Principal Component Analysis of a Real-World Cohort of Descemet Stripping Automated Endothelial Keratoplasty and Descemet Membrane Endothelial Keratoplasty Cases
Demonstration of a Powerful Data-Mining Technique for Identifying Areas of Research

Perone, Jean Marc; Goetz, Christophe; Zevering, Yinka; Derumigny, Alexis

DOI
10.1097/ICO.0000000000003584

Publication date
2025

Document Version
Final published version

Published in
Cornea

Principal Component Analysis of a Real-World Cohort of Descemet Stripping Automated Endothelial Keratoplasty and Descemet Membrane Endothelial Keratoplasty Cases: Demonstration of a Powerful Data-Mining Technique for Identifying Areas of Research

Jean-Marc Perone, MD, FEBO, Christophe Goetz, MD,† Yinka Zevering, PhD,† and Alexis Derumigny, PhD‡*

Purpose: Principal component analysis (PCA) is a descriptive exploratory statistical technique that is widely used in complex fields for data mining. However, it is rarely used in ophthalmology. We explored its research potential with a large series of eyes that underwent 3 keratoplasty techniques: Descemet membrane endothelial keratoplasty (DMEK), conventional Descemet stripping automated endothelial keratoplasty (ConDSAEK), or ultrathin-DSAEK (UT-DSAEK).

Methods: All consecutive DMEK/DSAEK cases conducted in 2016 to 2022 that had ≥24 months of follow-up were included. ConDSAEK and UT-DSAEK were defined as preoperative central graft thickness ≥130 and <130 μm, respectively. Seventy-six patient, disease, surgical practice, and temporal outcome variables were subjected to PCA, including preoperative anterior keratometry, the use of sulfur hexafluoride gas (SF6) versus air for primary tamponade, and postoperative best corrected visual acuity and endothelial cell density. Associations of interest that were revealed by PCA were assessed with the Welch *t* test or Pearson test.

Results: A total of 331 eyes were treated with DMEK (n = 165), ConDSAEK (n = 95), or UT-DSAEK (n = 71). PCA showed that ConDSAEK and UT-DSAEK clustered closely, including regarding postoperative best corrected visual acuity, and were clearly distinct from DMEK. PCA and follow-up univariate analyses suggested that in DMEK, 1) flatter preoperative anterior keratometry (average, K1, and K2) associated with more rebubbling (P = 0.004–0.089) and graft detachment (P = 0.007–0.022); 2) graft marking did not affect postoperative endothelial cell density; and 3) lower postoperative endothelial cell density associated with SF6 use (all $P > 0.001$) and longer surgery (P = 0.005–0.091). All associations are currently under additional investigation in our hospital.

Conclusions: PCA is a powerful technique that can rapidly reveal clinically relevant associations in complex ophthalmological datasets.

Key Words: principal component analysis, DMEK, DSAEK, ultrathin-DSAEK, anterior keratometry, sulfur hexafluoride, endothelial cell density, graft detachment

(*Cornea* 2025;44:209–220)

Principal component analysis (PCA) is a descriptive exploratory statistical technique that reduces the dimensionality of large complex datasets while minimizing information loss. This generates a simpler and, therefore, more interpretable and visualizable dataset in which the original information is concentrated in a few key variables. It is useful in several disciplines,[1] including in omics research.[2] To our knowledge, PCA has rarely been used in clinical ophthalmology and has never been used in the field of keratoplasty.

To assess the utility of PCA, we used it to examine 76 variables in our real-world series of Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK) cases. In DSAEK, the diseased corneal endothelium and its overlying Descemet membrane (DM) are replaced with a 100- to 200-μm-thick graft composed of endothelium, DM, and posterior stroma.[3] DMEK uses a similar graft, except it lacks stromal tissue and is, therefore, only 15-μm thick.[4] These techniques effectively restore visual acuity (VA) in patients with corneal endothelial diseases, including Fuchs endothelial corneal dystrophy (FECD) and pseudophakic bullous keratopathy (PBK), with fewer complications relative to penetrating keratoplasty, their predecessor technique.[5–8] DMEK yields better VA and other outcomes than DSAEK,[9] but the thin graft and its tendency to scroll make DMEK more challenging.[10] In particular, it is prone to graft detachment that

Received for publication January 12, 2024; revision received April 24, 2024; accepted April 24, 2024.

From the *Department of Ophthalmology, Metz-Thionville Regional Hospital Center, Mercy Hospital, Metz, France; †Clinical Research Support Unit, Metz-Thionville Regional Hospital Center, Mercy Hospital, Metz, France; and ‡Department of Applied Mathematics, Delft University of Technology, Delft, the Netherlands.

The authors have no funding or conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.corneajrnl.com).

Correspondence: Jean-Marc Perone, MD, FEBO, Department of Ophthalmology, Metz-Thionville Regional Hospital Center, Mercy Hospital, 1 allée du Château, CS 45001, 57085 Metz-Cedex 03, France (e-mail: jean-marc.perone@chr-metz-thionville.fr).

requires rebubbling. This may augment post-DMEK endothelial cell loss (ECL),[11–13] thereby promoting graft failure.[14]

To improve DSAEK/DMEK outcomes, surgical adjustments have been proposed. One involves removing stroma from the DSAEK graft with a microkeratome[15]: This generates ultrathin-DSAEK (UT-DSAEK) grafts whose central graft thickness (CGT) is <130 μm (or <100 μm, depending on the definition used).[16–18] However, while some studies suggest that UT-DSAEK yields superior VA to conventional DSAEK (ConDSAEK)[16,19,20] and may even approach the visual outcomes of DMEK,[16,17,21,22] other studies have not observed this.[23–26] Surgical adjustments in DMEK include using 20% sulfur hexafluoride gas (SF6) for the initial tamponade because these bubbles last twice as long as air bubbles.[27] Moreover, to prevent misorientation of the DMEK graft, eye banks or the surgeon marks the exposed DM with dermatological biopsy punches or gentian violet ink stamps or pens.[28–32] However, the impact of these approaches on DMEK outcomes is still being assessed.

Here, we used PCA to explore the relationships between DMEK, ConDSAEK, and UT-DSAEK in our real-world patient cohort in terms of 76 demographic and pre/peri/postoperative variables, including SF6 use and graft marking.

PATIENTS AND METHODS

Study Design and Ethics

This retrospective single-center cohort study was conducted in Regional Metz-Thionville Hospital Center, Grand Est, France. It was approved by the Ethics Committee of the French Society of Ophthalmology (Institute Review Board 00855 Société Française d'Ophtalmologie IRB#1), registered in ClinicalTrials.gov (Identifier: NCT04424550), and adhered to the Helsinki Declaration. All patients were informed before surgery that their surgery-related data might be used for research. All consented to this possibility. The consent procedure was conducted in accordance with the reference methodology MR-004 of the National Commission for Information Technology and Liberties of France (No. 588909 v1).

Patient Selection

The prospectively maintained medical records were searched for all consecutive adult (≥18 years) patients who underwent DSAEK or DMEK in September 2016 to February 2022 and were followed for at least 24 months. Eyes with ocular pathologies that could interfere with the final visual outcome (dry/atrophic macular degeneration, glaucoma, advanced diabetic retinopathy, and any maculopathy) or a history of retinal detachment or vitreomacular surgery were excluded.

Preoperative and Postoperative Examinations

Before surgery and on postoperative days 8 and 15, at months 1, 2, 3, 6, and 12, and yearly thereafter, all patients underwent standard ophthalmological examinations, including measurement of best corrected VA (BCVA) in logMAR. Preoperative endothelial cell density (ECD) was recorded by the eye bank. Where possible, postoperative ECD was measured with no-contact specular microscopy (NIDEK CEM-530 NIDEK Co, Ltd, Tokyo, Japan). While postoperative ECD could be measured at 6 months and later for most DMEK eyes, only a few such measurements were obtained for DSAEK eyes. Biomicroscopic eye examinations and anterior segment-optical coherence tomography (AS-OCT) (RS 3000; OCT RetinaScan Advance; NIDEK Co, Ltd) were also conducted. The preoperative CGT of the DSAEK grafts was measured just before and after thinning by the surgeon using an ultrasound pachymeter (Handy Pachymeter SP-100, Tomey Corporation, Nagoya, Japan).The postoperative CGT was measured by a specifically dedicated orthoptist, who applied AS-OCT while using calipers in the center of the graft at the echo location. Pre/postoperative central corneal thickness (CCT) was measured with non-contact ultrasonic pachymetry (Tono pachymeter NT-530P; Nidek Co, Gamagori Aichi, Japan).

