Department of Ophthalmology

Saarland University Medical Center Homburg/Saar

Director: Prof. Dr. Berthold Seitz

Qualitative and quantitative assessment of cornea guttata and endothelial cell loss in the human donor cornea.

Cumulative Dissertation for the Degree of Doctor of Medicine

of the Faculty of Medicine

UNIVERSITY OF SAARLAND

2022

submitted by

Tarek Safi

born on: 07.12.1994 in Tripoli, Lebanon

Table of Contents

1.1 Summary:

In contrast to penetrating keratoplasty (PKP), where all the layers of the donor cornea are transplanted, Descemet´s membrane endothelial keratoplasty (DMEK) designates the transplantation of only the Descemet´s membrane and the endothelial layer of the donor cornea which together form a thickness of 20-30 µm. The total number of corneal transplantations in Germany has drastically increased between the years 2001 to 2020 from 4730 to 9042 keratoplasties, whereby DMEKs have surpassed PKP since 2014. In light of this constantly growing number, the need to increase not only the number but also the quality of the donor corneas is paramount. Therefore, an essential part of the quality control of the donor corneas in the eye banks is ensuring a healthy and functional endothelial layer since it plays an important role in maintaining the clarity of the cornea and, thus, a satisfying postoperative visual acuity. Yet, postoperative complications related to endothelial failure still appear in 3% to 7% of cases. Accordingly, the purpose of the following publications was to enhance the quality control of the endothelial layer of the donor corneas. This was achieved by studying the impact of two important factors on the endothelial layer of the donor cornea: 1) The preparation and storage of the DMEK roll (DR), as well as 2) Cornea guttata (CG).

In the first paper, a novel, reproducible, and non-invasive method was introduced to quantify endothelial cell loss (ECL) of the DR caused by its preparation and storage for 5 days. After the preparation of DRs, the tissues were placed in their storage medium containing organ culture medium 1 without dextran. Non-invasive and reproducible ECL measurements followed both before the preparation and directly after the preparation, as well as on days 1, 2 and 5 after storage using inverted light microscopy.

The results showed an ECL of 11% due to the preparation procedure and 12% due to the 5 days storage of the tissues. Based on the results of these non-invasive measurements that proved to be highly reproducible with Cronbach's alpha values between 0.85 and 0.98, shipping of DRs after several days of storage to be used in a DMEK is debatable. To avoid excessive ECL and to maintain good quality of the donor endothelial layer, we recommend a storage time of the DR as short as possible.

The second paper determined the prevalence and severity of CG in grafts after PKP and investigated its clinical significance during the postoperative follow-up. In this retrospective study, it was shown that the prevalence of CG was 14.9%, the majority of which showed mild CG (G1) 13.6% and only 1.3% showed high-grade CG (G2 and G3). The mean corneal thickness, pleomorphism and polymegalism were significantly affected by high-grade CG. A progression to a higher grade of CG was detected in 16.8% of the CG cases during long term follow-up.

The third paper investigated the prevalence and severity of CG in grafts after DMEK and assessed its impact on various clinical parameters during the postoperative follow-up. CG appeared postoperatively in 18.7% of the grafts. 16.9% of those could be classified as mild CG (G1), and 1.9% as high-grade CG (G2 and G3). A significant clinical deterioration with increasing grades of CG was found in the following parameters: corrected distance visual acuity (CDVA), central corneal thickness, pleomorphism, and polymegalism. Fortunately, the second and third papers proved that only high-grade CG have a significant clinical impact on the patients during the postoperative follow-up examinations.

Due to the high prevalence of CG demonstrated in the above-mentioned papers and the negative impact of high-grade CG on the postoperative clinical parameters, the fourth paper aimed to detect CG in the donor corneas before their transplantation.

Therefore, semi-quantitative criteria for the detection of CG in the donor corneas in the eye bank were introduced. This retrospective study was able to show that three criteria that can be detected in the eye bank using inverted light microscopy seem to correlate with postoperative CG: 1) The presence of blebs (a small hyperdense thickening of the cell membrane), 2) the presence of cell membrane defects and interruptions, as well as 3) endothelial pictures with less than 50% of the cells having a hexagonal or circular shape.

1.2 Zusammenfassung:

Im Gegensatz zur perforierenden Keratoplastik (PKP), bei der alle Schichten der Spenderhornhaut transplantiert werden, bezeichnet die Descemet-Membrane Endothelial Keratoplasty (DMEK) nur die Transplantation der hinteren Korneaschichten. Das aus der Endothelschicht und der Descemet-Membran bestehende Transplantat hat eine Dicke von 20-30 µm. Die Gesamtzahl der Hornhauttransplantationen in Deutschland ist zwischen den Jahren 2001 und 2020 von 4730 auf 9042 Keratoplastiken drastisch angestiegen, wobei die DMEK seit 2014 die PKP zahlenmäßig überholt hat. Angesichts dieser stetig wachsenden Zahl ist es notwendig, nicht nur die Anzahl, sondern auch die Qualität der Spenderhornhäute zu erhöhen. Daher ist vor allem die Sicherstellung einer gesunden und funktionellen Endothelschicht ein wichtiger Bestandteil der Qualitätskontrolle zur Eignung der Spenderhornhäute für die Transplantation. Das Endothel ist dabei besonders für die Erhaltung der postoperativen Klarheit der Hornhaut verantwortlich. Dennoch kommt es in 3% bis 7% der Fälle zu postoperativen Komplikationen die in Zusammenhang mit einem Endothelversagen stehen. Das Ziel der folgenden Arbeit war es daher, die präoperative Qualitätskontrolle des Spenderendothels durch die Untersuchungen der folgenden Faktoren zu verbessern: 1) Die Durchführung der Präparation und Lagerung der DMEK-Rolle (DR) und 2) das Vorhandensein von Cornea guttata (CG).

In der ersten Arbeit wurde eine neuartige, reproduzierbare und nicht-invasive Methode zur Quantifizierung der Endothelzellverluste (ECL) der DR vorgestellt, die durch deren Präparation und Lagerung für 5 Tage verursacht werden. Nach der Vorbereitung der DR wurden die Gewebe in ihr Lagermedium (Organkulturmedium 1 ohne Dextran) gelegt. Mittels inversem Mikroskop wurden dabei nicht-invasive und reproduzierbare ECL-Messungen sowohl vor der Präparation als auch direkt nach der Präparation an

den Tagen 1, 2 und 5 nach der Lagerung durchgeführt. Die Ergebnisse zeigten eine ECL von 11% aufgrund des Präparationsverfahrens und 12% aufgrund der 5-tägigen Lagerung des Gewebes. Basierend auf die Ergebnisse dieser nicht-invasiven Messungen, die sich mit Cronbachs Alpha-Werten zwischen 0,85 und 0,98 als hoch reproduzierbar erwiesen haben, ist der Versand von DR nach mehrtägiger Lagerung zur Verwendung für eine DMEK umstritten. Um einen übermäßigen ECL zu vermeiden und eine gute Qualität der Spenderendothelschicht zu erhalten, empfehlen wir eine möglichst kurze Lagerungszeit der DR.

In der zweiten Arbeit wurden die Prävalenz und der Schweregrad von CG in Transplantaten nach PKP bestimmt und ihre klinische Bedeutung während der Nachuntersuchungen ermittelt. In dieser retrospektiven Studie zeigte sich, dass die Prävalenz von CG bei 14,9% lag. Dabei konnte in den meisten Fällen eine leichte CG (G1) 13,6% und nur in 1,3% eine hochgradige CG (G2 und G3) nachgewiesen werden. Die mittlere Hornhautdicke, der Pleomorphismus und der Polymegalismus waren bei der hochgradigen CG signifikant erhöht. Bei 16,8% der CG-Fälle wurde während der langfristigen Nachbeobachtungszeit eine Progression zu einem höheren CG-Grad festgestellt.

Die dritte Arbeit untersuchte die Prävalenz und den Schweregrad von CG in Transplantaten nach DMEK und bewertete ebenfalls ihren Einfluss auf verschiedene klinische Parameter während der postoperativen Nachsorge. CG traten postoperativ bei 18,7% der Transplantate auf. Dabei konnten 16,9% als leichte CG (G1) und 1,9% als hochgradige CG (G2 und G3) klassifiziert werden. Eine signifikante klinische Verschlechterung mit zunehmendem Grad der CG wurde bei folgenden Parametern

festgestellt: korrigierter Fernvisus (CDVA), zentrale Hornhautdicke, Pleomorphismus und Polymegalismus. Zusammenfassend zeigten die Studien zwei und drei, dass nur hochgradige CG bei den postoperativen Nachuntersuchungen einen signifikant negativen klinischen Einfluss auf die postoperativen Ergebnisse hatte.

Aufgrund der wie in den oben genannten Studien auftretenden hohen Prävalenzen von CG und deren negativen Einflusses in hochgradigen Stadien, zielte die vierte Arbeit darauf ab, CG in den Spenderhornhäuten präoperativ zu ermitteln. Dazu wurden semiquantitative Kriterien für den Nachweis von CG in den Spenderhornhäuten in der Hornhautbank untersucht. Es konnte gezeigt werden, dass drei verschiedene Kriterien, die in der Hornhautbank mit Hilfe des inversen Mikroskops erkannt werden können, mit dem Vorhandensein einer CG korrelieren: 1) das Vorhandensein von so genannten “Blebs“ (kleine fokale Verdichtungen der Zellmembran), 2) das Vorhandensein von Zellmembrandefekten und -unterbrechungen und 3) Endothelbilder, bei denen weniger als 50% der Zellen eine hexagonale oder kreisförmige Form aufwiesen.

2. Introduction and Purpose:

Corneal transplantation is a rapidly evolving field in ophthalmology. The two main types of corneal transplantation surgeries are 1) penetrating keratoplasty (PKP) whereby the patient's diseased cornea is excised and the donor cornea with all its layers is directly transplanted and 2) Descemet´s membrane endothelial keratoplasty (DMEK) which was introduced in 2006 [54, 55]. During DMEK, a DR consisting only of the Descemet´s membrane and endothelial layer of the donor cornea is previously prepared by stripping those layers from the rest of the corneal layers using a delicate preparation technique **(Figure 1)**. After removal of the defected endothelial layer of the patient's cornea, the previously prepared DR is transplanted on the stromal layer of the patient's cornea [82]. The number of corneal transplantations in Germany has increased drastically between the years 2001 to 2020 from 4730 to 9042 keratoplasties, whereby DMEKs have surpassed PKP since 2014 [25, and unpublished data of the German Keratoplasty Registry].

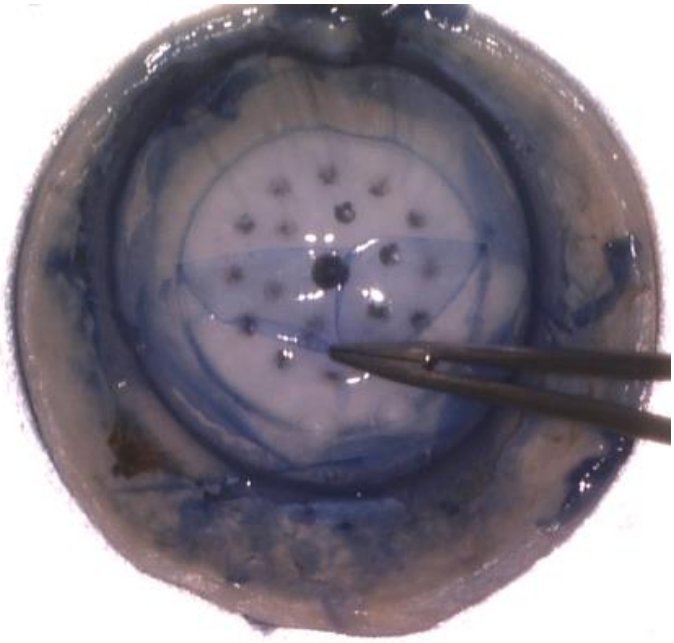


Figure 1: Stripping of the Descemet´s membrane and endothelial layer from the other layers of the cornea during DMEK preparation (self-created image).

The innermost layer of the cornea is called the endothelial layer **(Figure 2)**. It measures 5 µm in thickness and is formed by single-layered hexagonal endothelial cells. This layer plays a critical role in keeping the cornea clear and transparent by pumping out water from the cells through an active transport system of electrolytes and liquids [29, 65]. Through a membrane-bound Na^+-K^+-ATPases, the endothelium regulates the outflow of the aqueous humour from the stroma and provides a barrier function between the anterior chamber of the eye and the cornea [11].

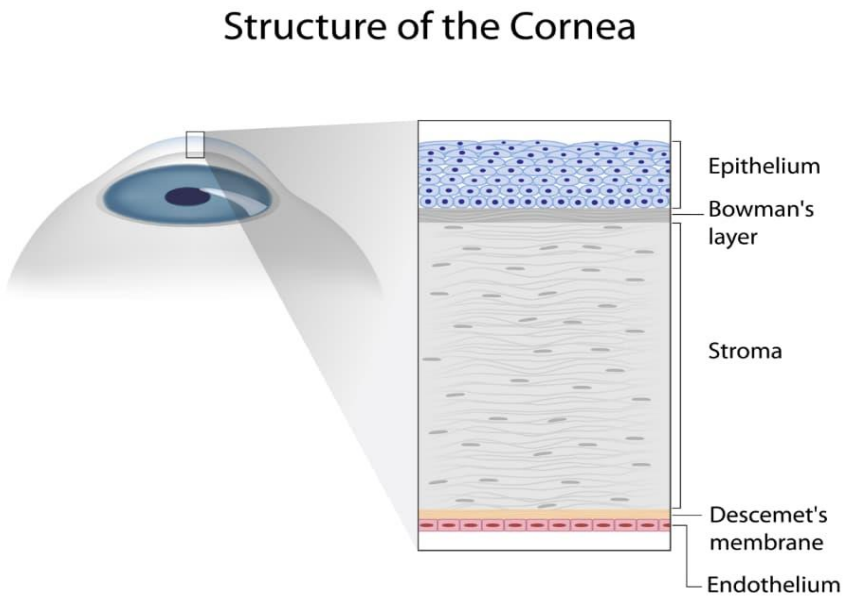


Figure 2: The layers of the cornea. This cross-section view of the cornea shows the 3 cell layers separated by Bowman's layer and Descemet's membrane. The multilayered epithelium is about 50 µm thick, the stroma is about 500 µm and the single layered endothelium is about 5 µm thick [26]. (Open access image distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license https://creativecommons.org/licenses/by/4.0/).

Since the endothelial cells are mostly non regenerative, damage to this layer leads to the disruption of the endothelial cell function leading eventually to corneal edema and

decompensation such as in Fuchs Endothelial Corneal Dystrophy (FECD) [95]. This dystrophy is the most common and important endothelial dystrophy. Its prevalence in the normal population varies a lot depending on the population studied and is higher in the older population affecting around 10% to 23% of the population above 60 years old [22, 101]. FECD is highlighted by the presence of widespread Cornea guttata (CG). CG are excrescences of the Descemet´s membrane made by accumulations of basement membrane and fibrillary collagens that disrupt the endothelial mosaic and usually appear in the central area of the cornea and then spread to the peripheral cornea in the advanced stages of the disease [29] **(Figure 3).** CG appear on the slit lamp as “beaten metal” [47] **(Figure 4).** As a diagnostic method for objective detection and quantification of CG, non-contact specular microscopy is typically used in daily clinical practice. It enables an enlarged in-vivo visualization of the reflected light from the endothelium [52] **(Figure 5).** However, this microscope can only be used on living patients, and cannot be applied on donor corneas.

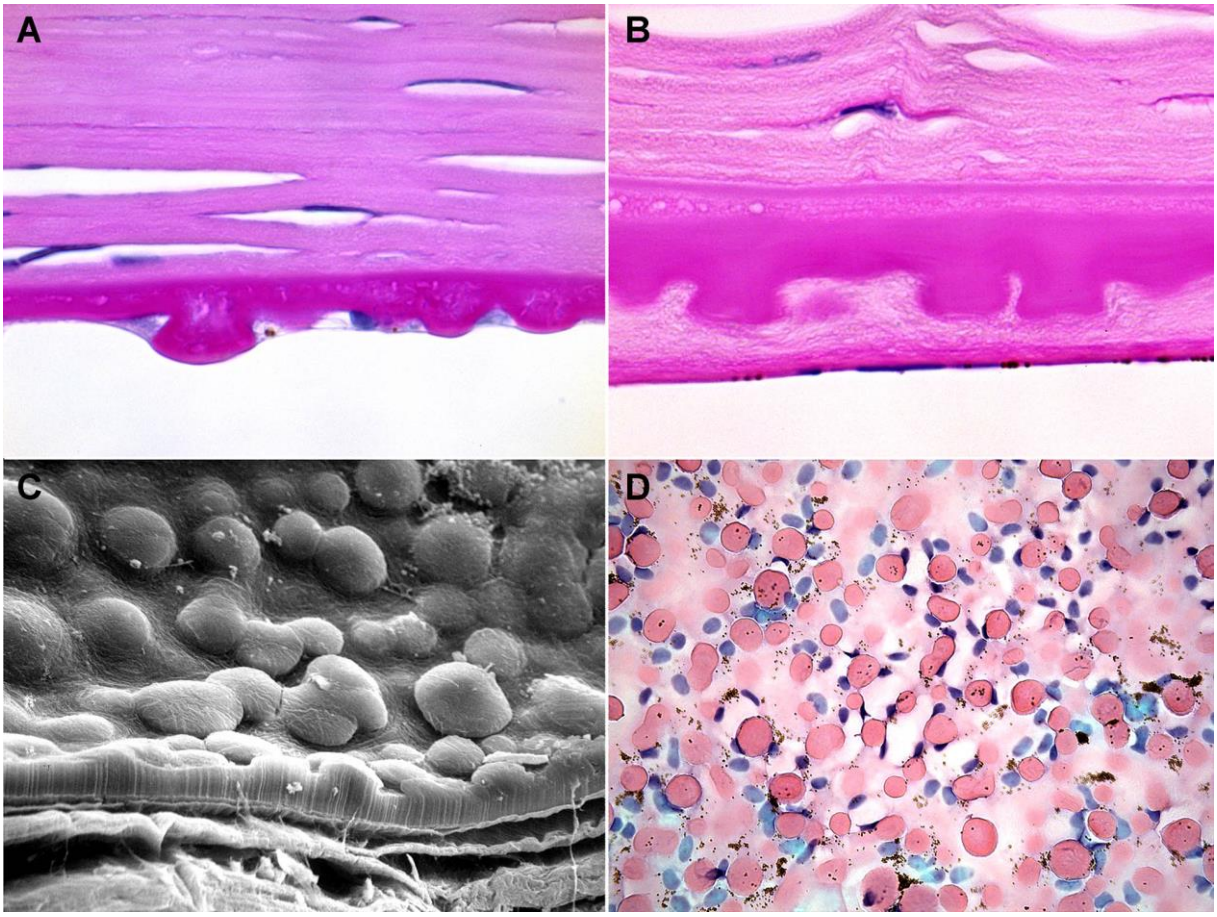


Figure 3: Fuchs endothelial corneal dystrophy (FECD). **A:** The Descemet´s membrane is irregular in caliber and studded with guttate excrescences. Some of the residual endothelial cells contain melanin granules. **B:** FECD, buried guttae. Guttae have been "buried" by a newly synthesized layer of extracellular matrix material. The endothelium is markedly atrophic. Buried guttae typically occur in the center of the cornea. This is also seen in pseudoexfoliation keratopathy whereby irregular thickening of Descemet's membrane is seen along with focal accumulations of locally produced pseudoexfoliation material onto or within Descemet's membrane [61] **C:** Mushroom or anvil-shaped excrescences disclosed by scanning electron microscopy covers the posterior surface of the Descemet´s membrane. The specimen is oriented epithelial side down. **D:** Flat preparation of the Descemet´s membrane stripped from patient with FECD during DSEK procedure. Many endothelial cells between round pink guttae contain melanin granules.
(A. PAS ×250, B. PAS ×250, SEM ×300, D. Whole mount flat preparation stained with H&E ×100, Courtesy Dr. R. C. Eagle, Jr. [21]).

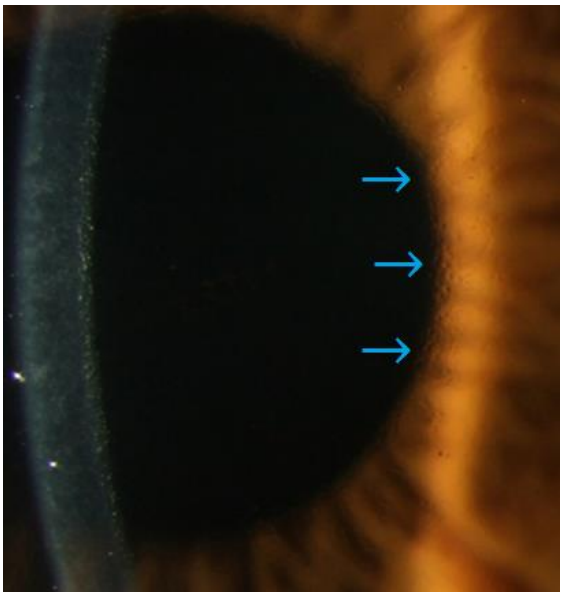


Figure 4: Guttae visible with the slit lamp. Arrows point to the "beaten metal" appearance typically seen in advanced Cornea guttata on the posterior surface of the cornea (self-created image).

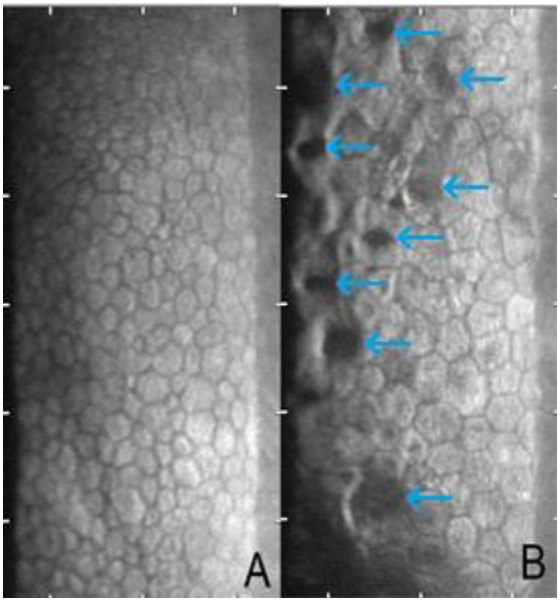


Figure 5: Endothelial layer illustrated by the specular microscopy after penetrating keratoplasty. **A:** Represents a healthy cornea. **B:** Represents a transplanted cornea having Cornea guttata indicated with the red arrows (self-created image).

