients showed stromal ripples.

Forty-five out of the 67 completely attached grafts remained attached over time, while 22/67 worsened at subsequent follow-up (12.6 ± 22.6 days) and of those, 15/22 required re-bubbling at a later stage. Grafts that remained attached showed stromal ripples in 18% of cases (8/45) that were mild in 13.5% (6/45). By contrast, attached grafts that showed worsening over time showed stromal ripples in 50% of cases (11/22) (mild in 41%, 9/22).

Among grafts completely attached at first follow-up, the presence of posterior stromal ripples was associated with worsening ($p = 0.014$, GLM) together with recipient age ($p = 0.043$), phaco-combined surgery ($p = 0.018$) and time at which AS-OCT was performed ($p = 0.033$, higher risk for earlier scan). Not significant was the type of tissue used ($p = 0.100$). Other parameters analysed (e.g., reason for graft and surgeon level) were largely not significant, therefore not kept in the model. Neither the presence of the PI nor the need for air release did influence DMEK graft attachment worsening over time (both $p = 1.00$, Fisher's exact test).

Fisher's exact test showed a significant difference in the distribution of stromal ripples between grafts that remained attached (18%) and those which detached (50%) (p = 0.021). Detailed AS-OCT characteristics of attached DMEK, which showed stability and worsening over time are reported in Figure 2.

3.3 | Partially detached grafts—AS-OCT factors associated with improvement/stability or worsening

80 DMEK grafts were analysed at this stage. Mean AS-OCT time after surgery was 3.4±2.4 days. Stromal ripples were evident in 56% of cases (23% mild and 34% moderate/severe). Central corneal involvement was present in 21% of cases, the paracentral cornea in 19%, ≤1/3 in 28% and 1/4 in 31%. On average, 2.1±1.2 quadrants were involved in detachment, with the superior quadrant involved (±others) in 40% of cases.19 grafts showed peripheral roll-in at the edges. 64% of patients still had some air left in the AC, with mean AC air fill of 22.2%±22.4% if present. Fifteen patients (19%) were re-bubbled at this stage (3.4±2.4 days after surgery), and the remaining 65 patients were followed up. Of the 65 eyes that did not undergo re-bubbling at this stage, 31 (48%) detached DMEK grafts showed stability (14/31) or even improvement (17/31) at subsequent follow-up, while the remaining 34 (52%) showed worsening of the detached area. Of this latter group, 22/34 required re-bubbling at a later stage. Detailed characteristics of grafts that showed stability/improvement or worsening are outlined in Figure 3.

Regression analysis was performed to analyse factors associated with DMEK graft detachment worsening versus stability/improvement. Detachment characteristics significantly associated with worsening at subsequent follow-up were: stromal ripples (p = 0.025), detachment axial extension (p = 0.003), degrees of detachment involvement (p = 0.029), peripheral roll-in shape (p = 0.033) and presence of air in the AC (p = 0.032). Surgeon level was not significant (p = 0.079). Other parameters analysed (e.g., number of quadrants involved, type of quadrant involved, recipient age, reason for graft, type of tissue used and phaco-combined procedures) were largely not significant, therefore not kept in the model. Neither the presence of the PI nor the need for air release did influence DMEK graft detachment worsening over time (p = 1.00 and p = 0.701, respectively, Fisher's exact test).

3.4 | Partially detached grafts—group-by-group comparison between stable/improved and worsened detachments

Fisher's exact test was used to compare the AS-OCT appearance of grafts that showed worsening at subsequent follow-up and those which showed stability/improvement. Different distribution between groups was found for moderate/severe stromal ripples (p = 0.026), detachment axial extension (p = 0.016) and presence of air in the AC (p = 0.021). Interestingly, the overall presence of none, mild and moderate/severe stromal ripples was just short of significance (p = 0.052), highlighting that mainly moderate/severe stromal ripples were distributed differently between groups. Also, the sole presence of central involvement was not different between groups (p = 0.262), while comparing detachment involving ≤1/3 of the surface area and >1/3 the difference was significant (p = 0.040). No differences were found in the number of quadrants involved (p = 0.077), in the type of quadrant involved (p = 0.197) and in the degrees of detachment extension (p = 0.426). Also, the presence of peripheral rolls showed no difference between groups (p = 0.584).