Surgical Techniques

DSAEK and DMEK were performed by the same experienced surgeon (J.-M.P.) as described.[33,34] The unprepared corneal grafts were stored in an organ culture medium and delivered after 2 days of deturgescence in deturgescence and shipment medium. The ECDs exceeded 2100 cells/mm^2. Most patients underwent surgery under general anesthesia. The remainder underwent peribulbar locoregional anesthesia. In all DMEK/DSAEK surgeries, the recipient epithelium was marked with a 9-mm trephine and a 9-mm-diameter disc of patient endothelium and DM was removed with an inverted Price–Sinskey hook (single-use PRICE hook #17302; MORIA SA, Anthony, France).

DSAEK Procedure

DSAEK was conducted as described.[16] The graft was thinned as much as possible just before surgery on an artificial chamber (Moria Single-Use Artificial Chamber, ref 19182; MORIA SA) with a 250-, 300-, or 350-μm-head rotational microkeratome (CBm turbine; MORIA SA). A second thinning was sometimes conducted (termed "double-cut"). The graft was trephined with a Hanna punch (ONE; MORIA SA) to produce an 8-mm-diameter disc. Another corneal incision was made opposite the first incision. The graft was placed on a Busin spatula (Single-Use Busin Spatula #17300; MORIA SA), introduced into the anterior chamber with Busin forceps (Single-Use Busin Forceps 23 G #17301; MORIA SA), and immediately centralized on the posterior surface of the recipient cornea by intracameral injection of a sterile air bubble.

DMEK Procedure

DMEK was conducted as described.[4] Just before surgery, the graft was tinted with Vision Blue (DORC International, Zuidland, The Netherlands) and trepanned with

an 8-mm Hanna microtrephine (Single-Use Busin Punch; MORIA SA), and the DM was dissected with disposable curved monofilament forceps. In the first quarter of our DMEK series, the graft was not marked for orientation. However, starting May 2017, the exposed DM of the graft was marked with a capital F or E with gentian violet ink from a sterile dermographic pen (Skin Marker; Devon, Japan). In December 2020, we added an asterisk (ie, F*) to further improve orientation. The ink marks disappeared within a day of surgery. The dissected graft was introduced into the DORC injectable system (DORC International, Zuidland, The Netherlands) with a glass cannula, injected into the anterior chamber, and unfolded with an external maneuver. A tamponade was immediately created to hold the graft against the host stroma. The tamponade was composed of sterile air until September 2020, at which point 20% SF6 in air was used.

Triple Procedure

If the patient was phakic, cataract surgery was performed before DMEK or DSAEK (termed "triple surgery") with a supracapsular technique[35] using Stellaris PC (Bausch and Lomb, Aliso Viejo, CA) and an intraocular lens (Zeiss CT Asphina 409 MV).

Postoperative Care and Complication Definitions

All patients were kept in the supine position for 12 postoperative hours and hospitalized in the Ophthalmology Unit for 3 days. All patients were then monitored by postsurgical visits at days 8 and 15, at months 1, 3, 6, and 12, and annually thereafter.

All patients applied 0.1% dexamethasone, neomycin, and polymyxin B drops (Maxidrol; ALCON, Rueil Malmaison, France; 4 four times daily) for 4 weeks. After tapering these drops, a 0.1% fluorometholone (Flucon; ALCON) regimen was administered for at least 12 months and then tapered. Patients who underwent triple-DMEK also received non-steroidal anti-inflammatory drug drops (indomethacin 0.1%; Chauvin, Montpellier, France; 4 times/day) for 4 weeks.

Irvine–Gass syndrome with VA loss was treated with oral acetazolamide (Diamox; Sanofi, Gentilly, France; 250 mg tablet 3 times/day) for 1 month and indomethacin 0.1% drops for 1 month as described above.

Allograft rejection (defined as a line of retrodescemetic precipitates) was treated for 1 month with Sterdex (dexamethasone with oxytetracycline; Thea, France; Clermont-Ferrand, 2 times/day) and the antiinflammatory corticosteroid eyedrop Maxidrol (ALCON; 12 times/day for 1 week and then 8, 6, and 4 times/day for the second, third, and fourth week, respectively).

A graft was defined as "significantly detached" if AS-OCT in the first postsurgical weeks showed that the detached area exceeded 20% of the graft and/or it threatened the visual axis. Rebubbling was conducted immediately under topical anesthesia (0.4% oxybuprocaine hydrochloride; Thea, Clermont-Ferrand, France) using sterile air and an operating microscope. If a fourth rebubbling session was needed, the graft was defined as a primary graft failure case and the patient was scheduled for repeat keratoplasty. Rebubbling was sometimes also conducted when more minor detachment was observed.

Other Definitions and Collected Variables

UT-DSAEK and ConDSAEK were defined as DSAEK with postcutting grafts that were ≤130 and >130 μm thick, respectively.[18] Seventy-six variables were collected from the prospectively maintained medical database. They were not selected for any reason other than that they were in our analytical DSAEK/DMEK database at the time of study.

The variables for the DMEK, UT-DSAEK, and ConDSAEK groups were as follows: patient age and sex; right/left eye; indication (FECD, PBK, second graft, and viral endothelial keratopathy); type of anesthesia (general vs. locoregional); use of triple-DMEK/DSAEK; whether the graft was a first-ever graft; graft donor age; surgery duration (from starting graft preparation to suture placement); postoperative Irvine–Gass syndrome; significant graft detachment; surgical success (clear cornea with graft in place and quantifiable VA over 1 postoperative year); preoperative and postoperative BCVA; and preoperative ECD.

The following variables were collected for the DMEK patients only: average anterior keratometry; K1; K2; axial length; whether the graft was not marked or marked with a capital F or E (single mark) or F* (double mark); whether SF6 was used for the tamponade instead of air; whether graft rejection, any rebubbling, multiple rebubbling, and primary graft failure occurred; and CCT before surgery and at all postoperative time points.

The following variables were collected for the UT-DSAEK and ConDSAEK patients only: cutting blade thickness; use of double cutting; and CGT before cutting, immediately after cutting, and at all postoperative time points.

Postoperative ECD was collected from all patients where possible, including a few DSAEK patients at 3 to 5 years.

Principal Component Analysis

PCA is conducted by computing a correlation matrix using pairwise complete observations.[1] This creates geometrical axes called principal components (PCs) that explain the total variation of the dataset in a hierarchical manner. The concept is best visualized with a 2-dimensional correlation circle (Fig. 1). Thus, the PC1 axis is shaped by the variables that both account for the most variation in the dataset and correlate with each other positively or negatively. These correlations determine the location of the center point of the PC1 axis (the origin): Thus, the variables that lie on or near PC1 to the left of the origin correlate positively with each other and negatively with the variables that lie on or near PC1 but to the right of the origin. The variables that lie furthest from the origin account most strongly for the variation represented by PC1. Similarly, the PC2 axis is shaped by the variables that account for the second largest source of