When CG progresses significantly and leads to the loss of a large number of endothelial cells, the remaining living cells cannot maintain the transparency of the cornea anymore leading to corneal edema and decrease of visual acuity. At this point CG is no more considered as an isolated finding anymore but a manifestation of FECD [15, 95, 96]. Unfortunately, until now, there is no clear and validated method for CG

detection in the donor cornea using inverted light microscopy in the eye bank. Therefore, each keratoplasty bears the risk of transplanting a donor cornea with guttae.

As part of the routine quality control of the endothelial layer of the donor corneas in the eye banks, the corneas are first examined using slit lamp examination for large defects and then assessed using inverted light microscopy examination, whereby a minimum of 6 pictures of the endothelial layer are taken in order to calculate the endothelial cell density (ECD). Only corneas having a minimum of 2000 cells/mm^2 for PKP and 2200 cells/mm^2 for DMEK are considered suitable for transplantation.

In contrast to PKP, we require a higher ECD for DMEK, because larger ECL is expected in DMEK at several timepoints: 1) preoperatively due to technical reasons during donor tissue preparation, 2) intraoperatively when the 20 µm thin DR is being unrolled and attached to the host stroma, as well as 3) postoperatively, especially if re-bubbling is performed due to incomplete postoperative graft attachment [5, 20, 73, 82, 98].

Despite the above-mentioned strict criteria set to ensure a satisfying quality of the endothelial layer, two main issues might negatively affect the status of this important layer, potentially leading to suboptimal postoperative results.

1. CG in the donor cornea. Until now, this entity cannot be detected preoperatively in the eye banks. First, the visual conditions associated with corneal evaluation using the slit-lamp are notably different than evaluating a donor cornea in vitro. The donor cornea must be examined while stored in its culture medium to maintain sterility, which leads to excessive light diffusion and refraction, significantly affecting the resolution and clarity of the reflected image. Also, the examined corneas are placed in organ culture medium 1 without dextran, which causes their swelling up to 1000–1500 µm leading to poor delineation of the endothelial cells and making the

typical “beaten metal” appearance of the guttae almost impossible to detect **(Figure 6)**. For the above-mentioned reasons, there are currently no clear criteria for the detection of CG in donor corneas. Furthermore, the impact of CG in donor corneas on the postoperative results is until now not studied in the literature.

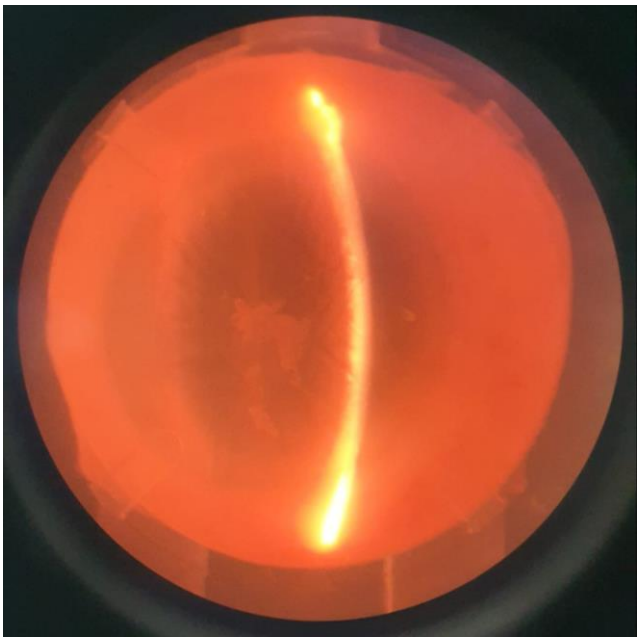


Figure 6: Slit lamp Photo of a donor cornea in organ culture medium 1. (self-created image)

2. The preparation and storage of the DR. Several studies mentioned in the literature assessed the effect of the preparation and the storage of the DR on the endothelial layer. However, the results of these studies varied from insignificant to detrimental [3, 37, 51, 58, 56, 72, 88]. Therefore, there was a need for a non-invasive and reproducible method to assess the impact of the preparation and storage of the DR on the quality of the endothelial layer.

In our studies we aimed at optimizing the quality of the donor endothelial cell layer in the eye bank in order to achieve the best results and to reduce the postoperative complications related to ECL and failure of the endothelial layer. In the first study, we

measured the impact of the preparation of the DR on the endothelial layer and quantified the resulting ECL using a new non-invasive and reproducible method. Subsequently, we assessed the consequences of the DR storage on the quality of the donor endothelium and on the ECL. After quantifying the ECL caused by the preparation of the DR, we investigated another potential cause of ECL and endothelial failure of the transplanted graft. In the second and third study we assessed the prevalence and impact of postoperative CG in the transplanted grafts after PKP and DMEK respectively. In light of the significant results of these studies, showing that the presence of high-grade CG negatively affected the clinical postoperative results, we were urged to conduced our fourth study aiming to define morphological semi-quantitative criteria that correlate with CG detection on the donor cornea before its transplantation.

3. Description of the Included Publications:

3.1 Publication 1: Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage

The ability to measure the endothelial cell loss (ECL) due to DMEK preparation before surgery allows the surgeon to better assess the quality of the graft, long term ECL and indirectly graft failure rates. According to the literature, the endothelial cell loss (ECL) after DR preparation varied from 0% to 23% in several studies [3, 51, 56, 58, 72]. To the best of our knowledge, there are 2 methods to measure the ECL after DR preparation. The first method involves complete stripping of the Descemet's membrane then manually unrolling it, and the usage of an inverted light microscope and enhanced image-analysis software titled "Fiji" to analyze the pictures taken and calculate the ECL. The ECL measured with this preparation method varied widely between different studies, i.e., from 9.3% [88] to 22.5% [37], and 12.44% in a study using the pneumodissection preparation method [3]. The second method involves measuring the ECL after partial peeling of the Descemet´s membrane, while leaving a small part of it attached to the stroma and the rest of the graft laid back against the stroma. The ECL caused by this preparation method directly after preparation varied from 0% directly after preparation [51], to 9.1% after 24 hours of storage [58], to 4.12% [58] and 23% [56] after 3 days of storage. In our study, a novel, reproducible, and non-invasive method was introduced to quantify endothelial cell loss (ECL) of the DR caused by its preparation and storage for 5 days. The donor corneas used in this study had a minimum ECD of 1800 cells/mm^2 and had no detectable endothelial pathologies. After the preparation of 30 DRs by stripping the Descemet´s membrane from the donor cornea (as usually done before DMEK), the tissues were, without further manipulation,

directly placed in their storage medium containing organ culture medium 1 without dextran. 5 corneoscleral discs that did not undergo any stripping were used as a control group and were directly placed in organ culture medium 1 without dextran. ECL measurements followed directly after the preparation, and on days 1, 2 and 5 after storage using inverted light microscopy. For each sample, each measurement was repeated 5 times to ensure reproducibility of the results and to minimize bias. For each single measurement, a minimum of 3 clear images was obtained, 1 from the center and 2 from the peripheries of the DR. Our results showed an ECL of 11% due to the preparation procedure and further 12% due to the 5 days storage of the tissues. A high reproducibility of the results was demonstrated by Cronbach's alpha values that varied between 0.85 and 0.98 on all measurement days. Therefore, in order to prevent excessive ECL and to maintain a good quality of the donor endothelial layer, shipping DRs after several days of storage to be used in a DMEK cannot be recommended.

3.2 Publication 2: Prevalence and impact of Cornea guttata in the graft following penetrating keratoplasty (PKP)

CG is a known complication detected after PKP. Nevertheless, to the best of our knowledge, no studies have been performed to estimate its prevalence and severity. Our research article aimed to determine the prevalence and severity of CG in grafts after PKP and investigated its clinical significance during follow-up. In this retrospective study, 1522 patients who underwent PKP performed in the Department of Ophthalmology at the Saarland University Medical Center (UKS, Homburg/Saar, Germany) were included. Postoperative follow-up examinations until September 2020 were included. The presence of CG was assessed using specular microscopy during every follow-up visit, and a detailed guttata grading system was established to classify the patients into several groups: Group 0 (G0) had no CG, Group 1 (G1) had mild CG, group 2 (G2) had moderate CG and group 3 (G3) had severe CG. The results of this study showed that the overall prevalence of postoperative CG on the graft was 14.9%. While the majority showed only a low-grade CG and belonged to G1 with a percentage of 13.6%, only 1.3% showed high-grade CG with G2 and G3 constituting 0.9% and 0.4% respectively. The mean corneal thickness, pleomorphism and polymegalism were significantly affected by high-grade CG with a $p<0.001$. During long-term follow-up examinations, a progression to a higher grade of CG was noted in 16.8% of the CG cases.

3.3 Publication 3: Prevalence and severity of Cornea guttata in the graft following Descemet Membrane Endothelial Keratoplasty (DMEK)

Similar to the above mentioned second publication, this third paper investigated the prevalence and severity of CG in grafts after DMEK instead of PKP and assessed its impact on various clinical parameters during postoperative follow-up. 664 patients were included in this retrospective study. The presence of CG was also assessed using specular microscopy during every follow-up visit, and the same detailed guttata grading system mentioned in the second publication was used to classify the patients into several groups: Group 0 (G0) had no CG, Group 1 (G1) had mild CG, group 2 (G2) had moderate CG and group 3 (G3) had severe CG. CG was postoperatively detected in 18.7% of the grafts. 16.9% could be classified as low-grade CG (G1), and 1.9% as high-grade CG (1.4% as G2 and 0.5% as G3). A significant clinical deterioration was found with increasing grades of CG concerning the following parameters: corrected distance visual acuity (CDVA), central corneal thickness, pleomorphism, and polymegalism with p values of 0.02, 0.02, 0.003 and 0.04, respectively. In conclusion, studies two and three showed that around 1-2% of the transplanted corneas have high-grade CG, and only high-grade CG have a significant clinical impact on the patients during the postoperative follow-up examinations.

3.4 Publication 4: Semiquantitative criteria in the eye bank that correlate with Cornea guttata in donor corneas

The prevalence of CG in the normal population varies a lot depending on the population studied and is higher in the older population reaching 10% to 23% [22, 101]. Despite careful preoperative examination of the endothelial layer of the donor corneas in the eye banks, CG can only be detected postoperatively (but not preoperatively) using specular microscopy. Therefore, each keratoplasty bears the risk of transplanting a donor cornea with guttae. Depending on the severity of the guttae, the consequences of transplanting such a diseased cornea vary from being completely asymptomatic to significantly affecting the visual acuity. Corneal decompensation may necessitate a repeat keratoplasty in severe cases [22]. The aforementioned studies 2 and 3 proved the significant negative impact of high-grade CG on the follow-up of corneal grafts. Therefore, in this research article we aimed to establish semi-quantitative criteria for the detection of CG in the donor corneas in the eye bank. Retrospectively, 262 patients who underwent keratoplasty were classified according to the postoperative CG grade (no CG, mild CG, and severe CG). After that, the corresponding 1582 preoperative donor corneal endothelial pictures of these patients were collected and analyzed using five potential morphological semi-quantitative criteria. Our results showed that three of these criteria that can be detected in the eye bank using inverted light microscopy seem to correlate with postoperative CG: The presence of blebs (a small hyperdense thickening of the cell membrane), the presence of cell membrane defects and interruptions, as well as endothelial pictures with less than 50% of the cells having a hexagonal or circular shape. As a conclusion, these 3 criteria seem to be predictive factors for the detection of preoperative CG.

4. Ongoing and Future Projects:

Currently we are taking our project one step further, as we are performing a study in our eye Bank implementing the novel method introduced in our first publication to measure the ECL of the prepared DR directly before transplanting it. This allows a detailed quantification of the ECL during the DR preparation and post DMEK while describing the clinical findings during the postoperative follow-up period.

On the other hand, we are currently also performing further studies together with researchers from the DFKI (German Research Center for Artificial Intelligence) aiming at improving the screening methods of the endothelial layer of donor corneas and thus facilitating the detection of CG in the eye bank. This project comprises >1000 patients and >5000 preoperative endothelial images, integrates more objective criteria and incorporates artificial intelligence (AI) as a more accurate and precise method of analyzing endothelial pictures in the eye bank. Creating an AI software that is able to precisely predict the risk of having CG in donor corneas would prevent the transplantation of such diseased donor corneas thus leading to a reduction in the rate of post-keratoplasty CG. The first step was the segmentation of the data, i.e., determination of the relevant (pixel) zones and “region of interests” within an image. During this step, the blurry and unanalyzable areas of the endothelial pictures were detected and eliminated, also the potentially defected cells having an abnormally large surface area were highlighted and marked. Afterwards, a hybrid classification algorithm was created to determine the quality of the corneas and to classify them according to the CG grade. A so-called "deep learning" method was used, i.e., a machine learning algorithm based on complex neural networks.

Finally, a decision support tool for the detection of CG was subsequently created combining the above-mentioned instruments into 2 components: (1) Graphical analytic

tools, whereby the endothelial images pass multiple OpenCV-based image processing steps including the Watershed transform algorithm. In this step, cell membranes are delineated, and abnormally large cells or cell depleted areas are marked in red. Several other cell representations such as “honeycomb” representation are created for an enhanced visualization of the endothelial layer. (2) Machine learning classifiers including Case-Based Reasoning were created to detect CG **(Figure 7)**. Preliminary unpublished results showed a performance comparable to humans and proved that the created decision support tool is able to improve the decision accuracy of the clinicians optimizing the classification process of preoperative cornea guttata.

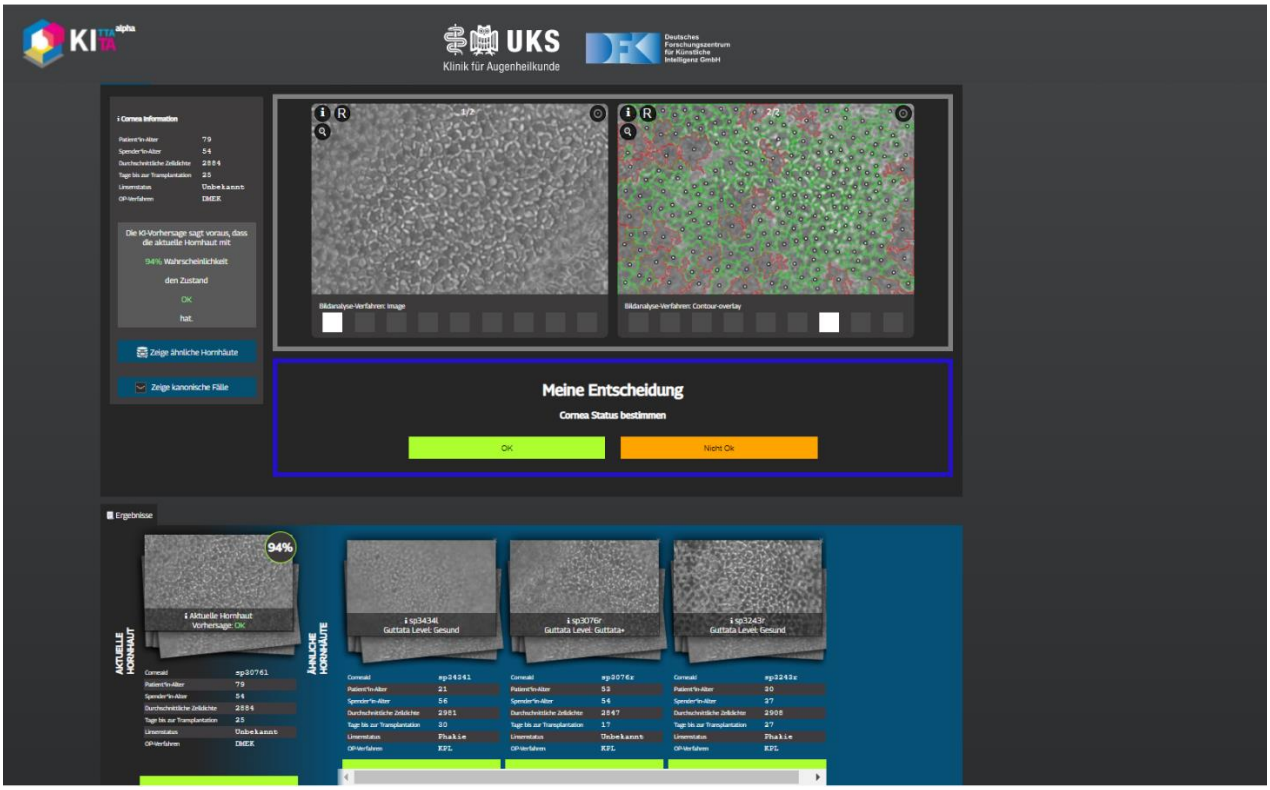


Figure 7: KIttool: decision support tool for the detection of CG integrating 2 components: (1) Graphical analytic tools, whereby endothelial cells are processed to generate several cell representations such as “honeycomb” representation for an enhanced visualization of the endothelial layer (EL). (2) Machine learning classifiers including Case-Based Reasoning were created to detect CG. (Self-created image)

5. Attachments:

5.1 Publication 1:

Reproducibility of Non-Invasive Endothelial Cell Loss Assessment of the Pre-Stripped DMEK Roll After Preparation and Storage

TAREK SAFI, BERTHOLD SEITZ, KOLJA BERG, KATJA SCHULZ, ACHIM LANGENBUCHER, AND LOAY DAAS

• PURPOSE: To present a novel, reproducible, and noninvasive method to quantify endothelial cell loss (ECL) of pre-stripped endothelial Descemet membrane lamellae (EDML) caused by its preparation and storage for 5 days.
• DESIGN: Prospective laboratory investigation.
• METHODS: Thirty EDML were stripped from corneoscleral discs and placed in a well plate containing organ culture medium 1 without dextran. An additional 5 corneoscleral discs were also placed in the same medium and served as a control group. Endothelial cell density (ECD) was measured without any additional manipulation by using spectral microscopy following an extensive protocol by which 3 clear images from the center and periphery were used for each measurement, and each measurement was repeated 5 times. ECD was measured before and directly after preparation and on days 1, 2, and 5 of storage.
• RESULTS: The average ECD of the 30 corneoscleral discs, which later underwent stripping, was 2,292 ± 308 cells/mm^2 vs 2,129 ± 222 cells/mm^2 for the 5 corneoscleral discs of the control group. The ECL of the control group was significantly lower than that of the EDML group ($P < .0001$), reaching ±2% versus 11 ± 5%, respectively, on day 0; 3% ± 4% versus 19 ± 10%, respectively, on day 1; 2% ± 2% versus 22% ± 11%, respectively on day 2; and 4% ± 3% versus 23% ± 9%, respectively, on day 5. Reproducibility of the results on all measurement days was good, with Cronbach alpha values ranging from 0.85 to 0.98.
• CONCLUSIONS: A highly reproducible, noninvasive method was presented for measuring the ECD of the EDML. Prestripped EDML lose a significant amount of cells, up to 11%, due to the preparation process and up to 23% after 5 days of storage. Therefore, shipping them after several days of storage to be used in a DMEK surgery cannot be recommended. (Am J Ophthalmol 2021;221:17–26. © 2020 Elsevier Inc. All rights reserved.)

AJO.com Supplemental Material available at AJO.com.

Accepted for publication Aug 3, 2020.

From the Department of Ophthalmology (T.S., B.S., K.B., K.S., A.L., L.D.), Saarland University Medical Center, Homburg, Germany.
Inquiries to Tarek Safi, Kirrberger Street 100, 66424 Homburg, Germany; e-mail: Tarek.Safi@uks.eu

CORNEAL TRANSPLANTATION IS A RAPIDLY evolving field in ophthalmology. One particular type of selective corneal transplantation is Descemet membrane endothelial keratoplasty (DMEK), which has been increasingly adopted and performed by surgeons[1] since its introduction in 2006.[2] Despite the many advances this technique has brought, preparation and handling of the delicate donor Descemet membrane (DM) is still presenting some technical challenges.[3] In fact, studies showed that endothelial cell density (ECD) decreases from 19% to 44% at 6 months after DMEK surgery in comparison to preoperative values.[4] For this reason, various methods of preparation have been developed to ensure the best possible outcomes.[4]

The ability to measure endothelial cell loss (ECL) due to DMEK preparation before surgery allows the surgeon to better assess the quality of the graft, the long-term ECL, and indirectly, the potential reason for graft failure rates.[5] However, there is not yet a standardized, noninvasive method to accurately and reliably measure the exact ECL caused by preparation and subsequent storage of the tissue. As far as the present authors are aware, 2 proposed methods currently exist to measure the ECD of the endothelial Descemet membrane lamellae (EDML).

The first method involves complete stripping of the DM, then manually unrolling it, and using inverted light microscopy and enhanced image analysis software titled "Fiji" on the open source "ImageJ" to calculate the ECD. Unfortunately, this is an invasive method, and the ECD measurement process involves manipulation of the DM by unrolling it, which probably results in additional cell loss and, thus, inaccurate results. In addition, this method raises questions about the validity and repeatability of its results, as the ECL measured varied widely between different studies, from 9.3% to 32%.[5–8]

The second method involves measuring the ECL after partially peeling the DM, leaving a small part of the DM attached to the stroma and the rest of the graft laid back against the stroma. Although this is a noninvasive method in regard to the DM as it does not cause additional manipulation of the tissue, it does not mimic the actual surgical preparation technique, where the tissue is totally stripped from the stroma and then placed in a storage medium while in the rolled configuration. The ECL calculated by this technique was not only low and may represent an

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2020.08.001

underestimation of the correct value, but it also varied between different studies, from 0% to 23% ECL.[9–11]

This paper presents a new method of measuring the ECD of the EDML, by which the ECL was calculated after the EDML preparation was completed and without any further manipulation of the rolled tissue. This method involves measuring the ECD by using specular microscopy by a well-trained technician directly after stripping the EDML and placing it in the storage medium. Previous studies have tried to use this technique, but the results were not accurate, showing no ECL after preparation.[6,9] This was due to the fact that DMEK lamellae exhibit a natural tendency to curl up into a scroll with the endothelial layer facing outward[12]; therefore, finding a clear, focused image to calculate the ECD using a specular microscope is considered extremely difficult. Hence, this research also focuses on the reproducibility and accuracy of the results using this technique.

Additionally, an evaluation of the ECL is made after 1, 2, and 5 days of storage in order to assess the feasibility of shipping pre-stripped and rolled donor EDML in cartridges which are ready to be injected into the anterior chamber of the eye during DMEK surgery.