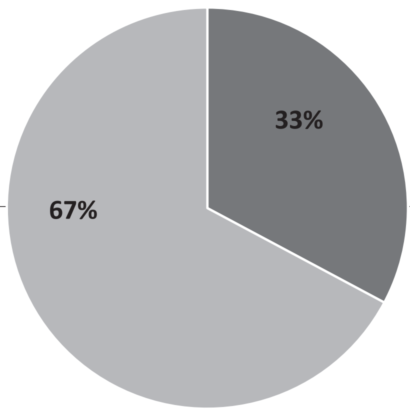
Central corneal thickness showed a significant difference between grafts that showed worsening (708±105 μm) and those which showed stability/improvement (663±103 μm) (p = 0.049, Mann–Whitney U test), while CV did not show any significant difference (62 335±24 600 μm^3 and 62 964±18 797 μm^3, respectively; p = 0.506).

3.5 | Stromal ripples

The presence of stromal ripples was significantly associated with the risk of graft detachment requiring re-bubbling at any time (p = 0.004, GLM), risk of worsening

Attached grafts at AS-OCT

Stability over time (n=45)	
Follow-up (mean)	3.0 ± 2.5 days
Stromal ripples	Mild: 13.3% Moderate/severe: 4.4%
Air in the AC	75.5%
Air / Amount air (if present)	75.5% / 44.1 ± 24.3%
Re-bubbling	At later stage: 0%

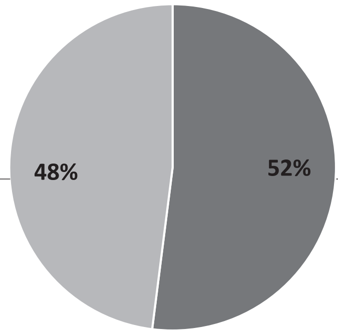


Worsening over time (n=22)	
Follow-up (mean)	1.2 ± 0.7 days
Stromal ripples	Mild: 41% Moderate/severe: 9%
Air in the AC	100%
Air / Amount air (if present)	100% / 46.1 ± 17.2%
Re-bubbling	At later stage: 68% (15)

FIGURE 2 Attached DMEK grafts at first available AS-OCT (n = 67). 67% of initially attached grafts showed stability over time (table on the left), while 33% detached at subsequent follow-up (on the right). AS-OCT characteristics of DMEK grafts in each group are highlighted in the corresponding table.

Detached grafts at AS-OCT

Stability/improvement over time (n=31)	
Follow-up (mean)	3.7 ± 2.5 days
Stromal ripples	Mild: 26% Moderate/severe: 13%
Axial extension	<¼: 52% Paracentral: 3% <1/3: 35% Central: 6%
Type quadrant	Superior ± any: 35% Lateral ± inferior: 19% Inferior only: 44%
Degrees	≤45°: 48% 136-180°: 0% 46-90°: 23% 181-270°: 10% 91-135°: 6% 271:360°: 10%
Peripheral roll	Mild: 6% Moderate/severe: 6%
Air / Amount air (if present)	52% / 36.9 ± 20.1%
Re-bubbling	Any stage: 0%



Worsening over time (n=34)	
Follow-up (mean)	2.3 ± 1.9 days
Stromal ripples	Mild: 24% Moderate/severe: 38%
Axial extension	<¼: 26% Paracentral: 26% <1/3: 29% Central: 18%
Type quadrant	Superior ± any: 29% Lateral ± inferior: 38% Inferior only: 26%
Degrees	≤45°: 38% 136-180°: 12% 46-90°: 29% 181-270°: 6% 91-135°: 3% 271:360°: 9%
Peripheral roll	Mild: 12% Moderate/severe: 12%
Air / Amount air (if present)	79% / 37.0 ± 18.0%
Re-bubbling	Any stage: 64.7%

FIGURE 3 Partially detached DMEK grafts at AS-OCT which did not undergo re-bubbling at this visit (n = 65). 48% of partially detached grafts showed stability or improvement over time (n = 31), while 52% showed worsening at subsequent follow-up (n = 34). AS-OCT characteristics of DMEK grafts in each group are highlighted in the corresponding table.