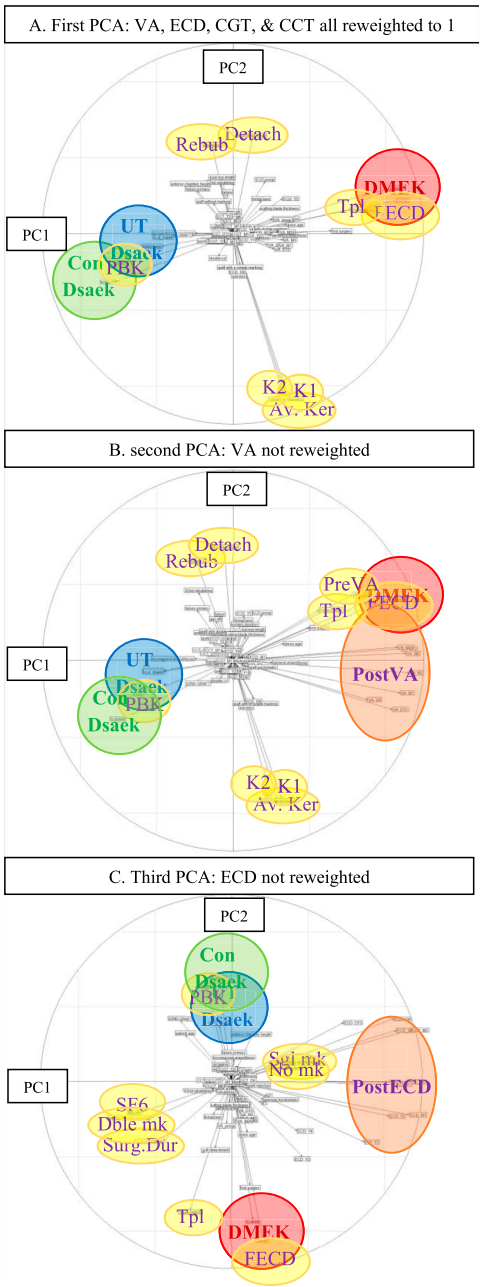

FIGURE 1. Correlation circle showing the contributions of the 76 study variables to PC1 and PC2 and their relationships with each other in (A) the first PCA (the multiple postoperative VA, ECD, CGT, and CCT time point data were reweighted to 1), (B) the second PCA (the multiple preoperative VA time point data were not reweighted), and (C) the third PCA (the multiple preoperative ECD time point data were not reweighted). Av. Ker, average anterior keratometry value; Dble mark, double marking of graft; Detach, graft detachment that affects VA or involves >1/3 of the graft; No mk, no marking of the graft; PostECD, postoperative ECD; PostVA, postoperative VA; PreVA, preoperative VA; Rebub, 1 or more rebubbling sessions; SF6, use of sulfur hexofluoride instead of air for tamponade; Sgl mk, single marking of the graft; Surg. Dur, surgery duration; Tpl, triple (phaco) surgery.

variation in the dataset. These variables do not correlate with the PC1-shaping variables. Consequently, PC2 lies orthogonal to PC1 on the correlation circle and also goes through the origin. As with PC1, the location of the variables that lie close to PC2 depends on how much PC2-encompassing variation they account for and how they correlate with each other. Thus, the correlation circle shows how the variables in the dataset relate to PC1 and PC2. The contribution of each variable to PC1 and PC2 can also be shown by factor-loading histograms. Biplots that show how sample groups relate to each other in terms of all other variables are generated by projecting the dataset on PC1 and PC2.

The remaining PCs are derived in a similar way. Thus, PC3 is shaped by the third largest source of variation and lies orthogonal to both PC1 and PC2 on a 3-dimensional graph. PC3 and the remaining PCs (ie, PC4 and PC5) often account for little information and are disregarded. A scree plot shows the amount of variation in the whole dataset that is explained by each PC.

Statistical Analysis

The patient variables were expressed as mean ± deviation or n (%). Three PCAs were conducted on the 76 variables by diagonalizing the correlation matrix by using the R base function eigen(). The R code that was used for the PCAs is available in the GitHub repository https://github.com/AlexisDerumigny/Reproducibility-Graft_cornea. Correlation circles, factor-loading histograms, biplots, and scree plots were made with package ggplot2 version 3.5.0 and package adegraphics version 1.0-21. Patient groups were compared with analysis of variance, Welch t test, or χ^2 test. PCA-revealed associations were confirmed with the Welch t test or Pearson test. All statistical analyses were conducted using R Statistical Software version 4.3.3 (2024-02-29). Significance was set to 0.05. P values were not adjusted for multiple comparisons because of the exploratory nature of the study.

RESULTS

Patient/Eye Characteristics in the DMEK, UT-DSAEK, and ConDSAEK Groups

In total, 331 eyes (331 patients) were treated with DMEK (n = 165), UT-DSAEK (n = 71), or ConDSAEK (n = 95).

The DSAEK groups did not differ from each other except that UT-DSAEK was conducted for FECD more often (34% vs. 18%, $P = 0.03$), involved triple-DSAEK more often (13% vs. 4%, $P = 0.024$), resulted in graft detachment more often (14% vs. 4%, $P = 0.047$), and, as expected, associated with lower postcutting preoperative CGT and postoperative CGT (all $P < 0.001$). Clinically significant differences in baseline or postoperative BCVA were not observed (Table 1).

Compared with the DSAEK patients overall, the DMEK patients were younger (72 vs. 76–77 years, $P < 0.001$). They were less likely to have PBK (4% vs. 41%–53%, $P < 0.001$) and undergo second graft (4% vs. 27%–28%, $P = 0.008$) and more likely to have FECD (93% vs.

TABLE 1. Demographic, Clinical, and Surgical Characteristics of the Eyes That Underwent DMEK, UT-DSAEK, and ConDSAEK (n = 331) and Their Changes in BCVA, CCT, CGT, and ECD After Surgery

	DMEK (n = 165)		UT-DSAEK (n = 71)		ConDSAEK (n = 95)		DMEK Versus UT Versus Con	UT Versus Con	DMEK Versus Total DSAEK
Characteristics	**n**	**Mean ± SD or n (%)**	**N**	**Mean ± SD or n (%)**	**n**	**Mean ± SD or n (%)**	***P****	***P*†**	***P*†**
Patient demographics and preoperative clinical characteristics									
Age, y	165	72 ± 9	71	76 ± 8	95	77 ± 10	<0.001	0.56	<0.001
Female sex	165	110 (67)	71	48 (68)	95	62 (65)	0.95	0.88	1.00
Right eye	165	84 (51)	71	34 (48)	95	57 (60)	0.24	0.16	0.55
Average keratometry, D	156	43.53 ± 1.62	Data not available for DSAEK patients						
K1, D	156	42.96 ± 1.66							
K2, D	156	44.16 ± 1.67							
Axial length, mm	143	23.60 ± 1.53							
Indications, graft donor age, and surgical details									
Indication	165		71		95				
PBK		7 (4)		29 (41)		50 (53)	<0.001	0.18	<0.001
FECD		153 (93)		24 (34)		17 (18)	<0.001	0.030	<0.001
Second graft		7 (4)		19 (27)		27 (28)	0.027	0.79	0.008
Viral keratopathy		0		0		1 (1)	1.000	1.000	1.000
Age of graft donor, y	164	75 ± 11	71	65 ± 14	95	68 ± 11	<0.001	0.16	<0.001
General anesthesia	165	161 (98)	71	52 (73)	95	77 (81)	<0.001	0.31	<0.001
Triple (phaco) procedure	165	72 (44)	71	9 (13)	95	4 (4)	<0.001	0.024	<0.001
First graft ever	165	157 (95)	71	48 (68)	95	64 (67)	<0.001	1.00	<0.001
DSAEK cuttg blade thick, μm	Not applicable for DMEK		71	356 ± 40	95	326 ± 37	—	<0.001	—
Double cut			71	2 (3)	95	10 (11)	—	0.11	—
Graft not marked	165	40 (24)	Not applicable for DSAEK						
Graft marked once	165	90 (55)							
Graft marked twice	165	35 (21)							
SF6 gas used for tamponade	165	41 (25)							
Surgery duration, min	165	35 ± 9	71	31 ± 9	95	33 ± 9	0.35	0.13	0.61
Postoperative complications									
Irvine–Gass syndrome	165	11 (7)	71	2 (3)	95	0 (0)	0.025	0.35	0.023
Graft rejection	165	2 (1)	Not available for DSAEK						
Significant graft detachment	165	25 (15)	71	10 (14)	95	4 (4)	0.025	0.047	0.085
Any rebubbling	165	57 (35)	Not available for DSAEK						
Multiple rebubbling	165	12 (7)							
Primary graft failure	165	6 (4)							
Surgical success‡	165	158 (96)	71	70 (99)	95	95 (100)	0.082	0.883	0.072
Preoperative and postoperative BCVA, logMAR									
Preop	136	0.68 ± 0.35	71	1.28 ± 0.45	95	1.33 ± 0.45	<0.001	0.51	<0.001
Day 8	164	0.80 ± 0.44	71	1.31 ± 0.38	88	1.25 ± 0.38	<0.001	0.33	<0.001
Day 15	165	0.52 ± 0.44	71	1.02 ± 0.42	95	1.00 ± 0.36	<0.001	0.72	<0.001
Month 1	161	0.31 ± 0.30	71	0.75 ± 0.38	95	0.82 ± 0.37	<0.001	0.20	<0.001
Month 3	160	0.18 ± 0.24	71	0.57 ± 0.34	95	0.57 ± 0.25	<0.001	0.96	<0.001
Month 6	158	0.12 ± 0.17	71	0.45 ± 0.33	95	0.47 ± 0.21	<0.001	0.75	<0.001
Month 12	157	0.09 ± 0.18	71	0.41 ± 0.34	95	0.41 ± 0.21	<0.001	0.91	<0.001
Month 24	154	0.07 ± 0.17	71	0.40 ± 0.37	95	0.39 ± 0.23	<0.001	0.88	<0.001
Preoperative and postoperative CCT, μm									
Preop	155	620 ± 63	71	637 ± 42	95	625 ± 55	0.71	0.35	0.33
Day 8	8	601 ± 41	Not applicable for DSAEK						
Day 15	18	614 ± 85							