MATERIAL AND METHODS

• **TISSUE SELECTION:** In this prospective laboratory investigation, tissues were provided by the Klaus Faber Center for Corneal Diseases, LIONS-Corneal Bank Saar-Lor-Lux, Trier/Westpfalz in the Department of Ophthalmology at Saarland University Medical Center (UKS), and they were deemed unsuitable for transplantation due to either positive serology or presence of certain diseases, or low ECD. Exclusion criteria included an ECD count of $<1{,}800$ cells/mm^2 or any endothelial pathology. Research consent was obtained for the use of these tissues. This research included a total of 35 corneoscleral discs, originally stored in organ culture medium without dextran.

• **TISSUE PREPARATION:** The ECD was measured directly before the preparation and stripping of the Descemet membrane from the corneoscleral discs by using a specular microscope (model 090-135.001; Leica Microsystems, Wetzlar, Germany). Five corneoscleral discs were placed directly in storage culture medium 1 (Biochrom AG, Berlin, Germany) without dextran. The preparation procedure of the remaining 30 EDML was performed by 1 well-trained surgeon (L.D.) using the technique described by Seitz and associates.[13]

At first, the 15-mm corneoscleral buttons were positioned epithelium-downward on a suction block, which is commonly used to prepare the EDML (Moria trephination system; Moria SA, Antony, France). In order to improve visualization, Blue Color Caps (BCC) (Croma GmbH, Leobendorf, Austria) staining was applied for approximately

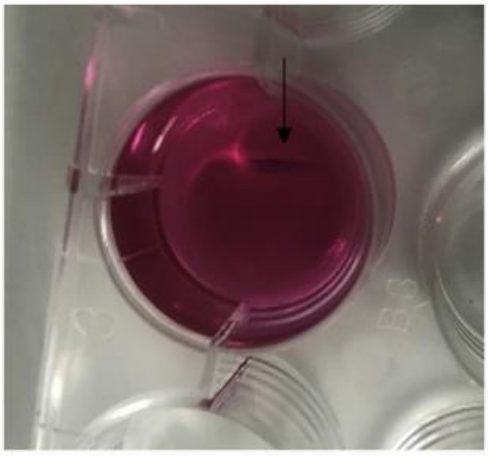


FIGURE 1. Endothelial Descemet membrane lamella placed in a well plate containing organ culture medium 1 without dextran, forming a rolled configuration (arrow).

60 seconds and then replaced with organ culture medium. Second, a superficial 7.5-mm mark was introduced by using the 7.5-mm trephine descemet's stripping automated endothelial keratoplasty (DSAEK) trephination system. Peripheral lamellar incisions of approximately 1.0-1.5 mm outside the 7.5-mm mark were made hexagonally or octagonally with a razor blade. Using a nontoothed forceps, the DM was grasped radially with very little extension, and its peripheral border was lifted circularly. Once one-third of the entire EDML was detached, pulling it from 3 sides toward the center was stopped, and it was then laid back in its original place. The last step was repeated twice after a 120-degree rotation of the suction block, leaving a small part of the central stroma attached. After that, lamellar trephination of the EDML with the 7.5-mm trephine was performed resulting in a curvilinear edge. After the peripheral circular part of the EDML was discarded, one-half of the forceps was used to lift the partially incarcerated margins of the graft within the trephination groove. Because most of the EDML was already stripped from the stroma, a complete separation was relatively easy and safe. However, the forceps was guided along the concavity of the corneoscleral button without touching the endothelium. Finally, a larger forceps was used to transfer the completely detached EDML to a well plate containing the storage medium. Because dextran was proven to have an unfavorable impact on the preservation of the pre-stripped DMEK tissue,[14] the storage medium used in this present study consisted of organ culture medium 1 without dextran. All the EDML took a scroll configuration shortly after preparation (Figure 1).

• **TISSUE MEASUREMENT PROTOCOL:** Without any further manipulation, the well plate containing the

FIGURE 2. Endothelial Descemet membrane lamella placed in a well plate and measured by specular microscopy (unit model 090-135.001; Leica Microsystems, Wetzlar, Germany).

EDML was placed under a specular microscope for measurement of the ECD (Figure 2). The measurements were performed by 2 well-trained technicians directly before and after the preparation as well as on days 1, 2, and 5 after preparation. For each sample, the measurement was repeated 5 times to ensure reproducibility of the results and to minimize bias. For each measurement, a minimum of 3 clear images were obtained from the center and 2 peripheries of the EDML roll as illustrated in Figure 3.

This step proved particularly difficult, because determining a clear and focused field of view was challenging and time-consuming because the EDML had a rolled configuration, therefore the focused field of view under the microscope was usually small. Taking measurements while the tissues are in organ culture medium and not balanced salt solution poses an additional challenge. After a clear picture was found, a square of 116.599-µm sides was placed on the image to delineate the area within which the cells were easiest to identify and thus could be counted (representing the region of interest [ROI]). Images were then analyzed by the software to automatically count the number of cells present within the ROI, followed by manual correction of the count by a trained technician to provide the most accurate results. Manual correction of the cell count consisted of adding cells that were not detected by the software and removing falsely counted cells. This step required expertise and knowledge of the cells' shape in the endothelial layer, based on the visualization and delineation of the cellular membranes or the nuclei of the endothelial cells, taking special care not to confuse granulations with nuclei. According to eye bank convention, cells that are only partially within the ROI are counted on 2 of the 4 borders and are neglected on the other 2 of the 4 borders. Figures 4, A through D, and 5, A and B, illustrate the above-described steps in detail. An estimate of the ECD is then automatically generated by the software based on the final number of cells counted. After each measurement, the container is gently removed from the microscope and then put back for the next measurement. Once all 5 measurements were completed, the sample was gently returned to the incubator.

• **STATISTICAL ANALYSIS:** All statistical analyses were performed using Excel (Microsoft, Redmond, Washington) and SPSS software (IBM, Armonk, New York). Descriptive statistics mean ± SD. The standardized Cronbach alpha value was calculated to assess the reproducibility of the results. The Mann-Whitney *U* test and Wilcoxon signed-rank test were used to identify statistically significant ECL after preparation and subsequent storage of the EDML. All statistics were 2-sided, and the alpha was set at .05.

RESULTS

IN TOTAL, 35 SAMPLES WERE USED IN THIS STUDY, OF WHICH 5 samples served as a control group. Dissection of the DM was performed successfully without tears in all 30 samples. The average ECD of the total number of the EDML samples and the control group before preparation was 2,292 ± 308 cells/mm^2 and 2,129 ± 222 cells/mm^2, respectively.

• **ECD MEASUREMENT AND ECL DUE TO PREPARATION AND STORAGE:** The ECD values presented on each measurement day are the average of the 30 individual ECD values of each EDML sample. Each individual ECD value is the average of the 5 measurements calculated on each day. Standard deviation (SD) values in Table 1 are calculated from the 30 individual ECD values. The average ECD of all the samples measured on each day was divided into a center region and 2 periphery regions and is shown in Table 1 along with the SD, median, minimum, and maximum measurement values.

The ECL was then calculated using ECD values on each day. Table 2 shows the average ECL of all the EDML samples measured on each day sorted into periphery and center measurements and also illustrates SDs, medians, and minimum and maximum values. The average percentage of ECL caused by preparation and subsequent storage of the EDML calculated on each day was 11% directly after preparation, 19% on day 1, 22% on day 2, and 23% on day 5. All ECL values calculated on each day were statistically significant compared with the mean ECD before preparation of the EDML (Table 2). The ECL values were then compared at each time point during storage and showed a 10% loss ($P < .001$) from day 0 to day 1 of storage, 4% loss ($P < .001$) from day 1 to day 2 and 1% loss ($P = .087$) from day 2 to 5.

In regard to the control group, the ECL was calculated on days 0 (directly after storage), 1, 2, and 5 and showed 1% ± 2%, 3% ± 4%, 2% ± 2%, and 4% ± 3%, respectively. *P* values of those losses were equal to .23, .23, .07, and .04, respectively. Compared with the control group, the

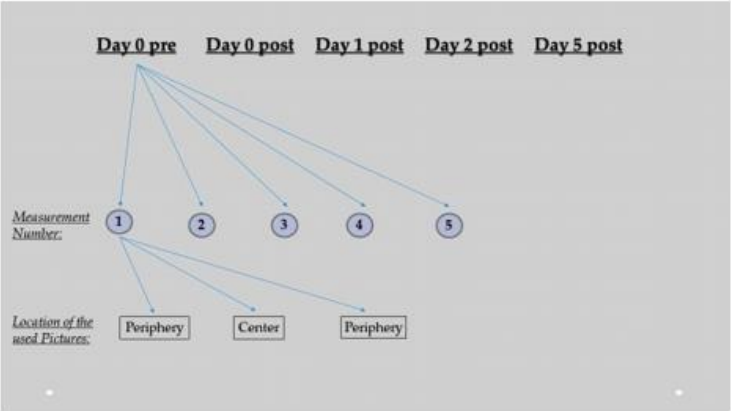


FIGURE 3. Illustration of the measurement protocol for each sample. EDML = endothelial Descemet membrane lamellae; pre = measurement made before preparation of the EDML; post = measurement made after preparation of the EDML.

EDML group had a statistically significantly bigger ECL with a P value $< .0001$ on all measurement days.

• **ECD AT THE CENTER VERSUS PERIPHERY OF THE EDML:** A comparison was made between the ECD values recorded at the periphery versus those at the center of the EDML. As shown in Table 3 and Figure 6, there were no statistically significant differences between the 2 groups on all measurement days, except for day 5.

• **REPRODUCIBILITY:** A standardized Cronbach alpha of the 5 measurements was calculated for each day across the total number of the EDML samples, and the results are shown in Table 4. Cronbach alpha values calculated in the peripheral and central areas ranged from .85 to .98 on all measurement days.

Furthermore, the SD of the repeated 5 measurements made for every EDML sample was calculated separately on each measurement day for the peripheral and central regions. The average of the 30 SD values obtained each day by the previous step was calculated and presented as a percentage of the mean ECD of that respective day. Those values ranged from 156 cells/mm^2 (or 8%) to 173 cells/mm^2 (or 10% of the mean ECD) on the peripheral region and from 146 cells/mm^2 (or 6%) to 138 cells/mm^2 (or 8% of the mean ECD) on the central region (Table 5).

DISCUSSION

THIS IS THE FIRST RESEARCH WITH THE ABILITY TO CALCULATE the ECL by using specular microscopy from reproducible noninvasive measurements of the ECD after performing the complete preparation procedure of the rolled EDML. So far, other methods have proven to be either invasive and might have represented an inaccurate overestimation of the ECL, or they did not mimic the complete preparation procedure and thus possibly represented an inaccurate underestimation of the ECL.

• **ECD MEASUREMENT AND ECL DUE TO PREPARATION AND STORAGE:** There are many differences among EDML preparation procedures, methods of measuring the ECD, storage media, and time frames used between the preparation of the EDML and the measurement of the ECD, which makes it, in turn, difficult to compare the published data regarding ECL caused by stripping of the DM and subsequent storage. In fact, different studies reached very different results regarding ECL caused by preparation of the EDML. Two studies showed no ECL at all, for example, Mayko and associates,[9] performed a preparation procedure in which the EDML was still partially attached to the stroma and supported by the corneoscleral rim, and Lie and associates,[15] who measured the ECD of the EDML after its complete stripping but the measurement protocol was not clearly explained in the study. An ECL of 9.3% was demonstrated in another study where the EDML was totally stripped and examined with the slit lamp microscope while in the injector tube.[6] However, another study showed an ECL of 12.4%, using the pneumodissection preparation method. A high ECL value, reaching 22.5%, was reported in another study where the EDML was examined by inverted microscopy after it was unrolled.[5] Finally, ECL ranged from 23% to 32% in studies where the measurement occurred after preparation and the tissue passed through the injector.[8,16]

The present novel, extensive, and detailed measurement protocol focused on accuracy and precision in the

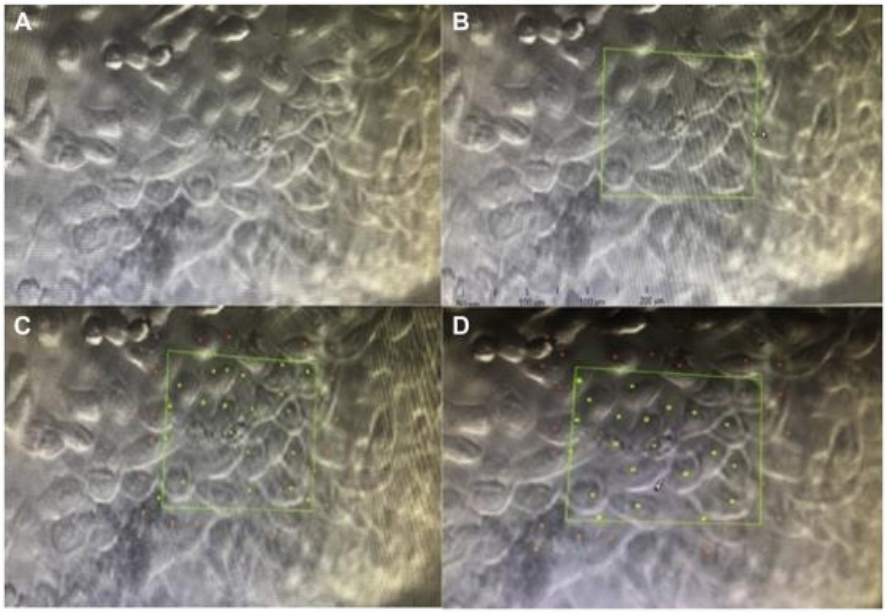


FIGURE 4. ECD measurement steps. (A) Step 1 involves finding a clear field of view. (B) Step 2 is adding a square (green box shows the region of interest) within which there is a sharply focused area containing cells. (C) Automatic generation of cell count within the square by the software; each cell is represented by a green dot. (D) Manual correction of The cell count was manually adjusted to include undetected cells (green dots) by the software and eliminating false dots that did not correspond to a clear cell. Cells that were only partially within the region of interest at the left and bottom borders were counted, but those at the right and top borders were not. ECD = endothelial cell density.

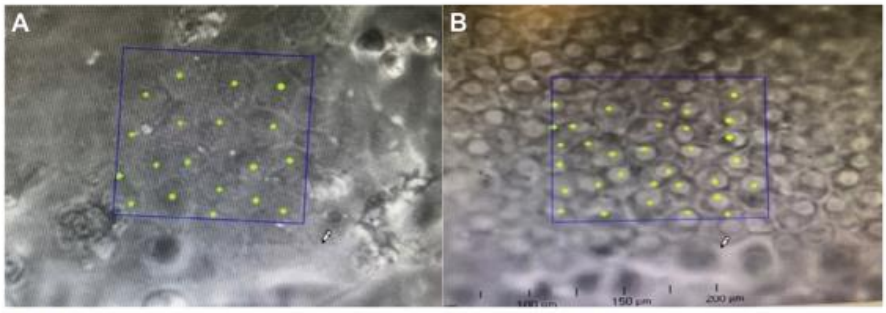


FIGURE 5. Cell identification methods. (A) Recognition of the cellular membranes that formed clear borders between the cells. The image quality is better when looking directly through the microscope. (B) Recognition of the cell nucleus. The blue box shows the region of interest, and each green dot represents one cell.

measurement of the ECD only after mimicking the complete preparation procedure of the EDML for DMEK surgery. Specular microscopy was used to assess the EDML noninvasively and without interfering with or affecting the storage and preservation of the samples. Our results showed an ECL of 11% caused by the preparation of the EDML alone. This value is acceptable and agrees with many other published articles using other measurement protocols.

The same problem of inconsistent results among different studies occurs when studying the ECL due to

TABLE 1. Average Values for Endothelial Descemet Membrane Lamellae Samples on Each Measurement Day[c]

ECD Timing and Location	Average	Median	SD	Minimum	Maximum
Day 0 pre-periphery[a]	2,295	2,263	±264	1,818	2,767
Day 0 pre-center	2,287	2,263	±232	1,796	2,789
Day 0 post-periphery[b]	2,032	1,971	±183	1,672	2,402
Day 0 post-center	2,048	1,971	±195	1,694	2,365
Day 1 post-periphery	1,831	1,862	±180	1,205	2,132
Day 1 post-center	1,847	1,825	±207	1,285	2,365
Day 2 post-periphery	1,769	1,789	±209	9,93	2,029
Day 2 post-center	1,763	1,752	±214	9,93	2,088
Day 5 post-periphery	1,703	1,679	±132	1,467	1,971
Day 5 post-center	1,720	1,679	±126	1,489	2,015

ECD = endothelial cell density; pre- = before stripping; post- = after stripping; SD = standard deviation.
[a]Measurements made before preparation of the endothelial Descemet membrane lamellae.
[b]Measurements made after preparation of the endothelial Descemet membrane lamellae.
[c]Average ECD, ±SD, and median, minimum, and maximum of the total number of the endothelial Descemet membrane lamellae samples on each measurement day. SD values were calculated from the 30 individual ECD values.

TABLE 2. Average Values for the Total Number of Endothelial Descemet membrane lamellae Samples on Each Measurement Day[a]

ECL Measurement Day	Average ECL %	Median	SD	Minimum	Maximum	*P* Value of ECL
Directly after preparation-center	10.3	10.7	±5.2	0.68	24.2	<.001
Directly after preparation-periphery	11.1	10.0	±5.0	5.34	21.4	<.001
Day 1 post-center[b]	18.8	17.0	±9.0	6.6	45.3	<.001
Day 1 post-periphery	19.4	14.9	±10.0	9.3	48.8	<.001
Day 2 post-center	22.3	19.7	±10.8	10.3	57.8	<.001
Day 2 post-periphery	22.1	16.4	±11.3	12.1	57.76	<.001
Day 5 post-center	22.7	19.5	±8.4	13.2	43.7	<.001
Day 5 post-periphery	22.9	18.3	±9.7	16.3	44.48	<.001

ECL = endothelial cell loss; pre- = before stripping; post- = after stripping; SD = standard deviation.
[a]Average ECL, ±SD, and median, minimum, and maximum of the total number of endothelial Descemet membrane lamellae samples on each measurement day. *P* values for the ECL were calculated on each day in comparison to the respective previous measurement day.
[b]Measurements were made after preparation of the endothelial Descemet membrane lamellae.

storage. Fewer studies assessed the ECL caused by storage of the EDML because many measurement protocols are invasive and thus do not allow for preservation of the tissue for multiple measurements. According to our results, the ECL increased steadily over time during storage of the EDML reaching 23% after 5 days of storage. Two published articles aimed to assess ECL caused by preparation and storage of the EDML, but both studies used noninvasive methods where the lamellae were only partially peeled and then laid back against the stroma of the corneoscleral disc. As a result of having the corneoscleral disc as a support and being only partially peeled off, the EDML might have lost a smaller amount of endothelial cells during the preparation and storage procedures than the actual complete stripping of the DM needed during DMEK surgery as well as the potential transportation in a glass cartridge.[10,11]

In the paper published by Muraine and associates,[10] an ECL of 4.12% was recorded after 3 days of storage, and in the paper published by Menzel-Severing and associates,[11] the ECL reached 9.1% after 24 hours of storage and 23% after 3 days of storage. Another interesting study was carried out by Bayyoud and associates,[17] in which 10 DM samples were completely stripped from the corneoscleral discs and stored in culture medium 1. In that study, the reported ECL was 4.1%, 4.8%, 9.4%, and 13.1% after 1, 4, 7, and 10 days, respectively, of storage of the EDML, but unfortunately the method and protocol of the ECD measurement was not included in the published article. Finally, Lie and associates[14] also reported a study in which 10 completely stripped EDML were preserved for up to 4 weeks in organ culture in a modified minimum essential medium (CorneaMax; Eurobio Scientific, Les Ulis, France), and the ECD

TABLE 3. Comparisons Between ECD Values Measured in the Peripheral and Central Regions of the Endothelial Descemet Membrane Lamellae

ECD	Differences in ECD Measurements Between Periphery and Center				
Measurement day	0 pre[a]	0 post[b]	1 post	2 post	5 post
P value	.68	.19	.75	.79	.02[c]

ECD = endothelial cell density; post = before stripping; pre = after stripping.
[a]Measurements made before preparation of the endothelial Descemet membrane lamellae.
[b]Measurements made after preparation of the endothelial Descemet membrane lamellae.
[c]Statistically significant.

TABLE 4. Standardized Cronbach Alpha Values

	Periphery	Center
Day 0 pre[a]	.95	.91
Day 0 post[b]	.93	.88
Day 1 post	.93	.85
Day 2 post	.95	.89
Day 5 post	.98	.97

post = before stripping; pre = after stripping.
[a]Measurements before preparation of the endothelial Descemet membrane lamellae.
[b]Measurements after preparation of the endothelial Descemet membrane lamellae.

was measured using inverted microscopy; yet again, the measurement protocol and whether it involved unrolling of the EDML was not mentioned in the published article. The study showed a mean ECD of 2,701 cells/mm^2 (±302) before and 2,719 (±322) cells/mm^2 immediately after the DM was stripped, and the authors noticed a decline from 2,604 ± 352 cells/mm^2 after 1 week of storage to 2,190 ± 768 cells/mm^2 after an additional 4 weeks of storage in organ culture. This would imply that there was no ECL caused by the stripping of the EDML, and only 3.6% and 18.9% of ECL occurred after 1 and 4 weeks, respectively, of storage, which seems too favorable in comparison to the results of the present study.

Two factors may be responsible for the ECL occurring during the storage of the EDML, first, because peeling off the stromal support during the preparation procedure, removing the stromal support of the endothelial layer makes it fragile and prone to additional cell loss during storage; and second, the storage medium itself, which is why we included the control group in our study to assess the ECL of the corneoscleral discs placed directly in culture medium 1 without dextran. According to the present results, there was a small amount of ECL (4%) after 5 days of storage that can be attributed to the storage medium itself. When the 2 study groups were compared, it was evident that the ECL on each day was much higher in the EDML group than in the control group, with a statistical significance of $P < .0001$. Consequently, the preparation procedure of the EDML plays an important role in the ECL during subsequent storage.

Another factor that might have partially contributed to the ECL in the present study was the low initial ECD values before stripping of the EDML. Krabcova and associates[18] concluded that samples with lower original ECD (<2,500 cells/mm^2) faced higher ECL after tissue storage.

The change in ECL during storage was also assessed, and it revealed a sharp increase in value during the first day of storage reaching 10%, followed by a gradual decrease of up to 4% during the second day of storage. Afterward, there was no statistically significant ECL from day 2 to 5, which indicates that the ECL occurring during storage takes place mainly within the first 48 hours.