TABLE 1 Risk of worsening (RW) and relative risk (RR) of DMEK graft detachment in attached and partially detached grafts based on evaluation of AS-OCT scans for the presence of posterior stromal ripples and detachment axial extension

		Improved/stable (n)	Worsened (n)	RW (%)	RR (95% CI)	p-Value
Risk of worsening						
Single AS-OCT feature						
Attached grafts						
Posterior stromal ripples	No	37	11	22.9%		
	Yes	8	11	57.9%	2.52 (1.3–4.9)	0.006
Partially detached grafts						
Axial extension	≤1/3 surface area	28	21	42.9%		
	>1/3 surface area	3	13	81.3%	1.9 (1.18–3.04)	0.008
Posterior stromal ripples	Mild/none	27	21	43.8%		
	Moderate/severe	4	13	76.5%	1.75 (1.09–2.81)	0.020
Combined AS-OCT features						
Partially detached grafts						
If moderate/severe posterior stromal ripples						
Axial extension	≤1/3 surface area	2	8	80%		
	>1/3 surface area	2	5	71.4%	0.89 (0.51–1.56)	0.682
If mild/none posterior stromal ripples						
Axial extension	≤1/3 surface area	26	13	33.3%		
	>1/3 surface area	1	8	88.8%	2.67 (1.4–5.06)	0.003

Note: The top part of the table describes the number of DMEK grafts showing stability/improvement or worsening and percentage of the risk of worsening based on the presence of a single risk factor (either posterior stromal ripples or detachment axial extension). Relative risk of worsening with 95% CI is also reported based on posterior stromal ripples and detachment axial extension. The bottom part of the table shows numbers of DMEK grafts showing stability/improvement or worsening and percentage of the risk of worsening when posterior stromal ripples (moderate/severe and mild/none) and detachment axial extension (≤1/3 and >1/3 of the graft surface area) are analysed together in partially detached grafts. Relative risk of worsening with 95% CI is reported as above. p-values always refer to comparisons with the row above.

previously attached grafts (p = 0.014) and risk of detachment worsening over time (p = 0.025).

We therefore evaluated if the reason for grafting was associated with their presence in the early postoperative time. No association was found between stromal ripples and the reason for grafting, both considering all types of stromal ripples (p = 0.401) and only the moderate/severe type (p = 0.196). Also, no correlation was found comparing DMEK only and triple procedure (p = 0.547). We also tested if the presence and severity of stromal ripples were associated with surgical time and this was not significant (p = 0.673, one-way ANOVA). However, given the retrospective nature of the study, surgical time available (from entrance to exit theatre) could have been influenced by the type of tissue used depending on the need for tissue preparation. So, the analysis was repeated for surgeon stripped and preloaded tissues separately and, again, the presence and degree of stromal ripples did show no association with surgical time ($p = 0.733$ and $p = 0.236$, respectively).

Overall the presence and degree of stromal ripples were associated with different levels of CCT. In the whole study population, at first available AS-OCT, when no stromal ripples were present CCT was 624.3 ± 80.2 μm, when stromal ripples were mild CCT was 707.6 ± 62.9 μm and CCT was 757.4 ± 125.1 μm when they were moderate/severe ($p < 0.001$ one-way ANOVA). Group-by-group comparison with Bonferroni correction showed significant differences between none and mild or moderate/severe ripples (both $p < 0.001$) while not significant was the difference between mild and moderate/severe ripples ($p = 0.081$). Also, CV changed in accordance with the presence of posterior stromal ripples with values of 63 640 ± 29 206 μm^3 when no ripples were present, 68 974 ± 26 104 μm^3 with mild stromal ripples and 85 121 ± 43 228 μm^3 with moderate/severe ripples, with significant differences among groups ($p = 0.003$). However, group-by-group comparison with Bonferroni correction showed that significant differences were only present between no and moderate/severe ripples ($p = 0.002$).

3.6 | Risk of worsening and relative risk

3.6.1 | Attached grafts

We calculated the risk of worsening (RW) for previously attached grafts based on the presence of stromal ripples. If no posterior stromal ripples were present, the absolute risk of graft detachment was 22.9% (11/48), the presence of any degree of stromal ripples increased this risk to 57.9% (11/19). Relative risk (RR) of graft detachment in patients with posterior stromal ripples at AS-OCT was 2.52 ($p = 0.006$).

3.6.2 | Partially detached grafts

We calculated the risk of graft detachment worsening for grafts showing detachments involving ≤1/3 or >1/3 of the axial surface area and in the presence or absence of moderate/severe stromal ripples. Results on RW and RR are shown in Table 1. Evaluation of the combined risk of worsening when posterior stromal ripples (moderate/severe and mild/none) and detachment axial extension (≤1/3 and >1/3 of the graft surface area) are analysed together in partially detached grafts is also shown in Table 1.