(*Continued*)

TABLE 1. (*Continued*) Demographic, Clinical, and Surgical Characteristics of the Eyes That Underwent DMEK, UT-DSAEK, and ConDSAEK (n = 331) and Their Changes in BCVA, CCT, CGT, and ECD After Surgery

	DMEK (n = 165)		UT-DSAEK (n = 71)		ConDSAEK (n = 95)		DMEK Versus UT Versus Con	UT Versus Con	DMEK Versus Total DSAEK
Characteristics	**n**	**Mean ± SD or n (%)**	**N**	**Mean ± SD or n (%)**	**n**	**Mean ± SD or n (%)**	***P****	***P*†**	***P*†**
Month 1	30	565 ± 68							
Month 3	54	552 ± 59							
Month 6	68	550 ± 57							
Year 1	84	547 ± 46							
Year 2	87	548 ± 47							
Preoperative and postoperative CGT, μm									
Immediately after cutting	Not applicable for DMEK		71	111 ± 18	95	188 ± 41	—	<0.001	—
Day 8			71	92 ± 25	92	171 ± 56	—	<0.001	—
Day 15			71	82 ± 20	94	149 ± 45	—	<0.001	—
Month 1			71	76 ± 18	95	137 ± 44	—	<0.001	—
Month 3			71	72 ± 18	95	129 ± 43	—	<0.001	—
Month 6			71	71 ± 17	95	130 ± 41	—	<0.001	—
Year 1			71	70 ± 17	95	131 ± 42	—	<0.001	—
Year 2			71	73 ± 20	95	130 ± 42	—	<0.001	—
Preoperative and postoperative ECD, cells/mm^2									
Preop (graft)	165	2555 ± 199	71	2540 ± 201	95	2445 ± 218	<0.001	0.004	0.003
Day 8	9	1638 ± 462	—	—	—	—	—	—	—
Day 15	10	1745 ± 469	—	—	—	—	—	—	—
Month 1	56	1583 ± 457	4	890 ± 105	—	—	—	—	—
Month 2	74	1332 ± 488	—	—	—	—	—	—	—
Month 3	109	1299 ± 481	7	1364 ± 538	9	1412 ± 397	0.76	0.85	0.45
Month 6	156	1268 ± 473	25	1289 ± 378	20	1224 ± 393	0.89	0.58	0.91
Year 1	144	1135 ± 451	23	1176 ± 316	27	1027 ± 390	0.41	0.14	0.54
Year 2	133	1051 ± 419	20	1031 ± 272	21	888 ± 310	0.21	0.12	0.12
Year 3	—	—	14	895 ± 246	14	875 ± 296	0.85	0.85	—
Year 4	—	—	7	904 ± 328	13	987 ± 333	0.50	0.60	—
Year 5	—	—	8	919 ± 207	12	949 ± 309	0.81	0.80	—

The data are presented as mean ± SD or n (%). Dashes (—) indicate missing values.

**P* values were determined with analysis of variance.

†*P* values were determined with the Welch *t* test or chi-squared test.

‡Surgical success was defined as a clear cornea with the graft in place and quantifiable VA over 1 yr of follow-up.

CCT, central corneal thickness (DMRK patients only); CGT, central graft thickness (DSAEK patients only); D, diopters; DSAEK cuttg blade thick, the thickness of the microkeratome blade used to prepare the DSAEK graft; double cut, the DSAEK graft was cut twice with the microkeratome; Preop, preoperative; triple procedure, phaco-DMEK or phaco-DSAEK.

18%–34%, $P < 0.001$) and undergo general anesthesia (98% vs. 73%–81%, $P < 0.001$) and the triple procedure (44% vs. 4%–13%, $P < 0.001$). Their graft donors were also older (75 vs. 65%–68% years, $P < 0.001$). They were more likely to develop Irvine–Gass syndrome (7% vs. 0%–3%, $P = 0.023$) and tended to develop significant graft detachment more often (15% vs. 4%–14%, $P = 0.085$). The baseline BCVA of the DMEK eyes was significantly lower (0.68 vs. 1.28–1.33 logMAR, $P < 0.001$), as was their BCVA at all postoperative time points (all $P < 0.001$) (Table 1).

Regarding the DMEK patients only, the average keratometry, K1, and K2 values were 43.53, 42.96, and 44.16 D, respectively, and the mean axial length was 23.60 mm. The graft was unmarked, marked once, and marked twice in 24%, 55%, and 21% of cases, respectively. SF6 was used instead of air for the tamponade in 25% of cases. Rebubbling and multiple rebubbling were conducted in 35% and 7% of all DMEK cases, respectively (Table 1).

Preoperative ECD was available for all patients. DMEK and DSAEK did not differ in terms of this variable at a clinically significant level (2555 vs. 2445–2540 cells/mm^2). Postoperative ECD was available for most DMEK patients at 12 and 24 months (87%–95%) but generally missing at earlier time points. Postoperative ECD was rarely obtained in the DSAEK patients (Table 1). Nonetheless, to demonstrate that the data-mining procedure of PCA can be effective even when there are several missing values, all of these data, including the few available year-3, -4, and -5 ECD data from DSAEK patients, were included in the PCAs.

Principal Component Analysis

Three PCAs were conducted on the 76 patient/surgery/follow-up variables in our database. These data are shown in Figures 1–2 and Supplemental Figures 1 to 3 (http://links.lww.com/ICO/B676, http://links.lww.com/ICO/B677, http://links.lww.com/ICO/B678).

First PCA: Reweighting of Postoperative VA, ECD, CGT, and CCT

Postoperative VA, ECD, DSAEK CGT, and DMEK CCT were all recorded at 7 to 11 time points. Since this artificially emphasizes their correlations with other variables, we first reweighted (normalized) the corresponding entries of the correlation matrix so that each of these 4 variables was counted as 1 variable. The scree plot of this first PCA (see Supplemental Fig. 1A, http://links.lww.com/ICO/B676) showed that PC1 and PC2 accounted for 11% and 9% of the total variation in the dataset, respectively. The correlation circle (Fig. 1A) showed that the reweighted variables all lay close to the origin (signaling little contribution to the correlation circle pattern) and that the variables that contributed most strongly to PC1 were 1) DMEK, FECD, and triple surgery, which were scattered near the right-hand side of PC1, and 2) UT-DSAEK, ConDSAEK, and PBK, which were located near the left-hand side of PC1. The factor-loading histogram depicts the same pattern in a different format (see Supplemental Fig. 2A, http://links.lww.com/ICO/B677). DMEK, FECD, triple surgery, UT-DSAEK, ConDSAEK, and PBK accounted for 49% of the variation represented by PC1. Thus, PC1 was driven largely by the keratoplasty type and their predominant indications. Triple surgery likely clustered with DMEK because it was conducted more often for DMEK (44%) than DSAEK (4%–13%) (Table 1).

Interestingly, PC2 was shaped most strongly by several DMEK variables, namely preoperative average keratometry, K1, and K2 at one end and graft detachment and rebubbling at the other (Fig. 1A and see Supplemental Fig. 2A, http://links.lww.com/ICO/B677). These variables accounted for 52% of the variation represented by PC2.

Thus, 1) DMEK, FECD, and triple surgery correlated strongly and positively with each other; 2) ConDSAEK, UT-DSAEK, and PBK correlated strongly and positively with each other and strongly negatively with DMEK/FECD/triple surgery; and 3) preoperative keratometry in DMEK correlated negatively with graft detachment and its surrogate measure rebubbling.

The PCA biplot shows the relationship between the samples in the DMEK, ConDSAEK, and UT-DSAEK groups in terms of their associations with all variables. The 2 DSAEK groups overlapped while DMEK clearly demonstrated a different distribution overall (Fig. 2A).