• **ECD AT THE CENTER VERSUS PERIPHERY OF THE EDML:** The normal human cornea shows a 5.8% ($P < .01$) and a 9.6% ($P < .001$) increase in ECD in the paracentral and peripheral regions of the cornea, respectively, in comparison with the central region. The paracentral region is defined as 2.7 ± 0.2 mm radial distance from the center and the peripheral region as 4.7 ± 0.2 mm from the center.[19] The present EDML samples had a diameter of 7.5 mm, corresponding to a maximal distance of 3.75 mm from the center. Thus, the peripheral region of the EDML in fact refers to the paracentral region of the cornea. However, the present results showed no clinically significant differences between the ECD in the center and periphery, although there was a statistically significant difference of 17 cells between the measurements of ECD in the periphery and center of the EDML on day 5, with a *P* value reaching .02. This did not seem to be of clinical significance.

• **REPRODUCIBILITY:** This research presents a new method of measuring the ECD after preparation and subsequent storage of the EDML in organ culture medium 1 without dextran. Hence, special attention was attributed to ensure internal consistency and reproducibility of the present results by following a strict measurement protocol that includes repeating all measurements 5 times and also

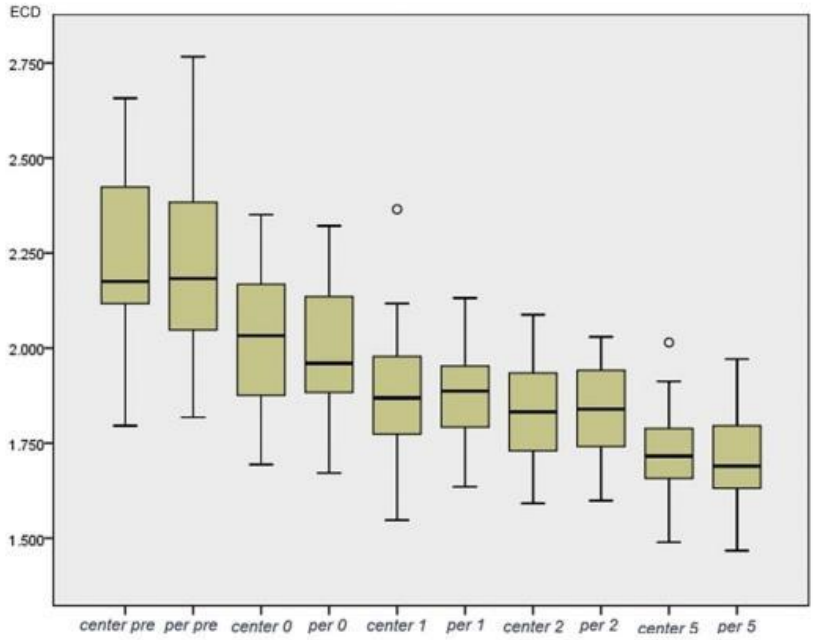


FIGURE 6. Comparison of center and periphery ECD measurements on each measurement day. ECD = endothelial cell density. pre = measurement done before preparation of the EDML. EDML = endothelial Descemet membrane lamellae. per = periphery.

TABLE 5. The Average of the Standard Deviation Values of the 5 Measurements Made for Each EDML Sample on Each Day and Its Representation as a Percentage of the Mean ECD[c]

Standard Deviation	Periphery (cells/mm^2)	Center (cells/mm^2)	% of the Mean ECD at the Periphery	% of the Mean ECD at the Center
Day 0 pre[a]	189	146	8	6
Day 0 post[b]	156	143	8	7
Day 1 post	161	151	9	8
Day 2 post	173	138	10	8
Day 5 post	137	125	9	7

ECD = endothelial cell density; EDML = endothelial Descemet membrane lamellae; pre = before stripping; post = after stripping.
[a]Measurements before preparation of the endothelial Descemet membrane lamellae.
[b]Measurements after preparation of the endothelial Descemet membrane lamellae.
[c]Average of the SD values of the 5 measurements made for each EDML sample on each day and their representation as percentages of the mean ECD.

by distinguishing between peripheral and central measurements. Standardized Cronbach's alpha values were calculated for each measurement day and revealed excellent values ranging from .85 to .98, implying very high reliability and reproducibility of the results.[20]

The SD of the 5 measurements done for each EDML sample was calculated as an additional parameter to assess the reproducibility of our results. The values ranged from 146 cells/mm^2 or 6% of the mean to 173 cells/mm^2 or 10% of the mean. These values are considered small and acceptable. They represent a minimal variation of the results around the mean on all measurement days and therefore indicate good reproducibility of the results.

• IMPACT OF STORAGE AND SHIPMENT OF THE EDML IN AN INJECTOR: Storage and shipment of the delicate

EDML in an injecting device is considered an additional manipulation step that will probably lead to additional cell loss. This seems to be particularly valid in view of the minimal amount of storage medium present inside the tube.

Assessing the cell count in a well plate noninvasively before shipment in an injector or storage bottle is not performed as a standard procedure until now, as this step is very challenging and requires high expertise and special training. However, this step would definitely be a beneficial and important factor in assessing the quality of the graft after preparation and just before its implantation, thus resulting in better patient care. Therefore, the present authors are currently conducting a similar research project in which measurements of the ECD of the EDML are performed in a well plate directly before implantation. The purpose of that study is to assess the impact of relative ECL of the EDML during preparation on the long-term endothelial cell loss after DMEK.

In conclusion, this study provides a new ECD measurement protocol for completely stripped EDML rolls that is highly reproducible. According to these results, there was a significant increase in the ECL of the EDML in comparison to the corneoscleral discs as measured by specular microscopy, reaching up to 23% versus 4% after 5 days of storage. This outcome contradicts shipping the pre-stripped DMEK in a glass cartridge around the world from an eye bank to a DMEK surgeon. The high quantity of ECL may endanger the post-operative state of the graft and thus the safety of the patient. Despite an initial success, repeat DMEK will be necessary mainly because of delayed endothelial insufficiency. Further research should focus on the post-operative long-term assessment of the pre-stripped DMEK grafts, which were shipped to the surgeon after several days of storage.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST and none were reported.

Funding/Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Financial disclosures: None of the authors have any financial disclosures in this study.

The authors thank the Klaus Faber Center for Corneal Diseases, LIONS Corneal Bank Saar-Lor-Lux, Trier/Westpfalz for their help in preserving and measuring the donor corneas.

REFERENCES

1. Eye Bank Association of America. 2016 Eye Banking Statistical Report 2017. Available at: http://restoresight.org/wp-content/uploads/2017/04/2016_Statistical_Report-Final-040717.pdf. Accessed November 15, 2019.
2. Melles G, Ong T, Ververs B, van der Wees J. Descemet membrane endothelial keratoplasty (DMEK). *Cornea* 2006;25(8):987–990.
3. Terry M. Endothelial keratoplasty. *Cornea* 2012;31(5):469–471.
4. Birbal R, Sikder S, Lie J, Groeneveld-van Beek E, Oellerich S, Melles G. Donor tissue preparation for Descemet membrane endothelial keratoplasty. *Cornea* 2018;37(1):128–135.
5. Jardine G, Holiman J, Stoeger C, Chamberlain W. Imaging and quantification of endothelial cell loss in eye bank prepared DMEK grafts using trainable segmentation software. *Curr Eye Res* 2014;39(9):894–901.
6. Tran K, Dye P, Odell K, et al. Evaluation and quality assessment of pre-stripped, preloaded Descemet membrane endothelial keratoplasty grafts. *Cornea* 2017;36(4):484–490.
7. Altaan S, Gupta A, Sidney L, Elalfy M, Agarwal A, Dua H. Endothelial cell loss following tissue harvesting by pneumodissection for endothelial keratoplasty: an ex vivo study. *Br J Ophthalmol* 2015;99(5):710–713.
8. Schallhorn J, Holiman J, Stoeger C, Chamberlain W. Quantification and patterns of endothelial cell loss due to eye bank preparation and injector method in Descemet membrane endothelial keratoplasty tissues. *Cornea* 2016;35(3):377–382.
9. Mayko Z, Benetz B, Menegay H, et al. Donor endothelial cell density measurements do not change immediately after DMEK preparation. *Cornea* 2016;35(12):1556–1561.
10. Muraine M, Gueudry J, He Z, Piselli S, Lefevre S, Toubeau D. Novel technique for the preparation of corneal grafts for Descemet membrane endothelial keratoplasty. *Am J Ophthalmol* 2013;156(5):851–859.
11. Menzel-Severing J, Walter P, Plum W, Kruse F, Salla S. Assessment of corneal endothelium during continued organ culture of pre-stripped human donor tissue for DMEK surgery. *Curr Eye Res* 2018;43(12):1439–1444.
12. Parekh M, Ruzza A, Ferrari S, Busin M, Ponzin D. Preloaded tissues for Descemet membrane endothelial keratoplasty. *Am J Ophthalmol* 2016;166:120–125.
13. Seitz B, DAAS L, Bischoff-Jung M, et al. Anatomy-based DMEK Wetlab in Homburg/Saar: novel aspects of donor preparation and host maneuvers to teach Descmet membrane endothelial keratoplasty. *Clin Anat* 2017;31(1):16–27.
14. Abdin A, Daas L, Pattmöller M, Suffo S, Langenbucher A, Seitz B. Negative impact of dextran in organ culture media for pre-stripped tissue preservation on DMEK (Descemet membrane endothelial keratoplasty) outcome. *Graefes Arch Clin Exp Ophthalmol* 2018;256(11):2135–2142.
15. Lie J, Birbal R, Ham L, van der Wees J, Melles G. Donor tissue preparation for Descemet membrane endothelial keratoplasty. *J Cataract Refract Surg* 2008;34(9):1578–1583.
16. Downes K, Tran K, Stoeger C, Chamberlain W. Cumulative endothelial cell loss in Descemet membrane endothelial keratoplasty grafts from preparation through insertion with glass injectors. *Cornea* 2018;37(6):698–704.
17. Bayyoud T, Röck D, Hofmann J, Bartz-Schmidt K, Yoeruek E. [Precut technique for Descemet's membrane endothelial keratoplasty, preparation and storage in organ culture]. *Klin*

Monatsbl Augenheilkd 2012;229(6):621–623 [article in German].
18. Krabcova I, Studeny P, Jirsova K. Endothelial cell density before and after the preparation of corneal lamellae for Descemet membrane endothelial keratoplasty with a stromal rim. *Cornea* 2011;30(12):1436–1441.
19. Amann J, Holley G, Lee S, Edelhauser H. Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. *Am J Ophthalmol* 2003;135(5):584–590.
20. Tavakol M, Dennick R. Making sense of Cronbach's alpha. *Int J Med Educ* 2011;2:53–55.

5.2 Publication 2:

CLINICAL SCIENCE

Prevalence and Impact of Cornea Guttata in the Graft After Penetrating Keratoplasty in Germany

Silvana Schönit, Amine Maamri, MD, Elena Zemova, MD, Cristian Munteanu, Tarek Safi, MD, Loay Daas, MD, and Berthold Seitz, MD, ML, FEBO

Purpose: The aim of this study was to analyze the prevalence and severity of corneal guttata (CG) in grafts after penetrating keratoplasty (PKP) and to determine its clinical significance.

Methods: This retrospective study included 1758 PKP performed in 1522 patients. In total, 6662 postoperative endothelial images revealed the prevalence and severity of CG (divided into categories G0 without CG and G1-G3 with increasing severity). Origin of the graft, postoperative corneal thickness, visual acuity, pleomorphism, polymegethism, and endothelial cell density (ECD) were analyzed.

Results: CG was detected in 14.9% of the grafts within 9 months after PKP, most of them were low-grade G1 (13.6%). Grafts from Homburg/Saar showed significantly less CG cases compared with other eye banks ($P = 0.034$). The mean corrected distance visual acuity (logMAR) did not differ between G1 (0.45 ± 0.31) and G0 (0.46 ± 0.31). The mean ECD was lower in G1 compared with G0 ($P < 0.001$). The mean corneal thickness was higher in G3 (597 ± 101 µm) compared with G0 (541 ± 65 µm) ($P < 0.001$). Pleomorphism and polymegethism were correlated with CG ($P < 0.001$). A progression of CG severity was detected in 13.5% of the cases during a follow-up time of 25.0 ± 19.9 months.

Conclusions: Our study suggested that CG are transplanted in 14.9% of PKP, most of which are low-grade CG not affecting the visual acuity but already leading to an increase in corneal thickness, loss of ECD, and alteration of endothelial cell morphology. In 13.5% of the cases, a progression was demonstrated in the postoperative course.

Key Words: cornea guttata, Fuchs dystrophy, penetrating keratoplasty, corneal transplantation, endothelium

(*Cornea* 2022;00:1–8)

The first description of corneal guttata (CG) traces back to Vogt[1] who detected "drop-like excrescences" in the slitlamp examination in 1921. This pathology appeared on the posterior surface of corneas, most commonly occurring in elderly individuals. CG designates accumulation of basement membrane and fibrillar collagens, resulting in a beaten metal appearance in specular microscopy interrupting endothelial tessellation. When this finding progresses and leads to the development of corneal edema, it typically contributes to Fuchs endothelial dystrophy (FECD) and is not considered as an isolated finding anymore.[2–5]

Guttae, which are the main clinical characteristic of this disease, affect the corneal endothelium which consists of a single layer of mostly hexagonal cells and result in loss of their function. Corneal endothelial cells (CECs) regulate the corneal deturgescence to prevent corneal edema.[6] Transport systems, such as the membrane-bound sodium potassium pump (Na+/K+-ATPase), control the water content of the cells through the active transport of electrolytes and liquids.[2] The loss of the CEC, for example, associated with FECD or with age, leads to alterations in the size (polymegethism) and shape (pleomorphism) of the remaining CECs. Because the endothelial cell density (ECD) declines to a critically low value, the remaining CECs are no longer able to maintain corneal deturgescence, resulting in corneal edema and visual loss.[3,6,7] Widespread guttae on the corneal transplant are therefore associated with increased frequency of graft failure.[8]

FECD was first described by Fuchs in 1910. It is characterized by decreased visual acuity, especially in the morning due to the decreased ability to pump water out of the cornea, leading to the formation of epithelial bullae and increasing corneal edema and opacity at the end-stage of the disease.[9] Despite the available medical therapy that consists of hyperosmolar eye drops that help deswell the cornea, corneal transplantation remains currently the only definitive treatment option for FECD. In the past decades, penetrating keratoplasty (PKP) was the main surgical therapy for FECD, but with the improvement of the minimally invasive posterior lamellar endothelial keratoplasty in 2006, this technique has replaced PKP as the treatment of choice.[10–12] In 2016, FECD was one of the main indications (46%) for keratoplasty in Germany.[13]

CG after PKP is, up to our knowledge, not evaluated in the literature in large studies. Therefore, the purpose of this study was to analyze the prevalence of CG in the transplanted corneal grafts after PKP, determine its severity, and assess its clinical significance.

MATERIALS AND METHODS

In this retrospective study, we analyzed various clinical findings of 1522 patients who underwent 1758 PKP

Received for publication August 8, 2021; revision received November 18, 2021; accepted November 22, 2021.
From the Department of Ophthalmology, Saarland University Medical Center (UKS), Homburg/Saar, Germany.
The authors have no funding or conflicts of interest to disclose.
Correspondence: Silvana Schönit, Saarland University Medical Center, Kirrberger Straße 100, Building 22, Homburg/Saar 66424, Germany (e-mail: Silvana.Schoenit@t-online.de).

performed in the Department of Ophthalmology at the Saarland University Medical Center (UKS, Homburg/Saar, Germany) between January 2009 and September 2019. Follow-up examinations up to September 2020 were included in the analysis. Donor corneas were provided internally by our in-house eye bank, *the Klaus Faber Center for Corneal Diseases incl. LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz*, and externally from multiple other eye banks. The study followed the tenets of the 1964 Declaration of Helsinki and was approved by the Ethics Commission of the German Medical Association (Deutsche Ärztekammer, 13/21).

The only inclusion criterion comprised any patient who underwent a PKP. The exclusion criteria included patients who did not have any analyzable postoperative endothelial pictures and patients lost during the follow-up.

The postoperative endothelial images were taken by specular microscopy (EM-3000; Tomey GmbH, Nagoya, Japan). All images were assessed by experts to determine the presence or absence of CG and its severity grade. A detailed objective CG grading system was used based on the publication of Huang et al[14] but with slight modifications: a fourth stage, including edema, was not entered in the evaluation because of the difficult assessment in specular microscopy. Since there were only 3 stages, the range for each category was expanded. Grade 1 CG indicates that less than 40% (mild) of the surface area of the endothelial image showed guttae. Grade 2 CG showed 40% to 80% (moderate) guttae and grade 3 showed more than 80% (severe) guttae. Figure 1 illustrates examples of the different CG grades post-PKP.

Patients were therefore classified into 4 distinct groups as follows: group 0 (G0) had no CG, group 1 (G1) had mild grade 1 CG, group 2 (G2) had moderate grade 2 CG, and group 3 (G3) had severe grade 3 CG.

The pleomorphism of the cells was described by the percentage of hexagonal CEC in the endothelial images. The polymegethism was represented by the coefficient of variation of the cell area (CV). Both values were assigned by specular microscope.

The uncorrected distance visual acuity (UDVA) and the corrected distance visual acuity (CDVA) were noted in logMAR. Central corneal thickness was measured with the Pentacam Scheimpflug tomography (OCULUS Optikgeräte GmbH, Wetzlar, Germany).

The donor age, the patient's sex and age at the time of surgery, the indication for PKP, and the eye bank origin of the donor corneas were also collected.

Data collection was performed using Microsoft Office Access. Data analysis was performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp, Armonk, NY). Furthermore, a significance level of $P < 0.05$ was set for all statistical tests, including χ^2 tests, post hoc tests, analysis of variance, Cox regression, log-rank test (Mantel–Cox), and *t* test.

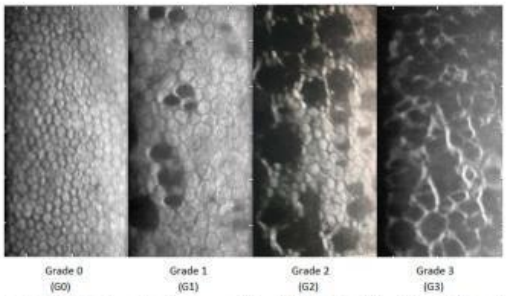


FIGURE 1. Specular images after PKP illustrating the different cornea guttata grades from no CG to grade 3 CG. (The full color version of this figure is available at www.corneajrnl.com.)

RESULTS

In total, 1522 patients were included in this study. Of these patients, 874 (57.4%) were male and 648 (42.6%) were female with a mean patient age of 55.3 ± 19.0 years at the time of surgery. The mean donor age was 66.3 ± 16.4 years. Keratoconus was the leading indication for PKP in 477 cases (27.1%), followed by corneal scars in 230 cases (13.1%), FECD in 200 cases (11.4%), immunological and nonimmunological transplant reaction in 161 cases (9.2%), endothelial decompensation in pseudophakic eyes in 160 cases (9.1%), infectious corneal ulcer in 125 cases (7.1%), high astigmatism in 71 cases (4.0%), corneal dystrophy other than FECD in 60 cases (3.4%), pellucid marginal degeneration in 27 cases (1.5%), ocular trauma in 23 cases (0.6%), and other indications in 224 cases (12.7%). In total, 6662 endothelial specular microscopy images were taken in the context of routine examinations after PKP.

Prevalence and Severity of CG

To determine the prevalence of CG immediately after PKP, we evaluated the first endothelial image within the first 9 months after the operation. In this time interval, 1424 PKP had postoperative endothelial images and therefore were used to calculate the prevalence of CG. In total, 14.9% of the transplanted corneas showed CG. Most of the CG cases were mild with G1 forming 13.6% (n = 193), G2 0.9% (n = 13), and G3 0.4% (n = 6).

The transplanted corneas were subdivided into 2 other groups, group A included grafts from our in-house *Klaus Faber Center for Corneal D. incl. LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz* (74.2%, n = 1056) and group B included grafts originating from various external eye banks (25.8%, n = 368).

Grafts from group A showed significantly less CG with only 13.9% of grafts affected compared with 17.6% of grafts in group B ($P = 0.034$). Severity classification of CG in group A versus B showed 12.7% versus 16.0% for G1, 1.0% versus 0.5% for G2, and 0.2% versus 1.1% for G3, respectively (Fig. 2).

In group B, external eye banks were included. The lowest rates of CG were found to be in eye bank 1 in the Netherlands (10%) and eye bank 2 in Germany (12.5%). The eye bank 3 (Germany) showed the highest percentage of CG

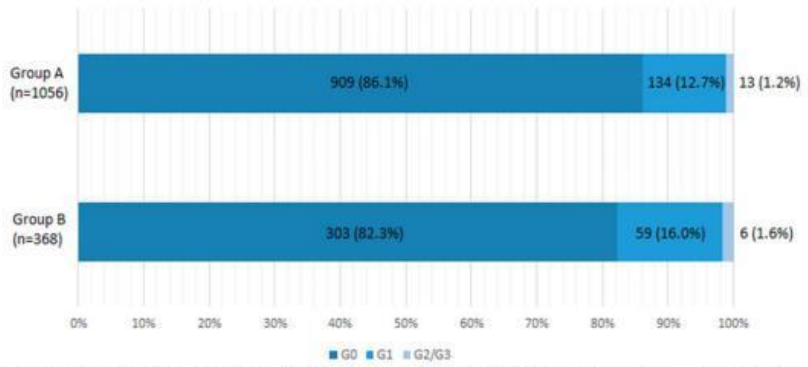


FIGURE 2. Prevalence of different guttae stages in the first examination within 9 months postoperative. Group A (n = 1056) included grafts from our in-house LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz and group B (n = 368) included grafts originating from various external eye banks ($P = 0.034$). G0, guttae grade 0; G1, guttae grade 1; G2, guttae grade 2; G3, guttae grade 3. (The full color version of this figure is available at www.corneajrnl.com.)

reaching 22.2% of the transplanted grafts, followed by eye bank 4 (Germany) with 19.4%. The differences between the eye banks within group B failed to reach a significant level ($P = 0.599$).

Impact of Inflammation

Because of the variant postoperative prevalences in the different eye banks, only the grafts from the eye bank Homburg/Saar were considered for the following prevalence analysis. Furthermore, we wanted to reduce the risk of confounding because of secondary CG caused by inflammation, which is why we created group C (304 operations) including all patients who had already undergone an allograft rejection in the history (89 cases) or in the current clinical course (139 cases) and recipients who had already undergone a PKP (239 cases). Group D (752 operations) consisted of the transplanted grafts without any of the mentioned risk factors. The prevalence of CG in the first examination within 9 months was with 17.4% in group C significantly higher compared with 12.5% in the group D without any risk factors ($P = 0.036$), suggesting the impact of inflammatory processes on the occurrence of CG post-PKP.