4 | DISCUSSION

Descemet's membrane endothelial keratoplasty graft detachment represents an important challenge in DMEK surgery, and early identification and treatment of those detachments that show worsening over time are essential to improve visual outcomes (Deng et al., 2018); (Dirisamer et al., 2012); (Yeh et al., 2013). Although some DMEK graft detachments may still improve at 6 months without repeated intracameral air injections (Yeh et al., 2013), only specific types of graft detachments, like laminar detachments, show a tendency to spontaneously reattach to the host stroma over time. Conversely, other types of graft detachments, such as peripheral rolls, do not show the same tendency (Bucher et al., 2015). Our study aimed at identifying those AS-OCT factors predictive of detachment worsening over time. Our results showed that posterior stromal ripples and detachment axial extension were associated with a higher risk of graft detachment requiring re-bubbling. Furthermore, in DMEK with apparent graft attachment in the early postoperative time, the presence of posterior stromal ripples was associated with a greater risk of subsequent graft detachment together with recipient age, phaco-combined surgery and time at which AS-OCT was performed. Lastly, posterior stromal ripples, detachment axial extension, degrees of detachment involvement, peripheral roll-in shape and the presence of air in the AC proved to be risk factors for graft detachment worsening over time.

Previous studies have already explored the role of some of these variables in increasing the risk for graft detachment. Axial extension of graft detachment was highlighted as a risk factor for graft detachment at 6 months (Yeh et al., 2013); (Dirisamer et al., 2012). In 2013, Yeh et al., 2013. evaluated the predictive role of graft detachment seen at the AS-OCT performed at 1 h, 1 and 3 months after surgery. In their study, minimal peripheral graft detachments were included in the definition of completely attached grafts, whereas partially attached grafts were divided into those involving <1/3 or >1/3 of the graft surface area. Interestingly, they found that graft appearance at 1 h after surgery had the greatest predictive value in determining graft attachment at 6 months. Specifically, detachments at 1 h correlated in 75% of cases with detachments at 6 months, while grafts attached at 1 h were attached at 6 months in 96% of cases. Their study also showed that detachments involving >1/3 of the graft surface area noted at 1 week, correlated with detachments at 6 months in only 31% of cases, and only when this finding was associated with detachment at 1 h, the risk of the graft remaining detached was 75% (Yeh et al., 2013). Although their indication was to examine the 1-h and 1-week AS-OCT together, performing an AS-OCT 1 h after surgery might not be a feasible option in many centres. In addition, while our data on detachments at a mean of approximately 3 days after surgery were similar to those found at 1 week in their study, with 46% of grafts showing complete attachment (versus 49%), 32% showing detachment in less than 1/3 of the graft surface area (vs 17%) and 22% showing larger detachments (vs 33%), we found a much higher rate of worsening over time in cases of large detachments (81% vs. 31% in their study) (Yeh et al., 2013).

Also recipient age (Oellerich et al., 2017); (Parker et al., 2020), phaco-combined procedures (Leon et al., 2018); (Gundlach et al., 2015) and peripheral roll-in shape were already associated with the risk of graft detachment (Bucher et al., 2015).

A new and interesting finding of our study was the association of posterior stromal ripples with the risk of graft detachment requiring re-bubbling, risk of worsening previously attached grafts and risk of detachment worsening over time. Not only was the presence of stromal ripples noted in those grafts that were initially attached and detached at subsequent follow-up, but the presence of moderate/severe stromal ripples was also found to overcome the protective role of detachment extension of ≤1/3 of the graft surface area, resulting in detachment worsening.

In other words, when moderate/severe stromal ripples were present, the risk of smaller graft detachment worsening was equal to the risk of larger detachments (80% and 71.4% for detachment of ≤1/3 or >1/3, respectively). For detachments involving less than 1/3 of the graft surface area and not involving the visual axis, Dirisamer et al. suggested waiting for spontaneous corneal clearance (Dirisamer et al., 2012); we found this to be true only in cases with no moderate/severe stromal ripples. Consequently, when posterior stromal ripples are present, patients should be monitored more closely and managed on an individual basis as is the case with larger graft detachments, taking into account surgeon and patient preferences (Dirisamer et al., 2012).