Second PCA: No Reweighting of Postoperative VA

Some studies suggest that UT-DSAEK yields better VA than ConDSAEK[16,19,20] and may even approach the performance of DMEK,[16,17,21,22] but other studies do not observe this.[23–26] To examine the relationships between postoperative VA, endothelial keratoplasty mode, and other variables, we repeated the PCA but without reweighting postoperative VA: This emphasizes any correlations between postoperative VA and the other variables. Postoperative ECD, CGT, and CCT continued to be reweighted to a single variable each. The scree plot showed that PC1 and PC2 accounted for 16% and 9% of the total variation, respectively (see Supplemental Fig. 1B, http://links.lww.com/ICO/B676). As intended, not normalizing postoperative VA greatly increased its importance: Postoperative VA now lay at the right-hand end of PC1

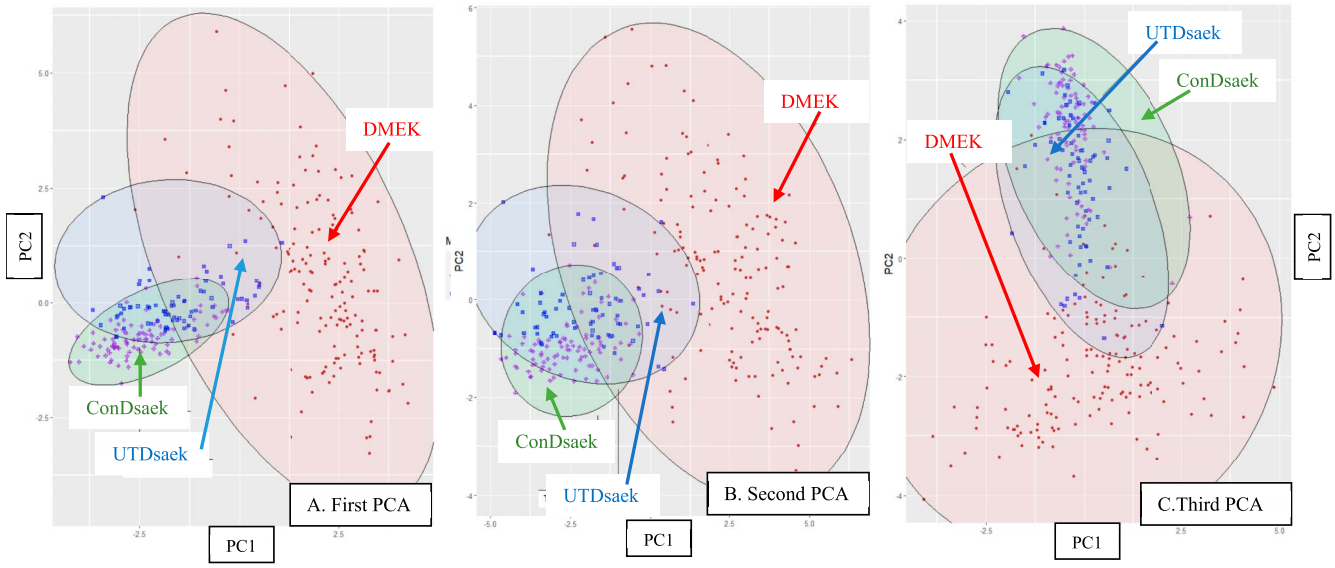

FIGURE 2. Biplot showing the orientation of the DMEK, UTDSAEK, and ConDSAEK groups relative to PC1 and PC2 and their relationships with each other in the (A) first, (B) second, and (C) third PCA.

in the correlation circle (Fig. 1B). However, emphasizing postoperative VA did not alter any of the associations seen in the first PCA: Furthermore, DMEK/FECD/triple surgery clustered together on PC1 across from ConDSAEK/UT-DSAEK/PBK, and PC2 showed the same negative association between keratometry and graft detachment/rebubbling. No new associations were observed. Postoperative VA clustered closely with DMEK/FECD/triple surgery but so did preoperative VA (Fig. 1B and Supplemental Fig. 2B, http://links.lww.com/ICO/B677). In the biplot, DMEK continued to cluster separately from the 2 DSAEK groups (Fig. 2B).

Thus, in our cohort, while DMEK correlated with better postoperative VA than either DSAEK groups, this is likely to reflect the lower preoperative VA in the DMEK patients, which correlates with postoperative VA.[36] This was also demonstrated by a plot showing the evolution of VA over time in the 3 groups (Fig. 3) and may reflect the fact that most DMEK patients had FECD while most DSAEK patients had PBK (Table 1). Thus, postoperative VA may be primarily driven by the keratoplasty indication, which shapes the preoperative VA.

Third PCA: No Reweighting of Postoperative ECD

Since DMEK is a difficult technique and associates with substantial ECL,[37] we asked whether we could identify potential risk factors of ECL in DMEK by not reweighting postoperative ECD (most of these values were from DMEK patients): This would emphasize any correlations between postoperative ECD and the other variables. Postoperative VA, CGT, and CCT all remained reweighted to 1 value each. The scree plot showed that PC1 and PC2 now accounted for 19% and 8% of the total variation, respectively (see Supplemental Fig. 1C, http://links.lww.com/ICO/B676). The correlation circle showed that, as intended, not reweighting postoperative ECD greatly increased the importance of this variable: Postoperative ECD now lay at the right-hand end of PC1, and interestingly, SF6 use, double marking, and surgery duration lay on the other side. Thus, postoperative ECD seemed to correlate negatively with these variables. PC2 showed the same pattern observed for PC1 in the first and second PCAs, namely DMEK/FECD/triple surgery at one end and ConDSAEK/UT-DSAEK/PBK at the other (Fig. 1C). All of these patterns were also shown by the factor-loading histogram (see Supplemental Fig. 2C, http://links.lww.com/ICO/B677). The biplot continued to show that the 2 DSAEK groups clustered closely while DMEK was clearly separate (Fig. 2C).

Thus, the 3 PCAs suggested that 1) lower preoperative anterior keratometry values in DMEK correlate with rebubbling and graft detachment and 2) SF6 use, double marking, and longer surgery duration in DMEK associate with lower postoperative ECD.

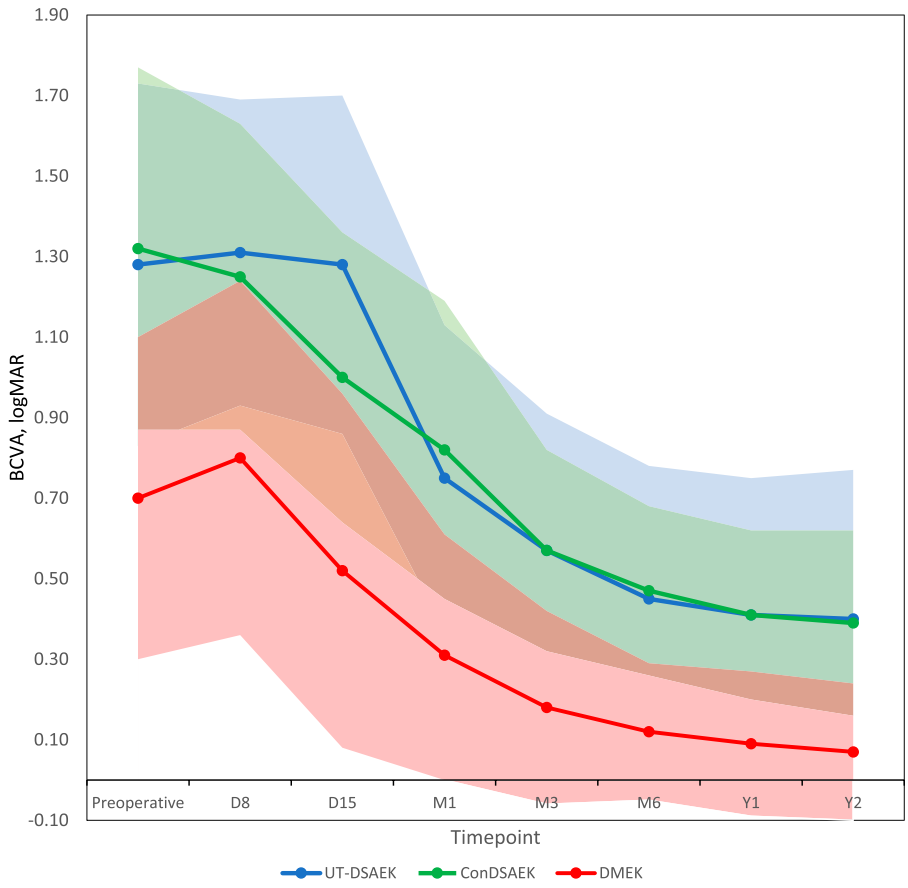

FIGURE 3. Postoperative change in BCVA in eyes that underwent DMEK, UT-DSAEK, or ConDSAEK. D, day; M, month; Y, year.