Progression of CG

For the analysis of CG progression, the whole population of 1758 PKP was included regardless of the date of the first endothelial examination. The prevalence of CG cases in the first examination was found to be 15.9% of the whole population (including the patients who did not have endothelial images within the first 9 months postoperatively). At the time point of maximum CG expression, 26.8% of the population showed CG. Overall, 69.5% of all CG cases appeared in the first postoperative year.

Progression from postoperative stage G0 to G1 occurred in 165 cases after an average time of 19.8 ± 1.2 months with a 95% confidence interval of 17.4 to 22.2 months postoperatively. Progression from a lower CG stage (G0 and G1) to G2 occurred in 51 cases after 30.3 ± 3.4 months with CI 95% (23.5; 37.0), and progression from a lower CG stage (G0, G1, and G2) to G3 occurred in 21 cases after an average time of 37.9 ± 6.0 months with CI 95% (26.1; 49.6) ($P < 0.001$). The progression is shown in Figure 3 as a Kaplan–Meier curve.

In total, 237 cases (13.5%) showed a progression either from no CG to the appearance of CG or from a lower to a higher grade of CG. By contrast, 1521 cases (86.5%) remained stable without progressing throughout the entire clinical course of 25.0 ± 19.9 months. Forty-four of the 279 cases (15.8%) that showed CG immediately underwent progression to higher stages. Not all high-graded CG showed expected intermediate stages in previous examinations.

Figure 4 illustrates the follow-up endothelial images of a patient who progressed from G1 to G3.

FECD and Postoperative CG

To check whether patients diagnosed with FECD had an increased risk of postoperative CG, we divided the cases of CG into 2 groups, patients diagnosed preoperatively with FECD (11.4%) and patients with other PKP indications (88.6%) and compared the 2 groups with a χ^2 test.

The prevalence at the time point of maximum CG expression differed in the group with preoperative diagnosis of FECD (31.5%) and the group of patients with other PKP indications (26.3%), but without a statistically significant difference ($P = 0.297$). Survival analysis with the log-rank test also showed no statistically significant differences ($P = 0.944$).

Impact of Age and Sex

The impact of the donor age on the CG prevalence and severity was also determined at the time point of maximum CG expression. For the analysis, we used the true donor

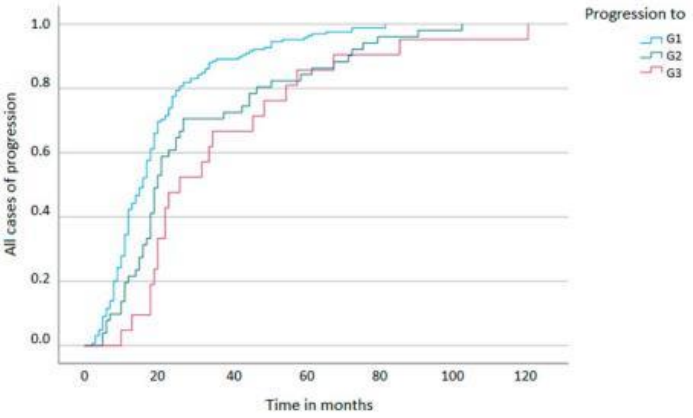


FIGURE 3. Kaplan–Meier curve shows all cases of progression recorded during the follow-up. The blue curve shows the progression from a lower guttae grade 0 (G0) to grade 1 (G1) in 165 cases, the green curve shows the progression from a lower guttae stage (G0 and G1) to grade 2 (G2) in 51 cases, and the red curve shows the progression from a lower guttae stage (G0, G1, and G2) to G3 in 21 cases ($P < 0.001$). (The full color version of this figure is available at www.corneajrnl.com.)

endothelium age which was determined as the donor age at the time of transplantation plus the postoperative time. The mean donor age in G0 CG was 67.2 ± 16.6 years, in contrast to 70.7 ± 15.4 years in the grafts with CG (G1, G2, and G3), and the difference was statistically significant using the *t* test ($P < 0.001$) and could be confirmed by using a Cox regression ($P = 0.002$; B = 0.004; SE = 0.001).

Furthermore, the mean donor age was 69.6 ± 16.1 years in mildly affected transplants with G1 CG, 74.7 ± 12.7 years in grafts with G2 CG, and 74.8 ± 9.3 years in G3 CG ($P = 0.002$).

The mean patient's age in G0 CG was 55.9 ± 33.1 years at the time of maximum expression and 58.9 ± 17.1 years in grafts with CG, revealing a significant impact of a higher patient's age on the prevalence of CG using the *t* test ($P = 0.012$). This significant difference was confirmed using Cox regression ($P = 0.015$; B = 0.006; SE = 0.003).

Female patients (28.2%) showed slightly higher percentages for CG than males (25.9%) at the point of maximum expression, but this difference was neither statistically significant at the mentioned time point ($P = 0.744$) nor on a survival analysis using the log-rank test ($P = 0.744$).

Clinical Impact of CG

We explored the influence of CG severity on different clinical parameters. The corresponding values were assigned to group G0, G1, G2, or G3 as mean of all the values recorded during the follow-up examinations. The mean values of the different groups were compared using post hoc tests.

No statistically significant difference was found in the mean UDVA in logMAR in G0 CG (0.84 ± 0.41) compared with G1 CG (0.83 ± 0.41) ($P = 0.72$). However, the visual acuity deteriorated to reach 1.1 ± 0.52 in the eyes with G3 CG, which was significantly worse than G0 CG ($P = 0.002$) (Fig. 5). The CDVA was 0.46 ± 0.31 in G0 CG and 0.45 ± 0.31 in G1 CG with a statistically insignificant difference ($P = 0.33$). CDVA increased to 0.51 ± 0.36 in G2 CG ($P = 0.84$) and 0.57 ± 0.32 in G3 CG ($P = 0.17$) but without statistical significance compared with G0 CG.

The mean central corneal thickness of the eyes with G0 CG was 541.4 ± 65.8 µm. This value was compared with G1, G2, and G3 CG: G1 CG had a thickness of 547.7± 64.5 µm ($P = 0.03$), G2 CG 582.7± 69.3 µm ($P < 0.001$), and G3 CG 597.5± 101.0 µm ($P < 0.001$).

ECD was 1802.3 ± 459.1 per mm² in G0 CG showing a statistically significant decrease to 1672.0 ± 446 per mm² in G1 CG ($P < 0.001$), 1689.0 ± 451.2 in G2 CG ($P = 0.033$), and 1639.4 ± 417.0 in G3 CG ($P = 0.055$).

CV increased significantly from a mean of 46.2 ± 14.5 in G0 CG, to 50.1 ± 16.8 in G1 CG, to 57.1 ± 16.2 in G2 CG, and reaching 65.4 ± 18.3 in G3 ($P < 0.001$).

The percentage of hexagonal cells in G0 CG was 40.3 ± 17.6%, this value significantly decreased to 36.6 ± 20.5 in G1, 30.2 ± 23.0 in G2, and 22.1 ± 29 in G3 compared with G0 ($P < 0.001$). Table 1 summarizes the impact of CG stages on the relevant clinical parameters.

Impact of Guttae on the Graft Survival

To investigate the impact of postoperative CG on the graft survival, the study collective was divided into 2 groups, 1 with CG (n = 212) and 1 without CG (n = 1212) in the first examination within 9 months. The overall graft survival rate at 36 months was 90.5% showing no significant difference between the group with CG (89.6%) and the group without CG (90.6%) using the log-rank test ($P = 0.591$). Furthermore, the survival of grafts with expression G0 or G1 CG was compared with grafts showing G2 or G3 CG in the first examination. In the G0/G1 group (n = 1405), the 36-month survival (90.6%) did not differ significantly ($P = 0.269$) from the G2/G3 group (84.2%, n = 19).

DISCUSSION

Our study showed a prevalence of 14.9% of CG after PKP. To the best of our knowledge, only 1 study by Nahum

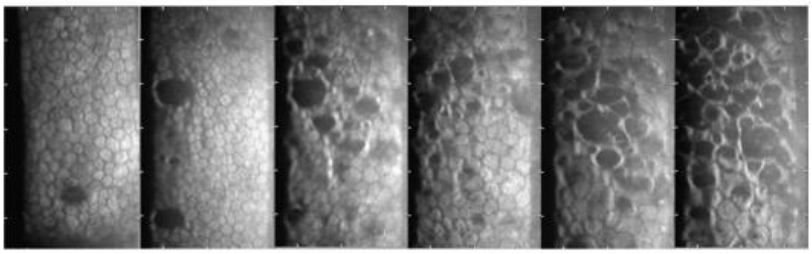


FIGURE 4. Clinical course illustrated with specular microscopy images of a male patient who underwent PKP in July 2014 at the age of 65 years. CG occurred for the first time 6 months after PKP and progressed during the follow-up from G1 in January 2015 to G3 in March 2020. G1, guttae grade 1; G3, guttae grade 3.

et al[15] examined the rate of CG after PKP, and their results showed a value of 10.5%, which was in line with our results. The study by Nahum et al[15] had 2 limitations. First, it included only 19 patients who underwent PKP, of which 2 had CG, and second, CG severity was not objectively assessed, it was only classified as isolated guttae and patches of CG.

Approximately 70% of all CG cases appeared within the first year after PKP, indicating that guttae were already present in the donor cornea at the time of the operation. However, despite the strict prescribed quality control measures by microscopic imaging of the endothelial layer and using the slit lamp to detect visible endothelial pathologies (eg, stromal opacities located on the central cornea), both low-grade and high-grade CG cases occur after PKP. The guidelines in Germany uniformly regulate the preoperative procedures and the standard values for the exclusion of donor corneas for transplantation, with a low ECD (<2000 cells/mm^2) or obviously altered morphology (eg, grafts with large central endothelial cell necrosis, severe pleomorphism or polymegethism, and high-grade vacuolization of endothelial cells).[16] Still, the risk for the appearance of postoperative CG varies in different eye banks as evidenced by the differing prevalence of postoperative CG from different eye banks. Kramp et al[17] found that only 46.3% of donor grafts at *LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz* were suitable for all types of keratoplasty. Most of the donor corneas were unsuitable for surgical use because of inadequate endothelial quality. It was also found that a high percentage of the discarded grafts were associated with a high donor age (>80 yrs).[17–19]

The occurrence of CG immediately after PKP suggest that guttae cannot always be reliably detected in the eye bank with the inverted light microscope. However, according to a study performed by Safi et al,[20] several morphological criteria that can be preoperatively detected using inverted light microscopy correlate with the appearance of postoperative CG. These criteria include the presence of blebs, cell

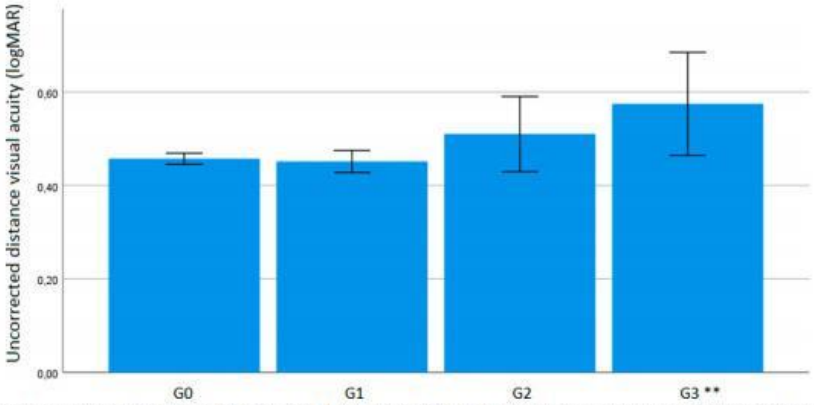


FIGURE 5. Impact of guttae severity grade on mean UDVA. A significant decrease in visual acuity was shown only for grade 3 CG compared with the group without CG. G0, guttae grade 0; G1, guttae grade 1; G2, guttae grade 2; G3, guttae grade 3, $^{**}P < 0.01$. (The full color version of this figure is available at www.corneajrnl.com.)

TABLE 1. Impact of Guttae Severity on Different Clinical Parameters

Clinical Parameter	G0	G1	G2	G3
Uncorrected visual acuity (logMAR)	0.84 ± 0.41	0.83 ± 0.41	0.86 ± 0.40	1.1 ± 0.52**
Best-corrected visual acuity (logMAR)	0.46 ± 0.31	0.45 ± 0.31	0.51 ± 0.36	0.57 ± 0.32
Density of endothelial cells (N/mm^2)	1802.3 ± 459.1	1672.0 ± 446.0***	1689.0 ± 451.2*	1639.4 ± 417.0
Corneal thickness at pupil center (µm)	541.4 ± 65.8	547.7 ± 64.5*	582.7 ± 69.3***	597.5 ± 101.0***
Coefficient of variation (polymegalism)	46.2 ± 14.5	50.1 ± 16.8***	57.1 ± 16.2***	65.4 ± 18.3***
Percentage of hexagonal-shaped cells in % (pleomorphism)	40.3 ± 17.6	36.6 ± 20.5***	30.2 ± 23.0***	22.1 ± 29.1***

G0, guttae grade 0; G1, guttae grade 1; G2, guttae grade 2; G3, guttae grade 3.
P value determined in comparison with G0: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

membrane defects and interruptions, and a low percentage of hexagonal and circular cells (<50%) in the donor endothelium.[20]

Furthermore, secondary CG cases could appear in the postoperative course in a previously healthy donor cornea because of surgical stress or damage to the endothelial cells, inflammatory process, or as an interaction of the graft with the unfamiliar environment in the patient's eye.[21]

Around 30% of CG cases were first detected after the first postoperative year, questioning the origin of these "late-appearing CG" cases. Although until now there is no definitive answer to this question, many possible explanations might clarify this phenomenon. 1) Since the postoperative microscopy images of the cornea did not examine the entire surface area of the cornea, but rather only a section of 0.25×0.54 mm^2, guttae may have been present immediately after PKP but simply have been missed during the first endothelial examinations. The implication of these assumptions is that the percentage of CG after PKP may in fact be higher than demonstrated in this study. 2) Since approximately 10% of CG did not appear until the third postoperative year, the origin of such cases might be a primary result of the natural aging process because CG occurs more often in older ages as demonstrated in several studies.[22,23] 3) The intraoperative and postoperative interactions between the donor graft and the recipient, including mild inflammatory reactions, could possibly lead to damages in the endothelial layer in the long term and thus the formation of CG. As evidenced by our prevalence analysis, inflammatory processes seem to affect the postoperative occurrence of guttae.

The influence of the preoperative diagnosis of FECD on the appearance of CG post-PKP was also analyzed and showed no increased risk of postoperative CG in patients diagnosed preoperatively with FECD. These results speak against a recurrence of FECD on the transplanted graft.

In our study, in most of corneas post-PKP (86.5%), no progression or late-onset CG was demonstrated over a period of 25.0 ± 19.9 months postoperatively. The Kaplan–Meier curve showed that a progression to a higher guttae stage took longer than a progression to a low-grade stage. The overlap of the G2 and G3 curves and the observed jumps between 2 CG grades with omission of an intermediate stage could be explained by the fact that some patients skipped a few follow-up examinations. Another possible explanation could be that only a low-grade affected area was detected in the previous endothelial image, whereas actually more severely affected areas had already been present in other unexamined areas.

Kitagawa et al[24] detected CG in 6.7% of a Singaporean population and significantly lower rates in a Japanese population (3.7%). Zoega et al[25] determined a prevalence of 15% to 23% CG in a cohort study of a White population. The 2 studies[24,25] were performed on populations over the age of 50 years and both showed higher prevalence of CG in female individuals. Higa et al[22] confirmed that female sex and older age are risk factors for the development of CG. Our study showed no correlation between the patient's sex and CG but a correlation between a higher patient's age and CG post-PKP. Considering donor age, a higher age was not only associated with a higher risk of post-PKP CG in general but also with a higher rate of high-graded CG in particular.

Moreover, our study suggested that the occurrence of CG leads to a worsening of several clinical parameters. The association of CG with decreased ECD could be demonstrated in several studies.[8,26,27] On the other hand, Nahum et al[15] did not determine significantly lower postoperative CEC in guttae-affected grafts after different keratoplasty procedures during the first 2 postoperative years. Our study showed a loss of CEC in corneas with G1 and G2 CG compared with the unaffected G0 CG corneas. Based on our CG grading system, an even lower ECD should be expected for G3 CG. However, this decrease could not be confirmed as statistically significant, probably because of the decreased precision of the specular microscope in counting the ECD in images containing large dark areas attributed to widespread CG. Nevertheless, this ECD decrease associated with a progression of CG grade was actually demonstrated in a study by Jackson et al[26] who found an inverse correlation between the number of guttae and ECD.

The visual acuity is known to be worse in patients with FECD.[9,28] Watanabe et al[29] detected a decreasing visual acuity with increasing guttae density in the endothelial images of patients with FECD. According to their study,[29] guttae increased intraocular forward light scattering leading to visual deterioration. This would be consistent with our observations showing a decrease in visual acuity at least in the advanced stages of CG, but only significant when measuring the uncorrected visual acuity. On the other hand,

Nahum et al[15] did not find any impact of CG on the visual acuity in the first 2 postoperative years in a population consisting mainly of mildly affected grafts. This study was also consistent with our results that showed no decrease in the visual acuity in G1 CG.

Huang et al[14] and Watanabe et al[29] showed a correlation between density of guttae and increasing central corneal thickness, which was compatible with our results. In our study, corneal thickness increased significantly with increasing grades of CG.

The increase in pleomorphism and polymegethism in patients with CG was demonstrated by Chiou et al.[4] In our study, CV, describing polymegethism, increased significantly from 46.2 ± 14.5 in G0 to 65.4 ± 18.3 in G3 and the percentage of hexagonal cells decreased significantly from 40.3% ± 17.6% in G0 to 22.1% ± 29.1% in G3. These results suggest that polymegethism and pleomorphism increased with worsening of the CG grade.

Our survival analysis was able to substantiate the statement of Borderie et al[8] showing that guttae generally do not affect the graft survival at the 36-month time point. A possible higher graft failure in high-graded guttae could not be demonstrated in a statistically significant way but would be consistent with our observations. The higher graft survival (90.5%) compared with the study by Borderie et al[8] (79.4%) may be due to the inclusion criteria of our study, which included only patients in whom postoperative endothelial images could be analyzed.

One of the limitations in our study was that the small surface area of the endothelial images captured and analyzed using a specular microscope. This might have led to false-negative results because guttae may have been absent in the small part of the cornea shown in the endothelial image but present in other areas of the cornea. To minimize the effect of this potential problem, all available images for a single cornea were considered for analysis and the endothelial images were taken in the central cornea as accurately as possible, where CG typically appears initially.[23,27] Another limitation was that the decreased precision of the computer-generated cell count in specular microscopy analysis of the endothelial images comprising large areas of guttae. Furthermore, the subjective refraction was performed by resident physicians during regular outpatient service and not by an optometrist in the setting of a prospective study. In addition, the data analysis was retrospective, and we included only spectacle-corrected visual acuity. Moreover, we had slightly less data available for the best-corrected visual acuity than for uncorrected visual acuity at stage G3, which might be the reason for the difference in statistical significance.

In conclusion, our study performed on 1758 PKP, including 6662 postoperative endothelial specular microscopy images, suggests that donor corneas containing CG are transplanted in 14.9% of PKP. Most of the CG are low-grade and do not seem to have an important clinical significance other than a mild increase in corneal thickness, loss of ECD, and an altered morphology of CEC. In addition, a progression of the CG grade could be demonstrated in 13.5% of the cases throughout an average of 25 months follow-up. Higher donor age was associated with higher risk for post-PKP CG in general and high-grade CG in particular. Further investigations are essential to establish a standardized method for the detection of high-grade CG in the eye bank preoperatively.

ACKNOWLEDGMENTS

We thank Mrs Christina Turner for her linguistic additions and corrections in this manuscript.

REFERENCES

1. Vogt A. Weitere Ergebnisse der Spaltlampenmikroskopie des vorderen Bulbusabschnittes: (Cornea, Vorderkammer, Iris, Linse, vorderer Glaskörper, Conjunctiva, Lidränder.) I. Abschnitt: Hornhaut. *Graefes Arch Clin Exp Ophthalmol*. 1921;106:63–103.
2. Adamis AP, Filatov V, Tripathi BJ, et al. Fuchs' endothelial dystrophy of the cornea. *Surv Ophthalmol*. 1993;38:149–168.
3. Wilson SE, Bourne WM. Fuchs' dystrophy. *Cornea*. 1988;7:2–18.
4. Chiou A, Kaufman S, Beuerman R, et al. Confocal microscopy in cornea guttata and Fuchs' endothelial dystrophy. *Br J Ophthalmol*. 1999;83:185–189.
5. Weiss JS, Møller HU, Aldave AJ, et al. IC3D classification of corneal dystrophies: edition 2. *Cornea*. 2015;34:117–159.
6. Borboli S, Colby K. Mechanisms of disease: Fuchs' endothelial dystrophy. *Ophthalmol Clin North Am*. 2002;15:17–25.
7. Yee RW, Matsuda M, Schultz RO, et al. Changes in the normal corneal endothelial cellular pattern as a function of age. *Curr Eye Res*. 1985;4:671–678.
8. Borderie VM, Sabolic V, Touzeau O, et al. Screening human donor corneas during organ culture for the presence of guttae. *Br J Ophthalmol*. 2001;85:272–276.
9. Fuchs E. Dystrophia epithelialis corneae. *Graefes Arch Clin Exp Ophthalmol*. 1910;76:478–508.
10. Tan DT, Dart JK, Holland EJ, et al. Corneal transplantation. *Lancet*. 2012;379:1749–1761.
11. Röck T, Landenberger J, Bramkamp M, et al. The evolution of corneal transplantation. *Ann Transpl*. 2017;22:749–754.
12. Seitz B, Daas L, Flockerzi E, et al. Descemet membrane endothelial keratoplasty DMEK–donor and recipient step by step [in German]. *Ophthalmologe*. 2020;117:811–828.
13. Flockerzi E, Maier P, Böhringer D, et al. Trends in corneal transplantation from 2001 to 2016 in Germany: a report of the DOG-Section cornea and its keratoplasty registry. *Am J Ophthalmol*. 2018;188:91–98.
14. Huang J, Tepelus TC, Baghdasaryan E, et al. Correlation between guttata severity and thickness of Descemet's membrane and the central cornea. *Curr Eye Res*. 2019;44:849–855.
15. Nahum Y, Canton V, Ponzin D, et al. Prevalence of guttae in the graft following corneal transplantation. *Br J Ophthalmol*. 2015;99:1660–1663.
16. Schroeter J, Maier P, Bednarz J, et al. Procedural guidelines. Good tissue practice for cornea banks [in german]. *Ophthalmologe*. 2009;106:265–276.
17. Kramp K, Suffo S, Laun D, et al. Analysis of factors influencing the suitability of donor corneas in the LIONS cornea bank Saar-Lor-Lux, Trier/Westpfalz from 2006 to 2016 [in German]. *Klin Monatsbl Augenheilkd*. 2020;237:1334–1342.
18. Martin C, Tschernig T, Loic H, et al. Corneae from body donors in anatomy department: valuable use for clinical transplantation and experimental research. *BMC Ophthalmol*. 2020;20:284.
19. Laun D, Suffo S, Kramp K, et al. How implementing a quality management system at the LIONS eye bank Saar-Lor-Lux, Trier/Western Palatinate from 2006 to 2016 impacted the rate and reasons for discarding human organ-cultured corneas [in German]. *Klin Monatsbl Augenheilkd*. 2021 [epub ahead of print].
20. Safi T, Daas L, Alexandersson J, et al. Semi-quantitative criteria in the eye bank that correlate with cornea guttata in donor corneas. *Klin Monatsbl Augenheilkd*. 2021;238:680–687.
21. Kitagawa K, Fujisawa A, Mizuno T, et al. Twenty-three cases of primary cornea guttata. *Jpn J Ophthalmol*. 2001;45:93–98.