What causes the stromal ripples to be present is still unclear. One study previously analysed the presence of corneal striae in various corneal diseases and in normal corneas (Grieve et al., 2017). Striae were observed in 82% of all corneas with at least one of the imaging modalities used in the study and in 38% of cases analysed with OCT. They identified corneal striae as ondulations in continuous lamellae mainly composed of collagen VI, lumican and keratocan, departing from the Descemet membrane and ending in the mild or anterior stroma. Although present at all corneal hydration levels, stromal hydration made them more visible (Grieve et al., 2017). If stromal ripples coincided with corneal striae, we would have expected them to be associated with the reason for grafting (higher frequency in BK), while our study did not find their presence to be associated with it. One might speculate they may be due to stromal oedema. Indeed, in our study, stromal ripples were associated with CCT. However, whether they appear first and lead to a problem with graft attachment and subsequent increase in CCT or they are a consequence of subclinical detachment, therefore of higher CCT in areas of apparent graft attachment is still not known. It is known that keratan sulphate (a major constituent of the posterior stroma) has a great capacity to absorb fluid, therefore, overhydration during surgery, leading to stromal ripples, could alter the negative imbibition pressure that normally facilitates graft adherence. Keratan sulphate also has the capacity to quickly loose the absorbed water, which is why oedema resolves quickly when endothelial pump function restarts after graft attachment (Edelhauser, 2006); (Costagliola et al., 2013). Unfortunately, we were unable to assess if posterior stromal ripples were present before surgery making them a preoperative risk factor for DMEK graft detachment, or if increased preoperative CCT was associated with their appearance, making them a consequence of high CCT, since most of our patients did not perform an AS-OCT immediately prior to surgery. In addition, no association was found between the presence and severity of stromal ripples and surgical time. However, we acknowledge that given the retrospective nature of the study, the only available surgical time was from entrance to exit theatre. This does not reflect the time of each surgical step. We might speculate that the time between recipient DM removal and donor tissue positioning could be more important in determining the level of stromal hydration, thus influencing the appearance of postoperative posterior stromal ripples. Further studies are needed to better understand how, why and when they develop and if any preoperative factors, such as corneal thickness or appearance immediately before surgery, or intraoperative factors, such as time of each surgical step or use of air, OVD or BSS, can predict their development. Should they be associated with preoperative stromal thickness, preoperative