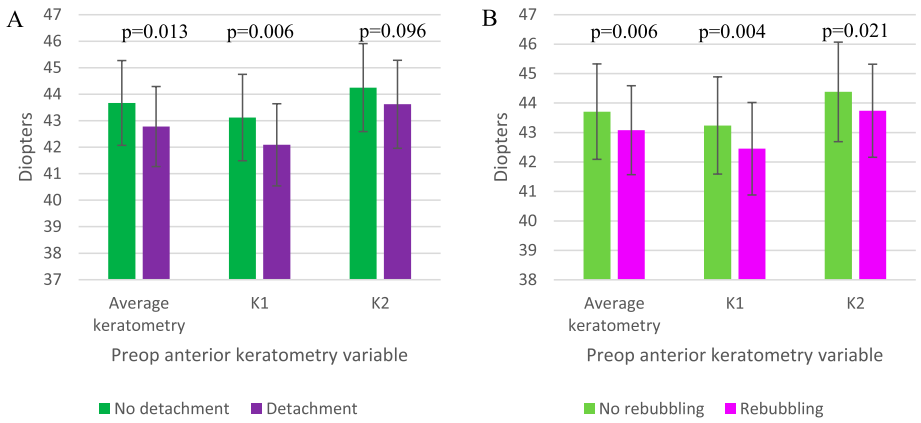

FIGURE 4. Association between graft detachment (A) or rebubbling (B) and preoperative anterior keratometry in DMEK. The data are shown as mean ± SD. All *P* values were determined with the Welch *t* test.

Univariate Analyses to Test PC2 Associations

Since the 2 PCAs revealed some interesting putative associations between variables in the DMEK group, we assessed these associations with univariate analyses.

Flatter Anterior Keratometry in DMEK Associates With More Frequent Graft Detachment and Rebubbling

The first and second PCAs suggested that graft detachment and its surrogate measure rebubbling correlated with preoperative anterior keratometry. Indeed, *t* tests showed that flatter preoperative anterior keratometry associated with significantly more graft detachment (P = 0.006–0.096) (Fig. 4A) and rebubbling (P = 0.004–0.021) (Fig. 4B).

Longer Surgery Duration and SF6 Use in DMEK Associate With Reduced Postoperative ECD

The third PCA showed that a longer surgery duration and SF6 use associated with lower postoperative ECD. Indeed, Spearman tests showed that longer surgery duration correlated weakly with lower ECD at 6, 12, and 24 months (r = −0.14, −0.23, and −0.18, respectively; P = 0.091, 0.005, and 0.034, respectively). Moreover, *t* tests showed that using SF6 rather than air for the primary tamponade strongly reduced 6-, 12-, and 24-month ECD (all $P < 0.0001$) (Fig. 5).

Double Marking of the Graft in DMEK Associates With Reduced Postoperative ECD but May Reflect Confounding

The third PCA suggested that double marking of the DMEK graft might also correlate negatively with postoperative ECD. Indeed, *t* tests showed that double marking associated with a significant reduction in ECD at 6, 12, and 24 months when compared with both no and single marking (all $P < 0.001$). These effects were not due to different preoperative ECDs: The no-mark, single-mark, and double-mark DMEK cases did not differ in preoperative ECD (Fig. 6). However, when we looked more closely at the data, we noted that SF6 use was introduced 3 months before double marking. Consequently, all 35 double-marked grafts (100%) underwent SF6 tamponade, and conversely, 35 of 41 SF6-treated grafts (85%) underwent double marking. Thus, we speculated that the high ECL in double-marked grafts may simply reflect concomitant treatment with SF6, which may be the real culprit. Therefore, we examined the 6 SF6-treated cases that did

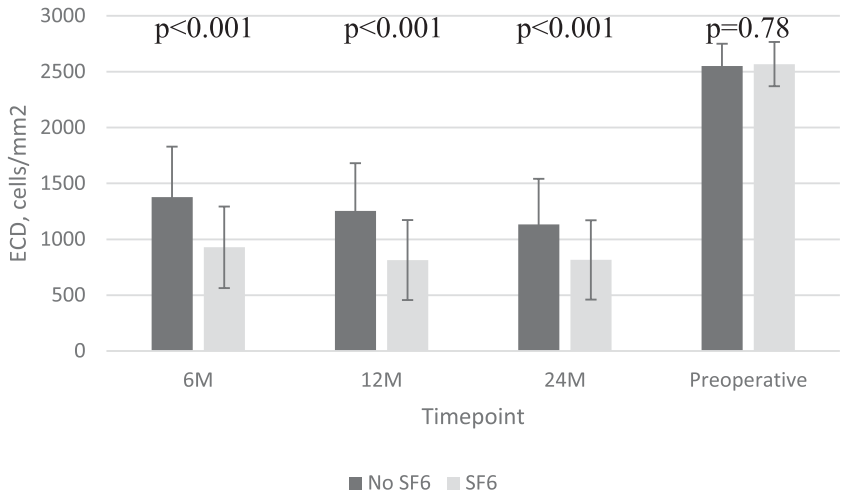

FIGURE 5. Effect of SF6 use on ECD at 6, 12, and 24 months after DMEK. The data are shown as mean ± SD. *P* values were determined by the Welch *t* test. M, months.

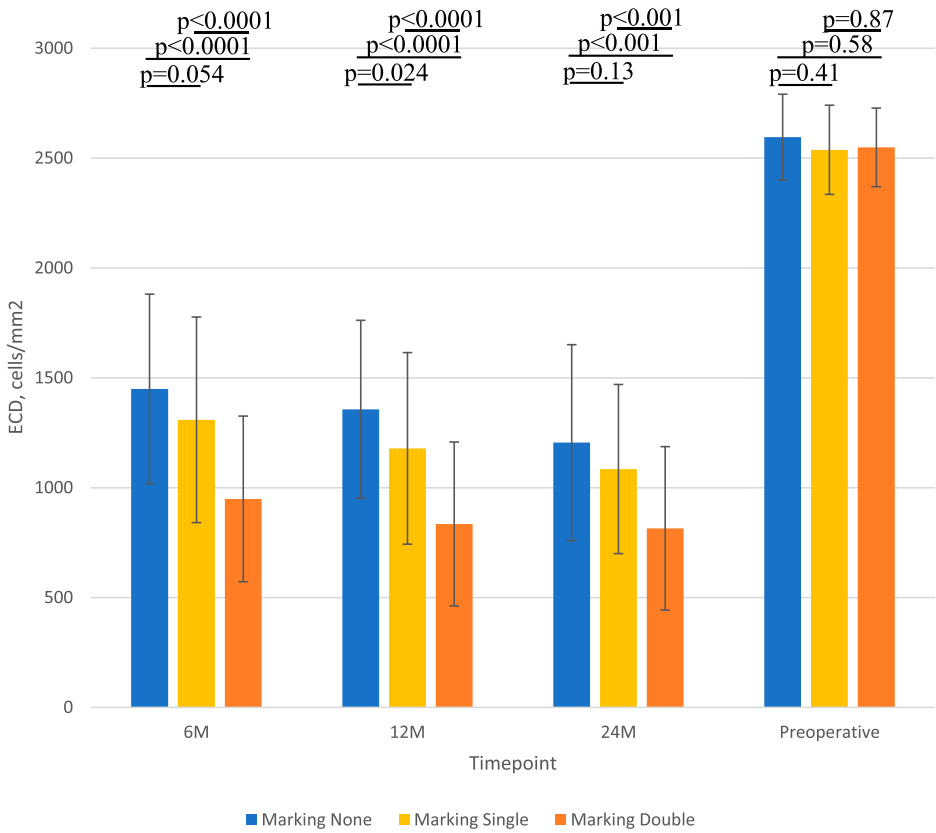

FIGURE 6. Effect of no marking, single marking, and double marking of the DMEK graft on ECD at 6, 12, and 24 months. The data are shown as mean ± SD. *P* values were determined by the Welch *t* test. M, months.

not undergo double marking. Two were early unmarked grafts that lacked postoperative ECD values. The remaining 4 underwent single marking and postoperative ECD measurements. Compared with the 86 single-marked eyes that underwent air tamponade, the 4 single-marked grafts that underwent SF6 tamponade exhibited lower postoperative ECD: At 6 months, the respective ECDs were 690 and 1341 cells/mm^2 (P = 0.0006). This was also observed at 1 year (644 vs. 1203 cells/^{2}, P = 0.0031). Two of the 4 SF6-treated single-marked grafts had 2-year ECD measurements (644 and 771 cells/mm^2), which were also lower than in the air-treated single-marked grafts (mean 1095 cells/mm^2, P = 0.221). By contrast, the 124 air-tamponaded cases had relatively similar postoperative ECDs regardless of whether they were single-marked or not marked: For example, the 6-month ECDs were 1341 and 1457 cells/mm^2, respectively (P = 0.111) (Fig. 7). These findings suggest that marking itself has at best very small