22. Higa A, Sakai H, Sawaguchi S, et al. Prevalence of and risk factors for cornea guttata in a population-based study in a southwestern island of Japan: the Kumejima study. *Arch Ophthalmol*. 2011;129:332–336.
23. Lorenzetti DW, Uotila MH, Parikh N, et al. Central cornea guttata. Incidence in the general population. *Am J Ophthalmol*. 1967;64:1155–1158.
24. Kitagawa K, Kojima M, Sasaki H, et al. Prevalence of primary cornea guttata and morphology of corneal endothelium in aging Japanese and Singaporean subjects. *Ophthalmic Res*. 2002;34:135–138.
25. Zoega GM, Arnarsson A, Sasaki H, et al. The 7-year cumulative incidence of cornea guttata and morphological changes in the corneal endothelium in the Reykjavik Eye Study. *Acta Ophthalmol*. 2013;91:212–218.
26. Jackson AJ, Robinson FO, Frazer DG, et al. Corneal guttata: a comparative clinical and specular micrographic study. *Eye (Lond)*. 1999;13(pt 6):737–743.
27. Giasson CJ, Solomon LD, Polse KA. Morphometry of corneal endothelium in patients with corneal guttata. *Ophthalmology*. 2007;114:1469–1475.
28. Wacker K, Grewing V, Fritz M, et al. Morphological and optical determinants of visual disability in Fuchs endothelial corneal dystrophy. *Cornea*. 2020;39:726–731.
29. Watanabe S, Oie Y, Fujimoto H, et al. Relationship between corneal guttae and quality of vision in patients with mild Fuchs' endothelial corneal dystrophy. *Ophthalmology*. 2015;122:2103–2109.

5.3 Publication 3:

Acta Ophthalmologica

ACTA OPHTHALMOLOGICA 2022

Prevalence and severity of cornea guttata in the graft following Descemet Membrane Endothelial Keratoplasty (DMEK)

Lena-Marie Schmitz, Tarek Safi, Cristian Munteanu, Berthold Seitz and Loay Daas

Department of Ophthalmology, Saarland University Medical Center (UKS), Homburg/Saar, Germany

ABSTRACT.

Purpose: **The aim of this study was to determine the prevalence and severity of cornea guttata (CG) in grafts after Descemet membrane endothelial keratoplasty (DMEK) and to investigate its impact on various clinical parameters during follow-up.**

Methods: **This retrospective study included 664 operations (DMEK and triple-DMEK) on 466 patients. The prevalence and progression of CG after the operation were examined using endothelial specular microscopy images. The severity grade of CG was classified into four grades: G0 without CG, G1 – G3 with increasing severity of CG. Clinical parameters such as central corneal thickness (CCT), visual acuity (VA), endothelial cell density (ECD), pleomorphism and polymegalism were examined during a postoperative follow-up time of 19.6 ± 15.8 months.**

Results: **Cornea guttata (CG) appeared postoperatively in 124 (18.7%) eyes. 112 (16.9%) could be classified as G1, 9 (1.4%) as G2 and only 3 (0.5%) as G3. The examination of clinical parameters showed significant differences between healthy and low-grade CG (G0/G1) and high-grade CG (G2/G3). A significant deterioration was found in the corrected distance visual acuity (CDVA) (p = 0.02). CCT showed an increase between G0 (534 ± 58 µm) and G2 (549 ± 71 µm)/G3 (558 ± 56 µm) with a p-value of 0.02. Additionally, a significant increase in pleomorphism (p = 0.003) and polymegalism (p = 0.04) was detected.**

Conclusion: **Cornea guttata (CG) prevalence after DMEK and triple-DMEK was found to be 18.7%, although most of these cases were classified as low-grade CG and showed no clinical significance. Around 1.9% were classified as high-grade CG and significantly affected several clinical parameters during the follow-up.**

Key words: cornea – cornea guttata – corneal dystrophy – DMEK – keratoplasty – specular microscopy

We would like to thank Mrs. Christina Turner for her linguistic additions and corrections in this manuscript.

Acta Ophthalmol.

doi: 10.1111/aos.15195

Introduction

The droplet-like changes in the posterior parts of the cornea known as 'cornea guttata' (CG, gutta = droplet) were first discovered and described by Vogt in 1921 (Vogt 1921; Son et al. 2014). Guttae represent accumulations and depositions of collagen and fibril fibers in the Descemet membrane, interrupting the tight connections of the endothelial cells. It is a commonly observed clinical finding in the slit lamp examination, especially in older patients. The appearance of the endothelium in the slit lamp can be described as 'hammered' metal (Lisch & Seitz 2012). It is frequently manifested as isolated guttae but also occurs in association with a genetically determined corneal dystrophy called Fuchs' endothelial corneal dystrophy (FECD). For an objective detection of the guttae, a non-contact specular microscopy can be used (McCarey et al. 2008). They could be identified as focally demarcated dark spots, which break through the endothelial cell layer (Seitz et al. 1997). The endothelial cells provide a barrier function between the anterior chamber of the eye and the cornea. Through membrane-bound Na^+-K^+-ATPases, the endothelium regulates the outflow of aqueous humour from the stroma (Bonanno 2012). In case of disease progression, it leads to an endothelial dysfunction associated with cell loss, morphological changes in size (polymegalism) and shape (pleomorphism) (Geroski et al. 1985; McCarey et al. 2008; Feizi 2018). It causes an oedematous swelling of the cornea, which is associated with a reduction in transparency, especially in the morning hours. From that point on, it is called a clinically manifested FECD (Fuchs 1910; Weiss et al. 2015; Wacker et al. 2019). The only curative therapy for FECD is corneal

transplantation. Today, the treatment of choice is Descemet membrane endothelial keratoplasty (DMEK) (Tan et al. 2012; Röck et al. 2017). This minimal invasive surgical technique, in which a lamella consisting only of the endothelium and the Descemet membrane is transplanted, was first published by Melles et al. (1998)). Since 2014, more than 50% of all keratoplasties in Germany have been performed as posterior lamellar keratoplasty (Flockerzi et al. 2018; Seitz et al. 2020). With the new triple procedure, cataract surgery can be performed simultaneously during DMEK (Schmidt et al. 2019).

Donor corneas that are suitable for a transplantation are preoperatively examined for possible pathologies and anomalies using the inverted light microscopy and the optical coherence tomography (Quintin et al. 2021). As per our in-house eye bank quality management protocol, the first step, is an examination of the cornea using a slit lamp microscope. The entire cornea is illuminated with different illumination directions by moving the slit lamp biomicroscope to detect stromal opacities and defects that are optically relevant (*e.g.* scars due to injuries) or stromal changes caused by infectious genesis (*e.g.* exposure keratopathy and scar after herpetic keratitis). Subsequent observations in so-called regressive light allow the assessment of the overall transparency of the corneal tissue. In addition, although very difficult to detect, the endothelial cell layer is also examined for the presence of guttae.

Next, an accurate evaluation of the donor endothelium is performed with an inverted light microscope. Each cornea is examined carefully in the centre as well as in the four peripheral quadrants. Prerequisites for a DMEK-graft is an endothelial cell density of ≥2200 cells/mm^2. In addition, the morphology of the cells is examined and analysed through the microscope focusing on the hexagonality and the size of the cells. The presence of necrotic and cell depleted surfaces is also taken into consideration as an important factor in the assessment of the donor corneas. Despite the strict quality controls, which prescribe and require a careful examination, CG can still be found on the grafts after transplantation. The presence of CG, depending on the size of the affected area in the grafts, is associated with a decrease in ECD, resulting in reduced graft survival (Borderie et al. 2001).

Up to our knowledge, there is a gap in the literature investigating this topic with large studies. Therefore, the purpose of this study was to assess the prevalence and clinical significance of CG in transplanted corneas post DMEK.

Materials and methods

Population

In this retrospective study, the medical records of 664 DMEK and triple-DMEK performed on 466 patients at the Department of Ophthalmology, Saarland University Medical Center (UKS, Homburg/Saar, Germany), were included. The inclusion criterion was any patient who underwent DMEK or triple-DMEK. Exclusion criteria included patients who did not have any analysable postoperative endothelial pictures or who had no postoperative follow-up examinations. Out of 710 surgeries performed during the study period, 46 surgeries could not be included in the study because of the exclusion criteria described above. The minimum follow-up time that could be included was 11 days. The maximum observation time was 83.2 months with a median of 14.4 months.

The study followed the tenets of the 1964 Declaration of Helsinki and was approved by the Ethics Commission of the German Medical Association (Identification Number: BU217/20).

Data collection

Postoperative endothelial cell images were taken using a non-contact specular microscope (EM-3000 ©; Tomey GmbH, Erlangen, Germany) at defined time intervals (T1 = 6 weeks, T2 = 6 months, T3 = 12 months, T4 = 2 years, T5 > 3 years) (Table 1) during the postoperative follow-up examinations. As a part of the assessment of the clinical significance of CG, corrected distance visual acuity (CDVA) in logMAR was recorded. To investigate the thickness of the cornea, the pachymetry in the area of the central corneal thickness (CCT) was determined with a non-contact specular microscope. The ECD, the percentage of hexagonal cells (6A) as a representation of pleomorphism and the cell variation coefficient (CV) as a representation of polymegalism were all automatically analysed and calculated by the above mentioned specular microscope directly after the endothelial images were taken.

As it is difficult to determine an optimal time-point for the analysis of the different clinical parameters related to the severity of CG, all follow-up examinations were included to obtain a larger number of clinical parameters per severity and to take into consideration their development process in the postoperative course. In each case, the respective classification of the CG at the time-point of examination was considered. A mean follow-up time of 20.1 ± 15.8 months could be determined.

The age of the donor at the time of donation and the age of the recipient at the time of surgery were also recorded. For this purpose, patients and donors were divided into 3 different groups (<60 years old (y), 60–80 y and >80 y). In this case, the final CG stage at the last included examination was used as a reference to show the maximum expression in relation to the age of donor and recipient.

CG grading system

The severity of postoperative CG was divided into four categories classified by using endothelial specular microscopy images. The evaluation of the endothelial images was performed by an experienced examiner from our

Table 1. Number of examinations per time-point (T1-T5) during the follow-up.

	T1	T2	T3	T4	T5
Corrected distance visual acuity (logMAR)	584	456	526	494	416
Density of endothelial cells (N/mm^2)	600	467	556	506	436
Corneal thickness at pupil centre (µm)	497	383	480	421	376
Coefficient of Variation (polymegalism)	600	467	556	506	436
Percentage of hexagonal-shaped cells in % (pleomorphism)	345	317	446	391	330

T = time of examination (T1 = 6 weeks, T2 = 6 months, T3 = 1 year, T4 = 2 years, T5 = +3 years).

ophthalmic clinic and was standardized with the aid of the predefined classification system. The estimation of the guttae covered area was calculated using an enhanced image-analysis software titled 'Fiji' on the open source 'ImageJ'. Grade 0 (G0) described a healthy cornea in which no CG was detected. In grade 1 (G1), less than 40% of the observed area was affected by CG. As G1 represents only a mild form of the disease with no clinical significance, we grouped G1 with G0 as 'healthy and low-grade CG' for the purpose of comparison with the more advanced form of the disease. Grade 2 (G2) showed an area of 40%–80% and Grade 3 (G3) described the highest grade with an area of more than 80% affected by CG. G2 and G3 were considered as high-grade CG. This classification was based on a publication from 2019 (Huang et al. 2019). Grade 1 and 2 used by Huang *et al.*, which were characterized by isolated and mild CG covering <20% and 20%–40% surface area, respectively, were merged in our study into one grade (G1) since they probably show the same clinical characteristics (Table 2).

All statistical analysis were performed using IBM® SPSS® Statistics (Version 22, International Business Machines Corporation (IBM), Armonk, New York, USA). In order to determine the time of onset of CG, the Kaplan–Meier survival analysis was executed. To investigate the significance between the target variables and CG grades chi-squared tests, post hoc tests and analysis of variance (ANOVA) were used. A p-level of <0.05 was used for all statistical tests.

Results

In total 664 surgeries were performed on 633 eyes, out of which 314 (49.6%) were left eyes and 319 (50.4%) were right eyes. Out of the total 664 surgeries performed on 633 eyes, 31 re-DMEKs were performed during our study period due to graft failure or rejection and were also included in the analysis. 218 (46.8%) of the patients were male and 248 (53.2%) were female with an average age of 69.1 ± 9.5 years on the day of surgery. Out of the total population 559 (84.2%) donor corneas were provided by our in-house *Klaus Faber Center for Corneal Diseases incl. LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz* and 105 (15.8%) corneas from various external eye banks (Mainz, Rostock and the German Society for Tissue Transplantation (DGFG)). The main indication for surgery was FECD with 94.9%. The follow-up time of all patients included in the study was 19.6 ± 15.8 months.

Prevalence and severity of guttae

In order to determine the prevalence of CG immediately postoperatively, the first postoperative endothelial examination was considered. It was found that 540 (81.3%) corneas belonged to G0. CG appeared in 124 (18.7%) eyes after an average time of 1.25 ± 2.2 months (Fig. 1). A total of 379 (57.1%) eyes underwent DMEK and 285 (42.9%) eyes underwent triple-DMEK. The prevalence of CG in DMEK was 75 (19.9%) versus 49 (17.2%) in triple-DMEK (p = 0.7). We could classify 112 (16.9%) as G1, 9 (1.4%) as G2 and 3 (0.5%) as G3.

Age and CG

To determine the influence of donor and recipient age on prevalence and severity, the status of the last examination with the maximum expression of CG was chosen.

The mean age of the patients with G0 at the time of surgery was 68.8 ± 9.4 years (median 69 years). In contrast, patients with CG had a mean age of 69.3 ± 9.3 years (median 70 years) with no statistical significance between the 2 groups (p = 1.0). Divided into 3 different age groups, 119 (17.9%) patients were under 60 y, 484 (72.9%) were 60–80 y, and 61 (9.2%) were over 80 y on the day of surgery. The prevalence of CG was 27 (22.7%) in the first, 112 (23.1%) in the second and 12 (19.7%) in the last group. There was no statistically significant difference in the prevalence of CG between the different age groups (p = 0.9) and the severity of the CG (p = 0.8, chi-square test) (Table 3).

In 658 cases, the age of the donor cornea could be traced, 49 (7.5%) donor corneas were aged under 60 y at the time of the donation, 402 (61.1%) were 60–80 y, and 207 (31.5%) were over 80 y. The percentage of CG in the donor corneas under 60 y, between 60 and 80 y and over 80 y was 22.4%, 20.2% and 28.5% respectively. Even if the prevalence was higher in the older ages, the differences failed to reach statistical significance (p = 0.2). Furthermore, there was no significant impact of donors age on the severity of CG (p = 0.7, chi-square test) (Table 4).

Progression of postoperative CG

To investigate the progression course of CG in the donor grafts, all the postoperative endothelial images were analysed and compared with the first one to screen for new emerging CG cases and for progression of a previously present minor CG. Figure 2 shows the Kaplan–Meier curves that represents all cases of progression, divided according to the maximum expression. The continuous curve indicates the appearance of new low-grade guttae (G1) on initially healthy corneas (G0) in 24 cases after an average time of 20.5 ± 2.1 months since the initial diagnosis. The dotted curve shows the progression of a healthy or low-grade CG G0/G1 to a higher grade CG G2 in 15 cases after an average time of 31.6 ± 3.9 months. The dashed curve presents the progression of guttae G0/G1/G2 to the highest stage of CG G3 in 17 cases after an average time of 37.1 ± 4.0 months. A statistical significance was found between the different time intervals of progression (p < 0,001).

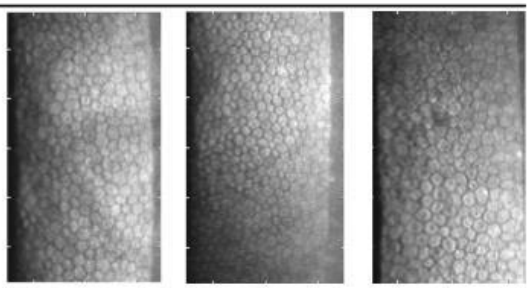
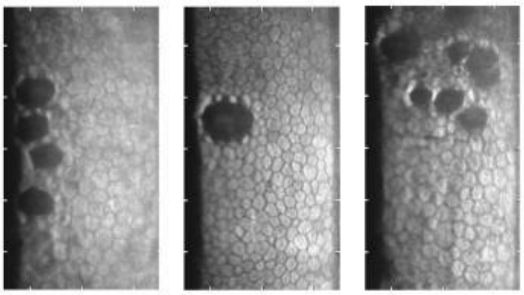
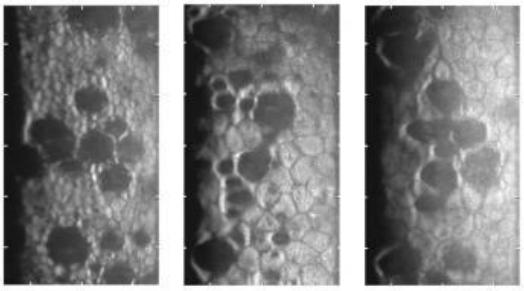
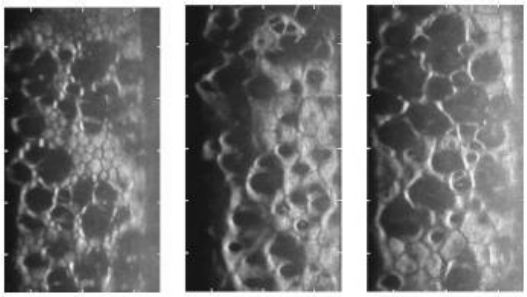
Overall, 56 (8.5%) cases showed a progression either from healthy corneas with G0 to corneas with any grade of CG or from a low-grade to a high-grade CG. At the end of the follow-up period, 513 (77.3%) of the population remained classified as a G0, 113 (17.0%) eyes showed G1, 18 (2.7%) eyes G2 and 20 (3.0%) eyes G3.

Graft survival/Graft rejection and CG

Out of the total of 664 surgeries performed, 631 (95.0%) grafts survived. Graft failure was observed in 33 (5.0%) eyes.

Divided into the 2 groups, with and without CG, no statistically significant higher graft failure could be determined. In the 513 eyes that were not affected by CG, graft failure was detected in 25 (4.8%) cases. In the 151 eyes affected by CG, regardless of when the CG was detected, 8 (5.3%) corneas were affected by graft failure. There was no significant difference between the two groups in terms of graft survival (p = 0.8, chi-square test).

Table 2. Classification of Cornea guttata based on the affected area after DMEK using non-contact specular microscopy images from grade 0 to grade 3.

Grade	Guttata			
G0	No guttae			
G1	<40% guttae			
G2	40%–80% guttae			
G3	>80% guttae			

Additionally, we investigated the association between graft rejection and CG. We found that in a total of 54 (8.1%) cases, an immune reaction was detected. In 38 (7.4%) of these cases, corneas without CG were affected by an immune reaction and in 16 (10.6%) cases corneas with CG were affected. No statistically significant difference was detected between the two groups($p = 0.2$, chi-square-test).

Furthermore, we investigated the correlation between endothelial cell morphology and graft rejection. Again,

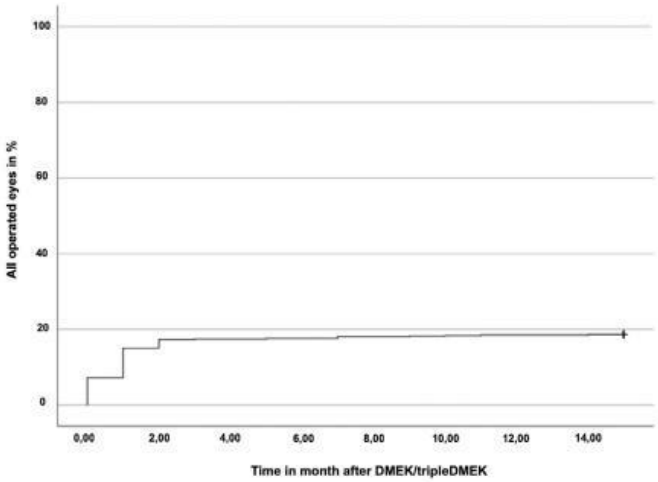


Fig. 1. Kaplan–Meier curve of the first cornea guttata (CG) detection immediately after DMEK/triple-DMEK.

Table 3. Comparison of different age groups of the recipient in relation to the cornea guttata grade (grade 0 (G0) – grade 3 (G3)) at the time of the last follow-up examination (final progression).

	G0	G1	G2	G3
<60 y	92 (77.3%)	24 (20.2%)	2 (1.7%)	1 (0.8%)
60–80 y	372 (76.9%)	80 (16.5%)	15 (3.1%)	17 (3.5%)
>80 y	49 (80.3%)	9 (14.8%)	1 (1.6%)	2 (3.3%)

Table 4. Comparison of different age groups of the donor in relation to the cornea guttata grade (grade 0 (G0) – grade 3 (G3)) at the time of the last follow-up examination (final progression).