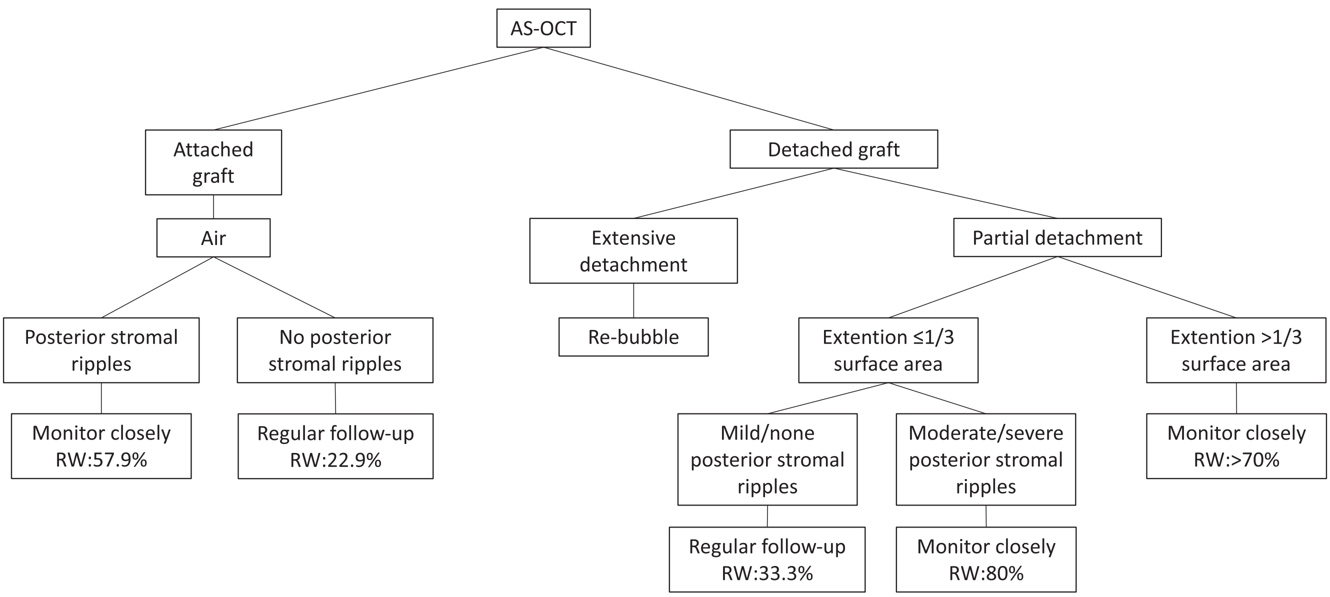


FIGURE 4 Flowchart showing the risk of worsening DMEK graft attachment over time based on the presence of stromal ripples and on detachment axial extension.

corneal deturgescence might reduce the risk of graft detachment.

The presence of air in the AC was surprisingly found to be associated with the risk of worsening over time. Longer air tamponade should be associated with decreased risk of graft detachment worsening over time. However, this likely represents the consequence of a limitation of our study, which is the different AS-OCT timing for our patients. Patients with earlier AS-OCT scans also had more frequent air left in the anterior chamber. Although earlier scans resulted associated with the risk of worsening only for previously attached grafts, the association between timing and air in the AC might have decreased its significance in other analyses.

Less conclusive and less easy to interpret were our results regarding the degree of detachment extension. A higher degree of extension of graft detachments was associated with the risk of worsening the detachment over time in the generalized linear model; however, in the group-by-group comparison of graft showing stability/improvement and worsening, it was not differently distributed, leading us to believe it might have a role only when combined with other parameters. Further studies are needed to clarify these points, ideally with the aid of artificial intelligence (AI) for more precise and faster measurements of graft detachments and their progression over time (Heslinga et al., 2020); (Hayashi et al., 2020).

There are limitations to our study, predominantly due to its retrospective nature. The timepoint of AS-OCT was not the same in all patients. However, this might reflect situations that are often encountered in daily practice, where patients might be given follow-up appointments at different time points. Also, all cases included in our study received air in the AC at the end of the surgery. Siebelmann et al. showed that the frequency and extension of graft detachment at any time point were greater with air tamponade compared with SF6 (Siebelmann et al., 2018); therefore, we are unable to determine if the findings of the present study can be applied to DMEK with SF6 gas injection.

Overall, the detachment rate in our study was high, reaching values up to 54% of grafts at first follow-up. However, as mentioned in the methods section, even very small areas of detachment were considered detached grafts as the aim was to follow their evolution over time, thus making this value not comparable with previous studies where different definitions of detached grafts were used. More than 1/3 axial extension at an average of approximately 3 days after surgery affected only 22% of eyes, which is in between the 9% and 33% rate of detachments involving >1/3 of the graft surface area found by Yeh t al at 1 h and 1 week postsurgery, respectively. (Yeh et al., 2013).

Despite limitations, we believe results from our study increase our knowledge of the natural history of graft detachment and provide new useful information, which can assist corneal surgeons in predicting DMEK graft detachment progression over time, especially in cases where AS-OCT at 1 h after surgery is not feasible.

To summarize, the flowchart below highlights the risk of worsening DMEK graft attachment over time in both attached and partially detached grafts based on the presence of stromal ripples and on detachment axial extension. We believe this scheme could be helpful in identifying which patients should be given regular or closely monitored follow-up based on their risk of worsening over time and highlight that when moderate/severe posterior stromal ripples are present, the risk of DMEK detachment worsening is invariably high (Figure 4).

Further studies using AI might help us in exactly defining the area and type of detachment to further improve our abilities to predict the detachment progression over time and to assist in making a timely clinical decision regarding if and when to re-bubble.

CONFLICT OF INTEREST

No conflicting relationship exists for any author.

ORCID

Giulia Coco https://orcid.org/0000-0002-2410-6366
Davide Romano https://orcid.org/0000-0002-8961-242X
Vito Romano https://orcid.org/0000-0002-5148-7643

REFERENCES

Bucher, F., Hos, D., Müller-Schwefe, S., Steven, P., Cursiefen, C. & Heindl, L.M. (2015) Spontaneous long-term course of persistent peripheral graft detachments after Descemet's membrane endothelial keratoplasty. *The British Journal of Ophthalmology*, 99, 768–772.