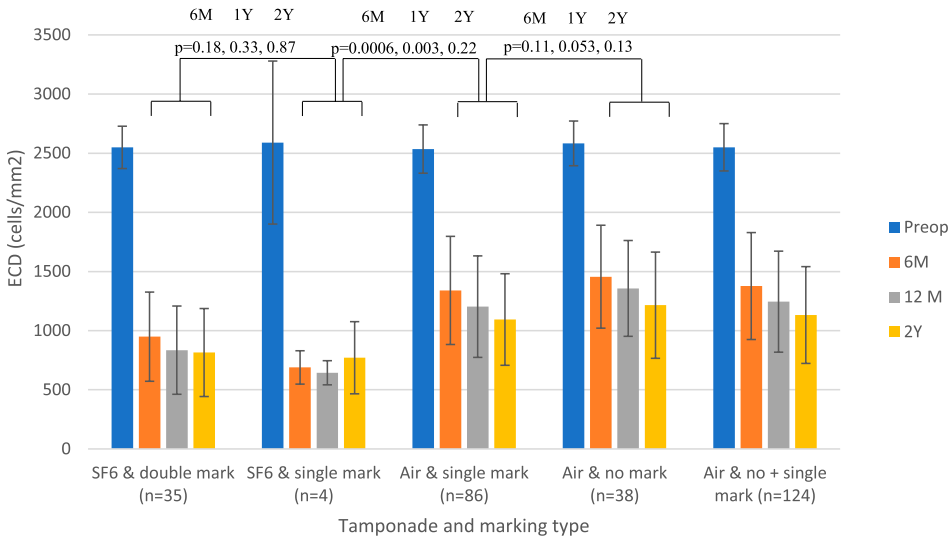

FIGURE 7. ECL at 6, 12, and 24 months associated with SF6 use rather than double marking. The data are shown as mean ± SD. *P* values were determined by the Welch *t* test. M, months; Y, year.

deleterious effects on the endothelium and that double marking of the graft is unlikely to cause significant ECL.

DISCUSSION

Our PCAs with 76 patient, graft, surgical, and outcome variables in a real-world keratoplasty cohort rapidly revealed 2 potential new associations between the variables, namely 1) flatter preoperative anterior keratometry in DMEK associated with more graft detachment and rebubbling and 2) SF6 use in DMEK associated with lower postoperative ECD compared with air.

To our knowledge, the role of preoperative keratometry in DMEK outcomes has not been assessed previously. We hypothesize that because the graft also has some curvature, a more curved cornea may promote the adherence of the graft to the stroma. We are conducting multivariate analyses to confirm our finding.

That SF6 use in DMEK associated with lower postoperative ECD was unexpected since a meta-analysis of 5 retrospective studies[27] and several more recent retrospective studies[12,38] suggest that SF6 is not toxic to endothelial cells in DMEK. We did note that SF6 use associated with less rebubbling (22% vs. 39% with air, which is a 56% reduction in the rebubbling rate), which is consistent with the previous studies on SF6 in DMEK (58% reduction).[39] Our findings are cause for concern because RCTs testing ECL after air and SF6 tamponade have not yet been conducted. Moreover, a study in cats, whose corneal endothelial cells do not regenerate (like in humans), showed that SF6 injection associates with greater corneal ECL than air.[40] While an in vitro study with immortalized corneal endothelial cell lines did not observe that SF6 increased ECL,[41] it should be noted that air itself is toxic to endothelial cells and the prolongation of the bubble caused by mixing air with SF6 may thus augment this effect.[31] These concerns about SF6 are widely held in the field, with the consequence that several corneal surgeons continue to prefer air tamponade in DMEK.[42] We are currently conducting additional studies to assess the endothelial safety of SF6 in our hands.

Our analyses also showed that longer surgery duration in DMEK associated with lower postoperative ECD. We also observed this association on univariate analysis in our previous study. This association likely reflects surgical difficulties, especially problematic graft dissection and unfolding, which not only extend the surgery duration but also decrease the ECD.[43]

Our PCA and follow-up *t* tests also suggest that DMEK graft marking does not affect the postoperative ECD. This is consistent with several studies by the Terry group[31,32,44] and confirms a study on DSAEK grafts that showed F (or F*) marking with gentian ink is not toxic to corneal endothelial cells.[45]

We also observed that UT-DSAEK and ConDSAEK did not differ markedly in terms of visual improvement and both were worse than DMEK in terms of visual outcomes. These results are in line with those of several other studies.[23–26] However, they contest the equally prolific studies that show that thin DSAEK grafts lead to better final BCVA[16,19,20] and may even approach DMEK outcomes.[16,17,21,22] Our recent cohort studies[33,46] suggest that these interstudy discrepancies may reflect variability in preoperative measurements of CGT because of factors such as the way the grafts were prepared: This can lead to artificially thick or thin preoperative grafts. These grafts are then classified as ConDSAEK and UT-DSAEK grafts, respectively, but, nonetheless, they all rapidly return to their constitutive physiological thickness after surgery and then undergo remodeling and thinning that is proportional to their constitutive thickness. The resulting graft thickness in turn directly shapes postoperative VA. Thus, preoperative measurement and classification biases in UT-DSAEK/ConDSAEK studies may explain the discrepancies in the field regarding VA outcomes.

Our PCAs were not able to detect any new associations between postoperative VA and other variables, which suggests that the keratoplasty indication and its effect on preoperative VA were the biggest factors that shaped postoperative VA after endothelial keratoplasty.

Study Strengths and Limitations

To our knowledge, this is the first time PCA has been applied to ophthalmological surgery. This technique is widely used in other fields to visualize high-dimensional data and enable other analyses, including clustering, and thus could be useful for researching complex fields like keratoplasty that involve a multitude of patient, disease, surgical practice, and temporal variables. Our study showed that it can quickly detect associations, even when applied to a relatively uncurated database with a large number of variables and missing data. However, the limitations of this technique should be understood. In particular, it can be difficult to interpret the data because of confounding with other variables. Thus, PCA should only be seen as illuminating possible associations that should then be verified with more precise methods.

CONCLUSIONS

PCA is a powerful method for identifying clinically relevant associations in ophthalmological research. Our novel findings regarding preoperative keratometry and SF6 use in DMEK are currently under additional investigation in our hospital.

REFERENCES

1. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. *Philos Trans A Math Phys Eng Sci*. 2016;374:20150202.
2. Kim S, Kang D, Huo Z, et al. Meta-analytic principal component analysis in integrative omics application. *Bioinformatics*. 2018;34:1321–1328.
3. Melles GR, Eggink FA, Lander F, et al. A surgical technique for posterior lamellar keratoplasty. *Cornea*. 1998;17:618–626.
4. Melles GRJ, Ong TS, Ververs B, et al. Descemet membrane endothelial keratoplasty (DMEK). *Cornea*. 2006;25:987–990.
5. Guechi O, Lhuillier L, Houmad N, et al. Visual outcomes following Descemet stripping automated endothelial keratoplasty for corneal endothelial dysfunction. *Eur J Ophthalmol*. 2017;27:513–519.
6. Melles GRJ, Ong TS, Ververs B, et al. Preliminary clinical results of Descemet membrane endothelial keratoplasty. *Am J Ophthalmol*. 2008; 145:222–227.
7. Price MO, Giebel AW, Fairchild KM, et al. Descemet's membrane endothelial keratoplasty: prospective multicenter study of visual and