	G0	G1	G2	G3
<60 y	38 (77.6%)	9 (18.4%)	1 (2.0%)	1 (2.0%)
60–80 y	321 (79.9%)	60 (14.9%)	10 (2.5%)	11 (2.7%)
>80 y	148 (71.5%)	44 (21.3%)	7 (3.4%)	8 (3.9%)

the CV-value was used as a parameter for polymegalism and the 6A-value as a parameter for pleomorphism. The mean CV-value was 59.8 ± 17.2 in the group of eyes affected by graft rejection. In the group of corneas without graft rejection, a mean value of 55.4 ± 17.6 could be found. No statistical significance could be demonstrated ($p = 0.9$, *t*-test). The comparison of 6A-values showed a mean value of 37.9 ± 20.2 in eyes with graft rejection and a value of 34.4 ± 18.9 in eyes without graft rejection. Again, no statistically significant difference could be detected ($p = 0.6$, *t*-test).

Clinical significance of CG

In order to analyse the clinical impact of CG on the transplanted corneas, several clinical parameters were compared in relation to the CG grades (Table 5).

There was a statistically significant deterioration in the CDVA with increasing grades of CG ($p = 0.02$). There was no statistically significant difference between G0 and eyes with G1 CG ($p = 0.06$).

A significant increase in the CCT was demonstrated with increasing CG grades ($p < 0.001$). When comparing the values of G0 with G2 ($p = 0.02$) and G3 ($p = 0.006$) and also G1 with G3 ($p = 0.04$), significantly higher CCT values could be evaluated. A significant decrease in endothelial cell density (ECD) could be observed with an increase in the CG grade ($p < 0.001$). Between G0 and G1, a statistically significant difference was reached ($p < 0.001$). Morphological cell changes were seen in high-grade CG stages, and significant differences were found for both the 6A-value ($p < 0.001$) and the CV-value ($p < 0.001$).The 6A-value decreased significantly from G0 to G2 CG by 10.9 ± 3.5% ($p = 0.01$) and from G0 to G3 CG by 13.9 ± 4.0% ($p = 0.003$). With a reduction of 12.4 ± 4.1%, a significant deterioration was also observed when comparing G1 with G3 ($p = 0.02$). Regarding the CV-value, an increase was found in the high-grade CG grades. Overall, a significant difference was found between G0 and G1 ($p < 0.001$)/G2 ($p = 0.04$).

Discussion

Postoperatively CG was detected in 18.7% of our population studied. 16.9% showed a mild G1, 1.4% a moderate G2 and only 0.5% a severe G3. They were detected in the first postoperative follow-up after a median time of 0.6 months (2.7 weeks). Due to this small timeframe between the date of the operation and CG detection, it can be assumed that CG may already have been present on the graft preoperatively. It should not be disregarded that intraoperative endothelial stress and interactions with the new host environment might also be potential risk factors for endothelial damage and CG in the early postoperative course. Despite the precise preoperative assessment of the donor corneas in the eye bank, many reasons mask the preoperative detection of grafts with CG. First, the visual conditions associated with corneal evaluation using the slit lamp are notably different than evaluating a cornea *in vivo*. The cornea must be examined while stored in its culture medium which leads to excessive light diffusion and refraction, significantly affecting the resolution and clarity of the reflected image. Also, the examined corneas are placed in organ culture medium 1 without dextran, which causes their swelling up to 1000–1500 µm so that the endothelial cells

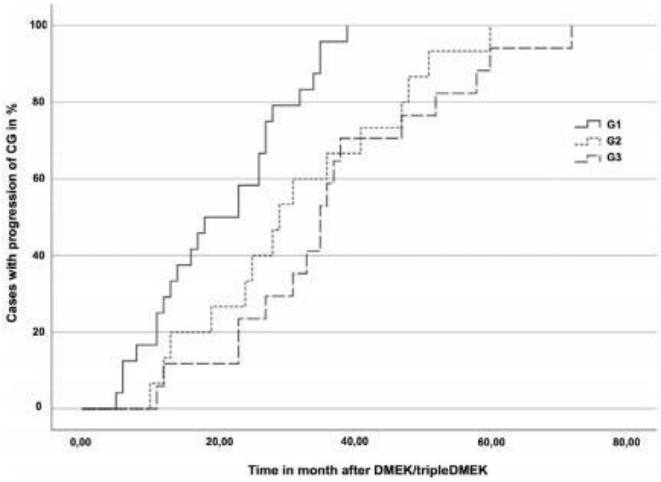


Fig. 2. Kaplan–Meier curves illustrating the progression of the cornea guttata (CG) during the follow-up to the maximum expression (continuous curve: Progression from healthy cornea (G0) to cornea guttata grade 1 (G1); dotted curve: Progression from healthy or low-grade cornea guttata (G0/G1) to cornea guttata grade 2 (G2); dashed curve: Progression from lower cornea guttata grades 0/1/2 (G0/G2/G2) to highest cornea guttata grade 3 (G3).

cannot be clearly delineated anymore and therefore makes it almost impossible to detect the typical hammered glass appearance of the guttae (Abdin et al. 2018). For the above-mentioned reasons, there are currently no clear criteria for the detection of CG in donor corneas. As a result, despite the close and strict inspection of donor corneas, CG cases still go unnoticed in many cases and are therefore transplanted during DMEK and penetrating keratoplasties. A retrospective study by Safi *et al.* investigated the morphological features that can be observed in the preoperative examination of donor corneas, that later on showed CG postoperatively. His study found that there was a higher incidence of CG in the donor grafts having cell membrane defects or small thickened areas of cell membrane called 'blebs'. Similarly, it was found that a proportion of less than 50% of the cells having a hexagonal or circular shape was also correlated with postoperative CG (Safi et al. 2021a, 2021b).

Comparing several studies done to determine the prevalence of CG in the general population, it was clear that the results varied a lot between different ethnic groups. A cross-sectional study performed in 2006 on the Reykjavik population in Iceland showed a prevalence of 7% CG in men and 11% in women. The mean age of the patients without CG was found to be 68 years and with CG 70 years (Zoega et al. 2006). In a prospective cohort study of a Caucasian population over a 7-year period, the cumulative incidence of primary CG was found to be 15%–23%. Only patients over 50 years, who had no potentially influential ocular diseases, were included in this study. It was also found that primary CG occurred earlier in the female gender (Zoega et al. 2013). Another Japanese population-based study performed in 2011 reported an overall prevalence of CG of 4.1%. Significant differences were shown in the prevalence between women (5.8%) and men (2.4%) (Higa 2011). This was also confirmed in a study by Krachmer *et al.* The results showed that women are more frequently and more severely affected by CG than men. The classification used in the above-mentioned study was based on the number and size of guttae detected in the slit lamp examination. The higher the number of guttae and the larger the area merged in mm, the higher the severity grade as follows:

- negative, 0 to 12 central CG;
- grade 1, greater than 12 central nonconfluent CG;
- grade 2, 1 to 2 mm of confluent central CG;
- grade 3, 2 to 5 mm of confluent central CG;
- grade 4, greater than 5 mm of confluent central CG;
- grade 4 + oedema, greater than 5 mm of confluent central CG with stromal or epithelial oedema.

In our study, we used a slightly modified version of the Huang *et al.*

Table 5. Comparison of different clinical parameters (mean ± standard deviation) in relation to the cornea guttata grade (grade 0 (G0) – grade 3 (G3)) during the follow-up time.

	G0	G1	G2	G3
Corrected distance visual acuity (logMAR)	0.24 ± 0.24 (n = 1902)	0.21 ± 0.19 (n = 467)	0.29 ± 0.25 (n = 64)	0.28 ± 0.17 (n = 43)
Density of endothelial cells (N/mm^2)	1502.5 ± 459.5 (n = 1998)	1407.6 ± 437.8*** (n = 472)	1492.0 ± 471.8 (n = 58)	1352.2 ± 559.4 (n = 37)
Corneal thickness at pupil centre (µm)	524.3 ± 58.3 (n = 1658)	530.0 ± 56.1 (n = 417)	549.7 ± 71.6* (n = 49)	558.3 ± 56.4** (n = 33)
Coefficient of variation (polymegalism)	53.4 ± 17.6 (n = 1998)	57.2 ± 18.0*** (n = 472)	59.8 ± 16.6** (n = 58)	59.2 ± 19.0 (n = 37)
Percentage of hexagonal-shaped cells in % (pleomorphism)	35.8 ± 17.4 (n = 1460)	34.2 ± 17.8 (n = 325)	24.8 ± 18.6* (n = 25)	21.8 ± 14.2** (n = 19)

Significant p-values compared with G0: *p < 0.05, **p < 0.01, ***p < 0.001.
n = number of examinations of the different CG severity levels G0-G3.

classification, which is based on the specular microscopy findings rather than slit lamp examination findings. Although these classifications are based on different examination tools, nevertheless, both classification systems are actually similar in terms of clinical significance. In the study by Krachmer *et al*. grade negative corresponds to grade 0 used in our classification, grade 1 corresponds to grade 1 in our study which represents mild clinically insignificant disease, grades 2 and 3 are comparable to grade 2 of our study indicating moderate disease progression and grade 4 and 4+ oedema are similar grade 3 of our study indicating widespread and severe disease progression. (Krachmer et al. 1978; Huang et al. 2019).

Furthermore, a correlation between higher age and CG was established from Higa *et al*. A direct comparison showed a prevalence of 2.8% in the group of participants aged 40–49 years, compared to 6.3% in the group of participants aged 70–79 years. No association was found though between the development of high-grade CG and increasing age (Higa 2011). In our study, similar results could be observed in terms of the prevalence of postoperative CG and age of the donor cornea. An increase in CG prevalence was found especially in the patients with older donor corneas aged above 80y (28.5%), compared with younger donor corneas aged under 60y (22.4%) and between 60 and 80y (20.2%), but with respect to the different CG grades 1–3, we did not find any correlation between recipient or donor age. It should be noted that, when looking at a retrospective study conducted by the Lions Eye Bank, the mean donor age from 2006 to 2016 was 70.3 ± 15.0 years. This study showed that the main reasons of non-suitable potential donors in the eye bank were a decreased endothelial cell quality and higher donor ages above 80 years (Kramp et al. 2020; Laun et al. 2021).

Up to our knowledge, there is only one study conducted in 2015 by Nahum *et al*. regarding CG prevalence after keratoplasty. It demonstrated a postoperative prevalence of only 4%. Of the 1116 included operations, only 19 cases were DMEK (Nahum et al. 2015). Due to this small amount of DMEK, this study has a very limited comparative value to our results.

Only 4.1% of our studied population developed CG after an initially inconspicuous postoperative endothelial cell image showing no guttae. Many potential interpretations could explain this phenomenon: (1) It could be the result of endothelial stress during surgery and during the postoperative course due to potential harmful interactions with the new host environment. (2) New CG cases could have emerged as part of a normal aging process. (3) It should be noted that only a small central section of the endothelium, measuring 0.25 mm × 0.54 mm, could be examined in a single specular microscopic image. Therefore, it might be possible that some CG cases were not detected in the first endothelial cell image typically at about 6 weeks after the transplantation. It should be noted that in 482 cases, an evaluable endothelial cell image could be provided in the first 6 weeks. It is possible that endothelial cell images in the first few weeks after the operation could not be analysed at this time due to the bad image quality, for example if the cells were not clearly defined due to corneal swelling after the operation.

In total, 8.5% of CG cases out of the whole population showed a progression. As a result, the percentage of high-grade CG increased from 1.9% to 5.7% at the end of the follow-up period. Comparing these results with the 7-year prevalence of CG of the Reykjavik study, similar trends can be noticed (Zoega et al. 2013). The progression of CG from G1 to G3 without the detection of an intermediate G2 stage can be explained by the fact that some patients skipped a few follow-up controls or the progression of the disease in the time between two control examinations might have been too fast.

Our study showed that graft rejection or even graft failure occurs only in rare cases after DMEK. Graft failure could be detected in a total of 5.0% of the eyes. There was no significant correlation between graft failure and pre-existing CG. These results fit with the findings of several studies, where a percentage of graft failure after DMEK of 3.1%–8.8% could be demonstrated (Guerra et al. 2011; Baydoun et al. 2015).

Regarding graft rejection, a larger difference between eyes with CG (10.6%) and eyes without CG (7.5%) could be shown, although no significance could be demonstrated. A possible explanation could be that the inflammatory process of the cornea could have an influence on CG. In the literature the occurrence of cornea pseudoguttata is described, which is a transient form of CG during an inflammatory process (Zantos & Holden 1981; Nakashima et al. 2007). Furthermore, it is described in the literature that there might be a correlation between morphological cell changes and the occurrence of graft rejections, which could be indicative of the inflammatory process already before the onset of the reaction (Monnereau et al. 2014). In our study, however, no significant difference in morphologic parameters could be observed, which might be due to the low number of immune reactions compared with unaffected corneas.

As demonstrated in our study, especially high-grade CG showed a significant influence on many clinical parameters. As expected, it was correlated with decreasing CDVA. Possible causes for the worsening of VA in eyes with a G2/G3 were explained in comparable studies. The increasing area covered by a CG leads to an increased irregularity of the posterior corneal surface. This results in higher corneal aberrations with forward scattering of light and an associated decrease in VA (Wacker et al. 2015; Oie et al. 2016). Regarding the corneal thickness, a positive correlation could be shown between increasing CG grades and increasing central corneal thickness. These results are in line with the study performed by Huang et al. (2019). The influence of high-grade CG was also reflected by the endothelial cell morphology. In fact, there was a significant decrease in the ECD, and an increase in pleomorphism and polymegalism in corneas with high-grade CG. The influence of CG on the morphology of the endothelium was also confirmed (Giasson et al. 2007). The consequences of such morphological changes may manifest as functional limitations of the endothelial layer (Lisch & Seitz 2012).

Three limiting factors may have affected the results of this study. (1) The limited ability of the specular microscopy to analyse only a very small surface area of the endothelial layer, which might have led to false-negative results. However, to reduce this limitation, all the postoperative follow-up images were taken into consideration in our study, and the

images were always taken in the centre of the cornea since early CG typically manifests in the central areas of the cornea and then spreads to the periphery (Lorenzetti et al. 1967; Giasson et al. 2007). (2) The decreasing precision of the specular microscopy analysis with increasing grades of CG due to possible swelling of the cornea (Huang et al. 2019). (3) In addition, a further limitation of the study was that not all endothelial cell images were analysable immediately postoperatively. This could have caused a distortion in the time of the first CG determination.

In summary, our study showed a prevalence of CG after DMEK of 18.7%. Most of the eyes, in which CG was confirmed, had only a mild G1 CG (16.9%). This low-grade CG showed almost no significant impact on the clinical outcomes of the operation, while high-grade CG (1.4% G2, 0.5% G3) demonstrated a reduced VA, increased CCT, polymegalism and pleomorphism. Increased donor age was found to be a risk factor for postoperative CG, whereas patient age and sex did not affect CG prevalence. The data provided in our study offer an impulse to establish further investigation methods in the preoperative donor cornea assessment to prevent the transplantation of high-grade CG affected corneas in the future.

References

Abdin A, Daas L, Pattmöller M, Suffo S, Langenbucher A & Seitz B (2018): Negative impact of dextran in organ culture media for pre-stripped tissue preservation on DMEK (Descemet membrane endothelial keratoplasty) outcome. Graefes Arch Clin Exp Ophthalmol **256**(11): 2135–2142.

Baydoun L, Ham L, Borderie V, Dapena I, Hou J, Frank LE, Oellerich S & Melles GR (2015): Endothelial survival after Descemet membrane endothelial keratoplasty: Effect of surgical indication and graft adherence status. JAMA Ophthalmol **133**(11): 1277–1285.

Bonanno JA (2012): Molecular mechanisms underlying the corneal endothelial pump. Exp Eye Res **95**(1): 2–7.

Borderie VM, Sabolic V, Touzeau O, Scheer S, Carvajal-Gonzalez S & Laroche L (2001): Screening human donor corneas during organ culture for the presence of guttae. Br J Ophthalmol **85**: 272–276.

Feizi S (2018): Corneal endothelial cell dysfunction: Etiologies and management. Ther Adv Ophthalmol **10**: 2515841418815802.

Flockerzi E, Maier P, Böhringer D et al. (2018): Trends in corneal transplantation from 2001 to 2016 in Germany: A report of the DOG-Section cornea and its keratoplasty registry. Am J Ophthalmol **188**: 91–98.

Fuchs E (1910): Dystrophia epithelialis corneae. Graefes Arch Clin Exp Ophthalmol **76**: 478–508.

Geroski DH, Matsuda M, Yee RW & Edelhauser HF (1985): Pump function of the human corneal endothelium. Effects of age and cornea guttata. Ophthalmology **92**(6): 759–763.

Giasson CJ, Solomon LD & Polse KA (2007): Morphometry of corneal endothelium in patients with corneal guttata. Ophthalmology **114**(8): 1469–1475.

Guerra FP, Anshu A, Price MO, Giebel AW & Price FW (2011): Descemet's membrane endothelial keratoplasty: prospective study of 1-year visual outcomes, graft survival, and endothelial cell loss. Ophthalmology **118**(12): 2368–2373.

Higa A (2011): Prevalence of and risk factors for cornea guttata in a population-based study in a southwestern Island of Japan: The Kumejima study. Arch Ophthalmol **129**(3): 332.

Huang J, Tepelus TC, Baghdasaryan E et al. (2019): Correlation between guttata severity and thickness of Descemet's membrane and the central cornea. Curr Eye Res **44**(8): 849–855.

Krachmer JH, Purcell JJ Jr, Young CW & Bucher KD (1978): Corneal endothelial dystrophy. A study of 64 families. Arch Ophthalmol **96**(11): 2036–2039.

Kramp K, Suffo S, Laun D, Bischoff-Jung M, Huber M, Langenbucher A & Seitz B (2020): Analyse von Einflussfaktoren auf die Eignung von kornealem Spendergewebe in der LIONS Hornhautbank Saar-Lor-Lux, Trier/Westpfalz von 2006 bis 2016 [Analysis of factors influencing the suitability of donor corneas in the LIONS cornea bank Saar-Lor-Lux, Trier/Westpfalz from 2006 to 2016]. Klin Monatsbl Augenheilkd **237**(11): 1334–1342.

Laun D, Suffo S, Kramp K, Bischoff M, Huber M, Langenbucher A & Seitz B (2021): How implementing a quality management system at the LIONS eye bank Saar-Lor-Lux, Trier/Western palatinate from 2006 to 2016 impacted the rate and reasons for discarding human organ-cultured corneas. Klin Monbl Augenheilkd (Epub ahead of print).

Lisch W & Seitz B (2012): Endotheliale Hornhautdystrophien (HD) – Diagnose und Therapie. Klin Monatsbl Augenheilkd **229** (6): 594–602.

Lorenzetti DW, Uotila MH, Parikh N & Kaufman HE (1967): Central cornea guttata: Incidence in the general population. Am J Ophthalmol **64**: 1155–1158.

McCarey BE, Edelhauser HF & Lynn MJ (2008): Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices and new intraocular drugs and solutions. Cornea **27**: 1–16.

Melles GR, Eggink FA, Lander F, Pels E, Rietveld FJ, Beekhuis WH & Binder PS (1998): A surgical technique for posterior lamellar keratoplasty. Cornea **17**(6): 618–626.

Monnereau C, Bruinsma M, Ham L, Baydoun L, Oellerich S & Melles GR (2014): Endothelial cell changes as an indicator for upcoming allograft rejection following Descemet membrane endothelial keratoplasty. Am J Ophthalmol **158**(3): 485–495.

Nahum Y, Canton V, Ponzin D & Busin M (2015): Prevalence of guttae in the graft following corneal transplantation. Br J Ophthalmol **99**(12): 1660–1663.

Nakashima Y, Yoshitomi F & Oshika T (2007): Clinical evaluation of cornea pseudoguttata. Br J Ophthalmol **91**(1): 22–25.

Oie Y, Watanabe S & Nishida K (2016): Evaluation of visual quality in patients with Fuchs endothelial corneal dystrophy. Cornea **35**: 55.

Quintin A, Hamon L, Mäurer S, Langenbucher A & Seitz B (2021): OCT application for sterile corneal graft screening in the eye bank. Klin Monatsbl Augenheilkd **238**(06): 688–692.

Röck T, Landenberger J, Bramkamp M, Bartz-Schmidt KU & Röck D (2017): The evolution of corneal transplantation. Ann Transplant **22**: 749–754.

Safi T, Daas L, Kiefer GL et al. (2021a): Semi-quantitative criteria in the eye bank that correlate with cornea guttata in donor corneas. Klin Monatsbl Augenheilkd **238** (6): 680–687.

Safi T, Seitz B, Berg K, Schulz K, Langenbucher A & Daas L (2021b): Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage. Am J Ophthalmol **221**: 17–26.

Schmidt I, Schlötzer-Schrehardt U, Langenbucher A, Eppig T, Hager T, Zimpfer A & Seitz B (2019): Ultrastructural findings in graft failure after Descemet membrane endothelial keratoplasty (DMEK) and new triple procedure. Medicine (Baltimore) **98** (19): e15493.

Seitz B, Daas L, Flockerzi E & Suffo S (2020): Descemet membrane endothelial keratoplasty DMEK – Spender und Empfänger Schritt für Schritt [Descemet membrane endothelial keratoplasty DMEK – Donor and recipient step by step]. Ophthalmologe **117**(8): 811–828.

Seitz B, Müller EE, Langenbucher A, Kus MM & Naumann GOH (1997): Reproduzierbarkeit und Validität eines neuen automatisierten Verfahrens der spiegelmikroskopischen Hornhautendothelanalyse [Reproducibility and validity of a new automatic method of specular microscopy analysis of corneal endothelium]. Ophthalmologe **94**(2): 127–135.

Son H-S, Villarreal G, Meng H, Eberhart CG & Jun AS (2014): On the origin of 'guttae'. Br J Ophthalmol **98**(9): 1308–1310.

Tan DT, Dart JK, Holland EJ & Kinoshita S (2012): Corneal transplantation. Lancet **379** (9827): 1749–1761.

Vogt A (1921): Weitere Ergebnisse der Spaltlampenmikroskopie des vorderen Bulbusabschnittes: (Cornea, Vorderkammer, Iris, Linse, vorderer Glaskörper, Conjunctiva, Lidränder.) I. Abschnitt: Hornhaut. Graefes Arch Clin Exp Ophthalmol **106**: 63–103.

Wacker K, McLaren JW, Amin SR, Baratz KH & Patel SV (2015): Corneal high-order aberrations and backscatter in Fuchs endothelial corneal dystrophy. Ophthalmology **122**(8): 1645–1652.