Chaurasia, S., Price, F.W., Gunderson, L. & Price, M.O. (2014) Descemet's membrane endothelial keratoplasty: Clinical results of single versus triple procedures (combined with cataract surgery). *Ophthalmology*, 121, 454–458.

Costagliola, C., Romano, V., Forbice, E., Angi, M., Pascotto, A., Boccia, T. et al. (2013) Corneal oedema and its medical treatment. *Clinical and Experimental Optometry*, 96(6), 529–535.

Dapena, I., Ham, L. & Melles, G.R.J. (2009) Endothelial keratoplasty: DSEK/DSAEK or DMEK - The thinner the better? *Current Opinion in Ophthalmology*, 20(4), 299–307.

Deng, S.X., Lee, W.B., Hammersmith, K.M., Kuo, A.N., Li, J.Y., Shen, J.F. et al. (2018) Descemet membrane endothelial keratoplasty: safety and outcomes: A report by the American Academy of ophthalmology. *Ophthalmology*, 125, 295–310.

Deshmukh, R., Nair, S., Ting, D.S.J., Agarwal, T., Beltz, J. & Vajpayee, R.B. (2022) Graft detachments in endothelial keratoplasty. *British Journal of Ophthalmology*, 106(1), 1–13.

Dirisamer, M., Van Dijk, K., Dapena, I., Ham, L., Oganes, O., Frank, L.E. et al. (2012) Prevention and management of graft detachment in Descemet membrane endothelial keratoplasty. *Archives of Ophthalmology*, 130, 280–291.

Edelhauser, H.F. (2006) The balance between corneal transparency and edema: The proctor lecture. *Investigative Ophthalmology & Visual Science*, 47, 1755–1767.

Gerber-Hollbach, N., Baydoun, L., López, E.F., Frank, L.E., Dapena, I., Liarakos, V.S. et al. (2017) Clinical outcome of rebubbling for graft detachment after Descemet membrane endothelial keratoplasty. *Cornea*, 36, 771–776.

Grieve, K., Ghoubay, D., Georgeon, C., Latour, G., Nahas, A., Plamann, K. et al. (2017) Stromal striae: A new insight into corneal physiology and mechanics. *Scientific Reports*, 7, 13584.

Gundlach, E., Maier, A.K.B., Tsangaridou, M.A., Riechardt, A.I., Brockmann, T., Bertelmann, E. et al. (2015) DMEK in phakic eyes: Targeted therapy or highway to cataract surgery? *Graefe's Archive for Clinical and Experimental Ophthalmology*, 253, 909–914.

Ham, L., van der Wees, J. & Melles, G.R.J. (2008) Causes of primary donor failure in Descemet membrane endothelial keratoplasty. *American Journal of Ophthalmology*, 145, 639–644.e1.

Hayashi, T., Tabuchi, H., Masumoto, H., Morita, S., Oyakawa, I., Inoda, S. et al. (2020) A deep learning approach in Rebubbling after Descemet's membrane endothelial keratoplasty. *Eye & Contact Lens*, 46, 121–126.

Heinzelmann, S., Böhringer, D., Eberwein, P., Reinhard, T. & Maier, P. (2016) Outcomes of Descemet membrane endothelial keratoplasty, Descemet stripping automated endothelial keratoplasty and penetrating keratoplasty from a single Centre study. *Graefe's Archive for Clinical and Experimental Ophthalmology*, 254, 515–522.

Heinzelmann, S., Böhringer, D., Haverkamp, C., Lapp, T., Eberwein, P., Reinhard, T. et al. (2018) Influence of postoperative intraocular pressure on graft detachment after Descemet membrane endothelial keratoplasty. *Cornea*, 37, 1347–1350.

Heslinga, F.G., Alberti, M., Pluim, J.P.W., Cabrerizo, J. & Veta, M. (2020) Quantifying graft detachment after Descemet's membrane endothelial keratoplasty with deep convolutional neural networks. *Translational Vision Science & Technology*, 9, 1–9.

Leon, P., Parekh, M., Nahum, Y., Mimouni, M., Giannaccare, G., Sapigni, L. et al. (2018) Factors associated with early graft detachment in primary Descemet membrane endothelial keratoplasty. *American Journal of Ophthalmology*, 187, 117–124.