refractive outcomes and endothelial survival. *Ophthalmology*. 2009;116:2361–2368.
8. Chen ES, Terry MA, Shamie N, et al. Precut tissue in Descemet's stripping automated endothelial keratoplasty. Donor characteristics and early postoperative complications. *Ophthalmology*. 2008;115:497–502.
9. Marques RE, Guerra PS, Sousa DC, et al. DMEK versus DSAEK for Fuchs' endothelial dystrophy: a meta-analysis. *Eur J Ophthalmol*. 2019;29:15–22.
10. Koo EH, Pineda R, Afshari N, et al. Learning Descemet membrane endothelial keratoplasty: a survey of U.S. Corneal surgeons. *Cornea*. 2020;39:590–593.
11. Gerber-Hollbach N, Baydoun L, López EF, et al. Clinical outcome of rebubbling for graft detachment after Descemet membrane endothelial keratoplasty. *Cornea*. 2017;36:771–776.
12. Hayashi T, Schrittenlocher S, Siebelmann S, et al. Risk factors for endothelial cell loss after Descemet membrane endothelial keratoplasty (DMEK). *Sci Rep*. 2020;10:11086–11089.
13. Dunker S, Winkens B, Van Den Biggelaar F, et al. Rebubbling and graft failure in Descemet membrane endothelial keratoplasty: a prospective Dutch registry study. *Br J Ophthalmol*. 2023;107:17–23.
14. Vasiliauskaitė I, Quilendrino R, Baydoun L, et al. Effect of six-month postoperative endothelial cell density on graft survival after Descemet membrane endothelial keratoplasty. *Ophthalmology*. 2021;128:1689–1698.
15. Busin M, Patel AK, Scorcia V, et al. Microkeratome-assisted preparation of ultrathin grafts for Descemet stripping automated endothelial keratoplasty. *Invest Ophthalmol Vis Sci*. 2012;53:521–524.
16. Busin M, Madi S, Santorum P, et al. Ultrathin Descemet's stripping automated endothelial keratoplasty with the microkeratome double-pass technique: two-year outcomes. *Ophthalmology*. 2013;120:1186–1194.
17. Mencucci R, Favuzza E, Marziali E, et al. Ultrathin Descemet stripping automated endothelial keratoplasty versus Descemet membrane endothelial keratoplasty: a fellow-eye comparison. *Eye Vis*. 2020;7:25.
18. Neff KD, Biber JM, Holland EJ. Comparison of central corneal graft thickness to visual acuity outcomes in endothelial keratoplasty. *Cornea*. 2011;30:388–391.
19. Dickman MM, Kruit PJ, Remeijer L, et al. A randomized multicenter clinical trial of ultrathin Descemet stripping automated endothelial keratoplasty (DSAEK) versus DSAEK. *Ophthalmology* 2016;123:2276–2284.
20. Droutsas K, Petrelli M, Miltsakakis D, et al. Visual outcomes of ultrathin-Descemet stripping endothelial keratoplasty versus Descemet stripping endothelial keratoplasty. *J Ophthalmol*. 2018;2018:5924058.
21. Dunker SL, Dickman MM, Wisse RPL, et al. Descemet membrane endothelial keratoplasty versus ultrathin Descemet stripping automated endothelial keratoplasty: a multicenter randomized controlled clinical trial. *Ophthalmology*. 2020;127:1152–1159.
22. Madi S, Leon P, Nahum Y, et al. Five-year outcomes of ultrathin Descemet stripping automated endothelial keratoplasty. *Cornea*. 2019;38:1192–1197.
23. Bhandari V, Reddy JK, Relekar K, et al. Descemet's stripping automated endothelial keratoplasty versus Descemet's membrane endothelial keratoplasty in the fellow eye for Fuchs endothelial dystrophy: a retrospective study. *Biomed Res Int*. 2015;2015:750567.
24. Chamberlain W, Lin CC, Austin A, et al. Descemet endothelial thickness comparison trial: a randomized trial comparing ultrathin Descemet stripping automated endothelial keratoplasty with Descemet membrane endothelial keratoplasty. *Ophthalmology* 2019;126:19–26.
25. Matsou A, Pujari R, Sarwar H, et al. Microthin descemet stripping automated endothelial keratoplasty versus Descemet membrane endothelial keratoplasty: a randomized clinical trial. *Cornea*. 2021;40:1117–1125.
26. Stuart AJ, Romano V, Virgili G, et al. Descemet's membrane endothelial keratoplasty (DMEK) versus Descemet's stripping automated endothelial keratoplasty (DSAEK) for corneal endothelial failure. *Cochrane Database Syst Rev*. 2018;6:CD012097.
27. Marques RE, Guerra PS, Sousa DC, et al. Sulfur hexafluoride 20% versus air 100% for anterior chamber tamponade in DMEK: a meta-analysis. *Cornea*. 2018;37:691–697.
28. Bachmann BO, Laaser K, Cursiefen C, et al. A method to confirm correct orientation of Descemet membrane during Descemet membrane endothelial keratoplasty. *Am J Ophthalmol*. 2010;149:922–925.e2.
29. Bhogal M, Maurino V, Allan BD. Use of a single peripheral triangular mark to ensure correct graft orientation in Descemet membrane endothelial keratoplasty. *J Cataract Refract Surg*. 2015;41:2022–2024.
30. Matsuzawa A, Hayashi T, Oyakawa I, et al. Use of four asymmetric marks to orient the donor graft during Descemet's membrane endothelial keratoplasty. *BMJ Open Ophthalmol*. 2017;1:e000080.
31. Terry MA, Straiko MD, Veldman PB, et al. Standardized DMEK technique: reducing complications using prestripped tissue, novel glass injector, and sulfur hexafluoride (SF6) gas. *Cornea*. 2015;34:845–852.
32. Veldman PB, Dye PK, Holiman JD, et al. Stamping an S on DMEK donor tissue to prevent upside-down grafts: laboratory validation and detailed preparation technique description. *Cornea*. 2015;34:1175–1178.
33. Perone J-M, Goetz C, Zevering Y, et al. Graft thickness at 6 months postoperatively predicts long-term visual acuity outcomes of Descemet stripping automated endothelial keratoplasty for Fuchs dystrophy and moderate phakic bullous keratopathy: a cohort study. *Cornea*. 2022;41:1362–1371.
34. Bloch F, Dinot V, Goetz C, et al. Ability of routinely collected clinical factors to predict good visual results after primary Descemet membrane endothelial keratoplasty: a cohort study. *BMC Ophthalmol*. 2022;22:350.
35. Perone JM, Goetz C, Zaidi M, et al. Supracapsular phacoemulsification: description of the "Garde à vous" technique and comparative clinical results. *J Fr Ophtalmol*. 2019;42:597–602.
36. Schrittenlocher S, Bachmann B, Tiurbe AM, et al. Impact of preoperative visual acuity on Descemet membrane endothelial keratoplasty (DMEK) outcome. *Graefes Arch Clin Exp Ophthalmol*. 2019;257:321–329.
37. Vasiliauskaitė I, Quilendrino R, Baydoun L, et al. Effect of six-month postoperative endothelial cell density on graft survival after Descemet membrane endothelial keratoplasty. *Ophthalmology*. 2021;128:1689–1698.
38. Inoda S, Hayashi T, Takahashi H, et al. Factors associated with endothelial cell density loss post Descemet membrane endothelial keratoplasty for bullous keratopathy in Asia. *PLoS One*. 2020;15:e0234202.
39. Marques RE, Guerra PS, Sousa DC, et al. Sulfur hexafluoride 20% versus air 100% for anterior chamber tamponade in DMEK: a meta-analysis. *Cornea*. 2018;37:691–697.
40. Landry H, Aminian A, Hoffart L, et al. Corneal endothelial toxicity of air and SF6. *Invest Ophthalmol Vis Sci*. 2011;52:2279–2286.
41. Hesse M, Kuerten D, Walter P, et al. The effect of air, SF6 and C3F8 on immortalized human corneal endothelial cells. *Acta Ophthalmol*. 2017;95:e284–e290.
42. Diab RA, Zarei-ghanavati M, Bardan AS, et al. Preferred practices of Descemet's Membrane Endothelial Keratoplasty (DMEK) surgeons in the UK: a national survey. *J EuCornea*. 2022;11:1–7.
43. Chaussard D, Bloch F, Elnar AA, et al. Identification of the preoperative and perioperative factors that predict postoperative endothelial cell density after Descemet membrane endothelial keratoplasty: a retrospective cohort study. *PLoS One*. 2022;17:e0264401–e0264418.
44. Veldman PB, Dye PK, Holiman JD, et al. The S-stamp in Descemet membrane endothelial keratoplasty safely eliminates upside-down graft implantation. *Ophthalmology*. 2016;123:161–164.
45. Stoeger C, Holiman J, Davis-Boozer D, et al. The endothelial safety of using a gentian violet dry-ink "S" stamp for precut corneal tissue. *Cornea*. 2012;31:801–803.
46. Neiter E, Sot M, Lhuillier L, et al. Relationship between preoperative corneal thickness and postoperative visual outcomes after posterior lamellar corneal transplant. *Int J Ophthalmic Pathol*. 2019;8:1.