López, E.F., Baydoun, L., Gerber-Hollbach, N., Dapena, I., Liarakos, V.S., Ham, L. et al. (2016) Rebubbling techniques for graft detachment after Descemet membrane endothelial keratoplasty. *Cornea*, 35, 759–764.

Mechels, K.B., Greenwood, M.D., Sudhagoni, R.G. & Berdahl, J.P. (2017) Influences on rebubble rate in Descemet's membrane endothelial keratoplasty. *Clinical Ophthalmology*, 11, 2139–2144.

Melles, G.R., Ong, T.S., Ververs, B. & van der Wees, J. (2006) Descemet membrane endothelial keratoplasty (DMEK). *Cornea*, 25, 987–990.

Moutsouris, K., Dapena, I., Ham, L., Balachandran, C., Oellerich, S. & Melles, G.R.J. (2011) Optical coherence tomography, scheimpflug imaging, and slit-lamp biomicroscopy in the early detection of graft detachment after Descemet membrane endothelial keratoplasty. *Cornea*, 30, 1369–1375.

Oellerich, S., Baydoun, L., Peraza-Nieves, J., Ilyas, A., Frank, L., Binder, P.S. et al. (2017) Multicenter study of 6-month clinical outcomes after Descemet membrane endothelial keratoplasty. *Cornea*, 36, 1467–1476.

Parekh, M., Leon, P., Ruzza, A., Borroni, D., Ferrari, S., Ponzin, D. et al. (2018) Graft detachment and rebubbling rate in Descemet membrane endothelial keratoplasty. *Survey of Ophthalmology*, 63, 245–250.

Parker, J.S., Ham, L., Parker, C.P., Parker, J.S., Dockery, P.W. & Melles, G.R.J. (2020) DMEK in super-seniors: clinical outcomes of Descemet membrane endothelial keratoplasty performed in patients ≥90 years old. *Current Eye Research*, 45, 1031–1035.

Röck, T., Bramkamp, M., Bartz-Schmidt, K.U., Röck, D. & Yörük, E. (2015) Causes that influence the detachment rate after Descemet membrane endothelial keratoplasty. *Graefe's Archive for Clinical and Experimental Ophthalmology*, 253, 2217–2222.

Rodríguez-Calvo De Mora, M.R., Groeneveld-Van Beek, E.A., Frank, L.E., Van DerWees, J., Oellerich, S., Bruinsma, M. et al. (2016) Association between graft storage time and donor age with endothelial cell density and graft adherence after Descemet membrane endothelial keratoplasty. *JAMA Ophthalmology*, 134, 91–94.

Romano, V., Kazaili, A., Pagano, L. et al. (2020) Eye bank versus surgeon prepared DMEK tissues: Influence on adhesion and rebubbling rate. *British Journal of Ophthalmology*, 106(2), 177–783.

Siebelmann, S., Ramos, S.L., Scholz, P., Matthaei, M., Schrittenlocher, S., Heindl, L.M. et al. (2018) Graft detachment pattern after Descemet membrane endothelial keratoplasty comparing air versus 20% SF6 tamponade. *Cornea*, 37, 834–839.

Stuart, A.J., Romano, V., Virgili, G. & Shortt, A.J. (2018) Descemet's membrane endothelial keratoplasty (DMEK) versus Descemet's stripping automated endothelial keratoplasty (DSAEK) for corneal endothelial failure. *Cochrane Database of Systematic Reviews*, 6(6), CD012097 John Wiley and Sons Ltd.

Tourtas, T., Schlomberg, J., Wessel, J.M., Bachmann, B.O., Schlötzer-Schrehardt, U. & Kruse, F.E. (2014) Graft adhesion in Descemet membrane endothelial keratoplasty dependent on size of removal of host's Descemet membrane. *JAMA Ophthalmology*, 132, 155–161.

Yeh, R.Y., Quilendrino, R., Musa, F.U., Liarakos, V.S., Dapena, I. & Melles, G.R.J. (2013) Predictive value of optical coherence tomography in graft attachment after Descemet's membrane endothelial keratoplasty. *Ophthalmology*, 120, 240–245.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Coco, G., Levis, H.J., Borgia, A., Romano, D., Pagano, L. & Virgili, G. et al. (2022) Posterior stromal ripples increase risk of Descemet's membrane endothelial keratoplasty graft detachment worsening over time. *Acta Ophthalmologica*, 00, 1–10. Available from: https://doi.org/10.1111/aos.15250