Wacker K, Reinhard T & Maier P (2019): Pathogenese, Diagnose und Klinik der Fuchs-Endotheldystrophie [Pathogenesis and diagnostic evaluation of Fuchs' endothelial corneal dystrophy]. Ophthalmologe **116**(3): 221–227.

Weiss JS, Møller HU, Aldave AJ et al. (2015): IC3D classification of corneal dystrophies-edition 2. Cornea **34**(2): 117–159.

Zantos SG & Holden BA (1981): Guttate endothelial changes with anterior eye inflammation. Br J Ophthalmol **65**(2): 101–103.

Zoega GM, Arnarsson A, Sasaki H, Söderberg PG & Jonasson F (2013): The 7-year cumulative incidence of cornea guttata and morphological changes in the corneal endothelium in the Reykjavik eye study. Acta Ophthalmol **91**(3): 212–218.

Zoega GM, Fujisawa A, Sasaki H, Kubota A, Sasaki K, Kitagawa K & Jonasson F (2006): Prevalence and risk factors for cornea guttata in the Reykjavik eye study. Ophthalmology **113**(4): 565–569.

Received on January 3rd, 2022.
Accepted on May 14th, 2022.

Correspondence:
Lena-Marie Schmitz
Saarland University Medical Center
Kirrberger Straße 100, Building 22, 66424
Homburg/Saar, Germany
Tel.: 00496841/1622387
Fax: 00496841/22400
Email: schmitz.lena-marie@web.de

5.4 Publication 4:

Klinische Studie

Thieme

Semiquantitative Criteria in the Eye Bank That Correlate with Cornea Guttata in Donor Corneas

Semiquantitative Kriterien in der Hornhautbank korrelieren mit Cornea guttata in Spenderhornhäuten

Authors
Tarek Safi[1], Loay Daas[1], Gian-Luca Kiefer[2], Mansi Sharma[2], Alassane Ndiaye[2], Matthieu Deru[2], Jan Alexandersson[2], Berthold Seitz[1]

Affiliations
1 Department of Ophthalmology, Saarland University Hospital and Saarland University Faculty of Medicine, Homburg, Germany
2 Department of Cognitive Assistants, German Research Centre for Artificial Intelligence Saarbrucken Branch, Saarbrucken, Germany

Key words
cornea guttata, donor cornea, inverted light microscopy, keratoplasty, eye bank

Schlüsselwörter
Cornea guttata, Spenderhornhaut, inverse Lichtmikroskopie, Keratoplastik, Hornhautbank

received 28.1.2021
accepted 28.4.2021

Bibliography
Klin Monatsbl Augenheilkd 2021; 238: 680–687
DOI 10.1055/a-1498-1846
ISSN 0023-2165

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Correspondence
Tarek Safi, M.D.
Saarland University Hospital and Saarland University Faculty of Medicine, Department of Ophthalmology
Kirrberger Straße, building 22, 66424 Homburg, Germany
Phone: +49 6841 16 22 387, Fax: +49 6841 16 22 400
tarek.safi@uks.eu

ABSTRACT

Background Cornea guttata may not be recognized in the eye bank and recent studies have displayed that guttae are transplanted in about 15% of cases in varying severities. The purpose of this study was to establish semiquantitative criteria for the detection of cornea guttata in donor corneas in the eye bank.

Methods In this retrospective cohort study, preoperative endothelial pictures of donor corneas were collected and classified according to the post-penetrating keratoplasty cornea guttata grade into three distinct groups: group 1 consists of healthy corneas with no guttae (guttata grade 0); group 2 constitutes corneas with mild asymptomatic cornea guttata (guttata grade +); and group 3 comprises corneas with advanced widespread cornea guttata (guttata grade ++/+++/++++). The preoperative pictures of each group were then individually analyzed using the following five semiquantitative criteria: The number and the area of the cell-depleted surfaces, the presence of less than 50% of the cells having a hexagonal or a circular shape, the presence of cell membrane defects and interruptions, the presence of blebs in the cell membrane, and the presence of groups of cells with a distinct whitish color.

Results In total, 262 patients were included in this study, with a total number of 1582 preoperative donor corneal endothelial pictures. Out of those pictures, groups 1, 2, and 3 encompassed 995 (62.9%), 411 (26.0%), and 176 (11.1%) pictures, respectively. Three out of the five eye bank criteria were found to correlate with postoperative cornea guttata with a highly significant p value of < 0.001. These three criteria are the presence of less than 50% of the cells having a hexagonal or a circular shape, the presence of cell membrane defects and interruptions and, the presence of blebs. The presence of groups of cells with a distinct whitish color was only a weak predictive factor for cornea guttata ($p = 0.069$). There was no statistically significant correlation between the number and the area of cell-depleted surfaces and postoperative cornea guttata with a $p = 0.181$.

Conclusion Three semiquantitative criteria that can be detected in the eye bank using inverted light microscopy seem to correlate with postoperative cornea guttata: The presence of blebs, the presence of cell membrane defects and interruptions, as well as endothelial pictures with less than 50% of the cells having a hexagonal of circular shape. The presence of groups of cells with a distinct whitish color appears to be a weak predictor of cornea guttata.

ZUSAMMENFASSUNG

Hintergrund Cornea guttata wird in der Hornhautbank möglicherweise nicht erkannt und neuere Studien haben gezeigt, dass Cornea guttata in etwa 15% der Fälle in unterschiedlichen Schweregraden transplantiert werden. Ziel dieser Studie war es, semiquantitative Kriterien für den Nachweis von Cornea guttata in Spenderhornhäuten in der Hornhautbank zu etablieren.

Methoden In dieser retrospektiven Kohortenstudie wurden präoperative Endothelbilder von Spenderhornhäuten gesammelt und entsprechend dem Grad der Cornea guttata nach perforierender Keratoplastik in 3 verschiedene Gruppen eingeteilt: Gruppe 1 besteht aus gesunden Hornhäuten ohne Cornea guttata (Cornea-guttata-Grad 0), Gruppe 2 aus Hornhäuten mit leichter, asymptomatischer Cornea guttata (Cornea-guttata-Grad +) und Gruppe 3 umfasst Hornhäute mit fortgeschrittener, ausgedehnter Cornea guttata (Cornea-guttata-Grad ++/+++/++++). Die präoperativen Bilder jeder Gruppe wurden dann einzeln anhand der folgenden 5 semiquantitativen Kriterien analysiert: die Anzahl und die Fläche der zelldepletierten Flächen, das Vorhandensein von weniger als 50% der Zellen, die eine hexagonale oder kreisförmige Form haben, das Vorhandensein von Zellmembrandefekten und -unterbrechungen, das Vorhandensein von Blebs in der Zellmembran und das Vorhandensein von Zellgruppen mit einer deutlichen weißlichen Farbe.

Ergebnisse Insgesamt wurden 262 Patienten in diese Studie eingeschlossen, mit einer Gesamtzahl von 1582 präoperativen Spenderhornhaut-Endothelbildern. Von diesen Bildern umfassten die Gruppen 1, 2 und 3 jeweils 995 (62,9%), 411 (26,0%) und 176 (11,1%) Bilder. Es wurde festgestellt, dass 3 der 5 Kriterien der Hornhautbank mit postoperativen Cornea guttata mit einem hochsignifikanten p-Wert von < 0,001 korreliert sind. Diese 3 Kriterien sind: das Vorhandensein von weniger als 50% der Zellen, die eine hexagonale oder kreisförmige Form haben, das Vorhandensein von Zellmembrandefekten und -unterbrechungen und das Vorhandensein von Blebs. Das Vorhandensein einer Gruppe von Zellen mit einer deutlichen weißlichen Farbe war nur ein schwacher prädiktiver Faktor für Cornea guttata (p = 0,069). Es bestand kein statistisch signifikanter Zusammenhang zwischen der Anzahl und der Fläche der zelldepletierten Flächen und der postoperativen Cornea guttata mit p = 0,181.

Schlussfolgerung Drei semiquantitative Kriterien, die in der Hornhautbank mittels inverser Lichtmikroskopie detektiert werden können, scheinen mit postoperativer Cornea guttata zu korrelieren: das Vorhandensein von Blebs, das Vorhandensein von Zellmembrandefekten und -unterbrechungen sowie Endothelbilder, bei denen weniger als 50% der Zellen eine hexagonale oder zirkuläre Form aufweisen. Das Vorhandensein von Zellgruppen mit einer deutlichen weißlichen Farbe scheint ein schwacher Prädiktor für Cornea guttata zu sein.

Introduction

Advances in keratoplasty procedures have led to a significant increase in the number of keratoplasties [1] and to improvements in the postoperative results, such as visual acuity, graft rejection rates, and complication rates [2,3]. However, regardless of whether penetrating keratoplasty (PKP), DSAEK, or DMEK is applied, one complication is always possible, and until now, we have not been able to prevent it; that is cornea guttata. Guttae are endothelial excrescences which disrupt the endothelial mosaic and usually first appear in the central area of the cornea and then spread to the peripheral cornea in the advanced disease [4]. Since corneal endothelial cells are responsible for actively pumping out fluid and maintaining the cornea's transparency, in the presence of many guttae, the endothelial cells lose their function and cannot hold the cornea's transparency in the long run. This often leads to irreversible visual impairment, sometimes so severe that a repeat transplantation of a new cornea becomes necessary [4–7]. The prevalence of cornea guttata in the normal population varies a lot depending on the population studied [8–12] and is higher in the older population [11]. Therefore, during every keratoplasty, there is a risk of transplanting a donor cornea with guttae. Depending on the severity of the guttae, the consequences of transplanting such a diseased cornea vary from completely asymptomatic, to significantly affecting the visual acuity, to corneal decompensation necessitating a repeat keratoplasty in severe cases [12].

The rate of postoperative cornea guttata varies in the literature from 4.44 [10] to 25.6% [13]. In a large-scale retrospective study, we found a certain degree of cornea guttata in post-keratoplasty patients (around 15% after PKP and 25% after DMEK). Around 3% of the cases showed widespread guttae (++ or +++) (unpublished data). The ability to detect guttae in the donor cornea during the eye bank examination can be of great importance to prevent its transplantation. To our knowledge, there are, so far, no definitive criteria to detect cornea guttata in the eye bank. Unfortunately, guttae detection in the donor corneal tissue is very challenging and is mainly restricted to only few advanced cases. So far, very few studies investigated the clinical implications of postoperative cornea guttata. One study showed that postoperative cornea guttata was clinically insignificant, when mild and widespread guttae were considered as one group [10]. Another study also showed similar results to the abovementioned study, but when widespread guttae were considered alone as a group (apart from mild guttae), it was found that they significantly reduced graft survival, discouraging the transplantation of donor corneas with widespread guttae [13].

Therefore, the objective of this research article is to set semiquantitative criteria for the detection of cornea guttata in the donor corneas using inverted light microscopy in the eye bank. This was achieved by creating five potential semiquantitative criteria that might be predictive of cornea guttata and applying them to three groups of donor endothelial pictures: the first group comprises donor corneas that did not have cornea guttata following keratoplasty, the second group had mild asymptomatic guttae

following keratoplasty, and the third group had advanced widespread guttae following keratoplasty. The results of these five criteria were compared statistically between the three groups in order to test their validity.

Methods

Collection of data

In this retrospective cohort study, medical records of all consecutive 711 patients who underwent PKP between February 2017 and December 2018 were reviewed. Out of these, 262 patients were included in this study. Main inclusion criterion was the availability of preoperative endothelial pictures of the donor cornea as well as postoperative endothelial pictures. The patients received transplants provided by the Klaus Faber Center for Corneal Diseases incl. LIONS-Corneal Bank Saar-Lor-Lux, Trier/Westpfalz at the Department of Ophthalmology, Saarland University Medical Center (UKS).

All grafts were preserved according to the guidelines of the European Eye Bank Association (EEBA). The donor corneas have been stored in organ culture medium I at 34 °C. During their routine evaluation, the corneas are shortly placed in a hypotonic balanced salt solution for a maximum of 5 minutes in order to take a minimum of six pictures to evaluate the endothelial cell density. Only corneas with more than 2000 cells/mm² were eligible for PKP and more than 2200 cells/mm² for DMEK. The preoperative endothelial images were taken and stored by an inverted light microscope (Leica 090–135.001; Leica Microsystems, Wetzlar, Germany). In total, 1582 pictures were available. Each of these pictures was analyzed using the five semiquantitative criteria discussed below.

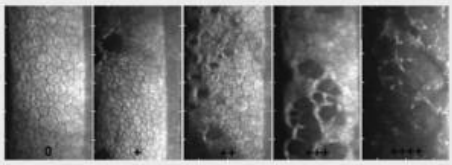
The cornea guttata grade was assessed using endothelial pictures taken between 6 weeks and up to 3 months postoperatively by specular microscopy (Tomey specular microscope EM-3000; Tomey Corp., Nagoya, Japan) [14–16]. The patients were classified into three groups depending on the severity of the postoperative cornea guttata. Group 1 consists of healthy patients with no guttae, group 2 constitutes patients with mild asymptomatic cornea guttata, and group 3 comprises patients with advanced widespread cornea guttata, see below.

This study followed the guidelines of the Helsinki declaration and was approved by the local ethics committee (ID: 197/20).

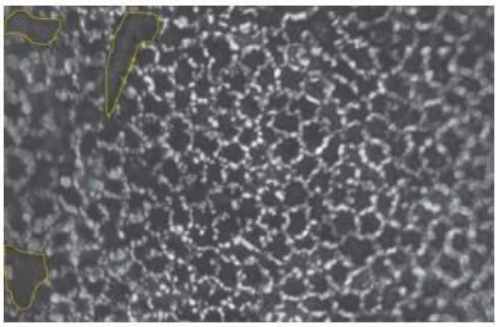
Guttata grading system

In this study, a detailed objective guttata grading system was used to classify the patients into the three distinct groups depending on the severity of the detected postoperative guttata. This grading system is composed of five categories as illustrated in ▸ **Fig. 1**:

- 0 whereby no guttae are detected,
- + whereby guttae cover less than 20% of the surface area of the postoperative endothelial pictures,
- ++ whereby guttae cover between 20 and 40% of the surface area of the postoperative endothelial pictures,
- +++ whereby guttae cover between 40 and 60% of the surface area of the postoperative endothelial pictures, and
- ++++ whereby guttae cover more than 60% of the surface area of the postoperative endothelial pictures.



▸ **Fig. 1** Illustration of the postoperative guttata grading system.



▸ **Fig. 2** Application of criterion 1 on an endothelial picture of a donor cornea showing 3 cell-depleted surfaces demarcated in yellow with a total surface area of 11 541 pixels (4519 µm²).

This grading system was also used by Huang et al. [17].

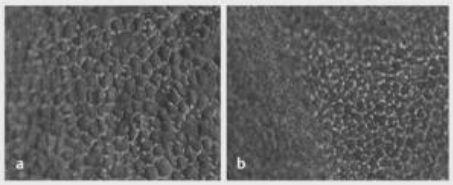
For the present study, group 1 included patients having guttata 0, group 2 included patients having guttata +, and group 3 included patients having guttata ++, +++, and ++++.

Five semiquantitative criteria

After a detailed analysis of the preoperative endothelial pictures, a team of experts in this field discussed all the aspects of the endothelial cells' morphology that might potentially have a correlation with cornea guttata and organized them into a set of 5 semiquantitative criteria for the potential detection of cornea guttata presented as the following:

Criterion 1

The first criterion was the number and the area of the cell-depleted surfaces. The total surface area of a preoperative endothelial picture is 0.13 mm², corresponding to 717 × 463 = 331 971 pixels. One cell-depleted surface is defined as an area not containing endothelial cells and measuring at least 1500 pixels (587 µm²), corresponding to approximately the size of 2 endothelial cells. The area of such surfaces was calculated using an enhanced image analysis software titled "Fiji" on the open source "ImageJ". ▸ **Fig. 2** illustrates an example showing 3 cell-depleted surfaces with a total acellular surface area of 5043 + 3928 + 2570 = 11 541 pixels (4519 µm²).



▸ **Fig. 3** Criterion 2: a Endothelial picture of a donor cornea with more than 50% of its cells with a hexagonal or a circular shape. b Endothelial picture of a donor cornea with less than 50% of its cells having a hexagonal or a circular shape. a A score of 0 on criterion 2 and b a score of 1 are shown.

Criterion 2

The second criterion was the cell shape. For this criterion, each picture was assigned a number of either 0 or 1, where 0 indicates that more than 50% of the cells in the picture have a hexagonal or a circular shape, and 1 indicates that less than 50% of the cells have a hexagonal or circular shape but are pleomorphic. ▸ **Fig. 3a** illustrates a picture with a score of 0 and ▸ **Fig. 3b** illustrates a picture with a score of 1. The determination of the percentage for this criterion was based on an estimation done by the examiner.

Criterion 3

The third studied criterion was the cell membrane morphology. A picture was assigned a score of 0 when more than 50% of the cells was clearly visible without defects or interruptions, 1 was assigned when more than 50% of the cells had minor cell membrane defects and interruptions that did not affect the clarity and delineation of the cells, and 2 was assigned when more than 50% of the cells had major cell membrane defects and interruptions that affected the identification and delineation of the cells. An example of this criterion is shown in ▸ **Fig. 4** where a has a score of 0, b has a score of 1, and c has a score of 2. The determination of the percentage for this criterion was based on an estimation done by the examiner.

Criterion 4

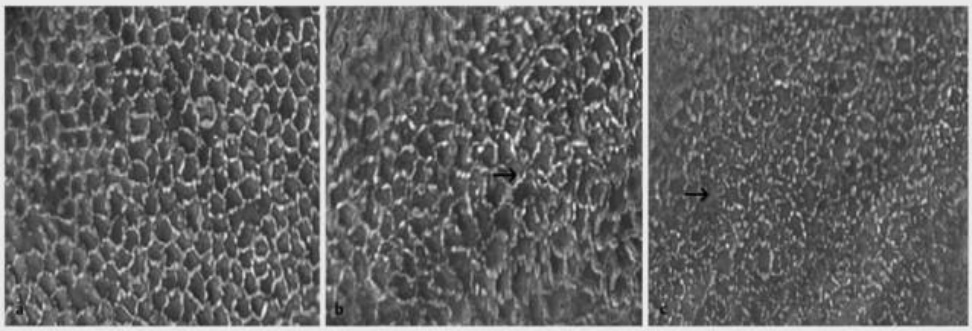
Criterion number 4 was the presence of so-called "blebs". A bleb was defined as a small thickening of the cell membrane that

1. sometimes projects into the cytoplasm;
2. usually has hyperdense characteristics represented as a white dot.

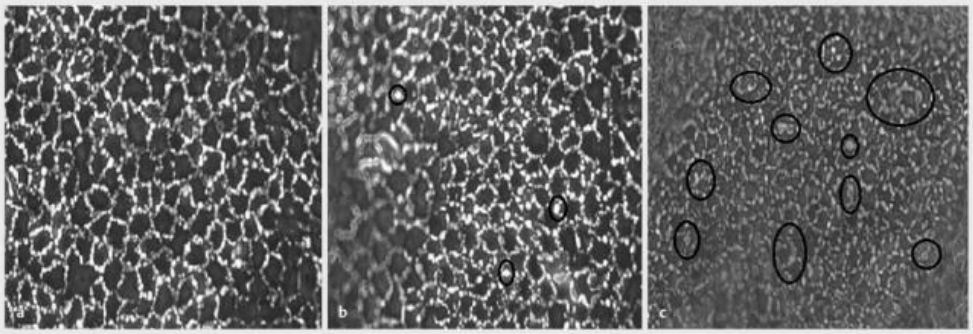
A picture is considered to have a score of 0 when it contains only a few or no blebs and 1 when more than 50% of the cells have small blebs that do not affect the clarity and delineation of the cells, and 2 when more than 50% of the cells have significant blebs affecting the clarity and delineation of the cells. Scores 0, 1, and 2 are illustrated in ▸ **Fig. 5a–c**, respectively. The determination of the percentage for this criterion was based on an estimation done by the examiner.

Criterion 5

The fifth and last criterion was the presence of a color difference in a group of cells. In this criterion, the detection of a group of cells having a different whitish color (i.e., cytoplasm appears "white" instead of "black" on the picture) than the rest of the cells is considered potentially unhealthy and might also constitute a predictive factor of cornea guttata. Pictures containing cells having such a group of cells are assigned a score of 1, otherwise a score of 0 is given. ▸ **Fig. 6** visualizes the presence of a white colored group of cells in a picture scoring 1 on this criterion. The presence of this group of cells was assessed by the examiner.



▸ **Fig. 4** Endothelial pictures of 3 donor corneas illustrating criterion 3. a, b, and c show scores of 0, 1, and 2 on the third criterion, respectively. The 0 indicates that more than 50% of the cells have an intact cell membrane, 1 indicates that more than 50% of the cells have minor cell membrane defects and interruptions, and 2 indicates that more than 50% of the cells have major cell membrane defects and interruptions. The arrows show examples of cell membrane defects and interruptions.



▶ **Fig. 5** Endothelial pictures of 3 donor corneas illustrating criterion 4. **a**, **b** and **c** have scores of 0, 1, and 2 on criterion 4, respectively. The black circles represent a few examples of blebs. A score of 0 indicates few or no blebs, a score of 1 indicates that more than 50% of the cells have minor blebs, and a score of 2 indicates that more than 50% of the cells have major blebs affecting the clarity and delineation of the cells.

Statistical analysis

All statistical analyses were performed using Excel (Microsoft, Redmond, Washington) and SPSS software version 25 (IBM, Armonk, New York). For the categorical and nominal variables, the chi-square test (two-tailed) was used, and for the ordinal variables, Somers' D test was used. An ANOVA test was performed on the normally distributed continuous variables to determine the difference between the guttata grades. For the non-normally distributed variables, the Kruskal-Wallis test was used instead. For correlations related to normally distributed variables, the Pearson correlation test was used. The Spearman rho correlation test was used otherwise, if the variables where not normally distributed. All statistics were two-sided, and the alpha was set at 0.05.

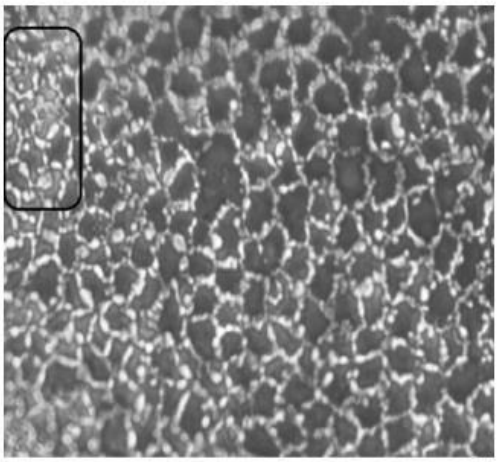
Results

In total, 262 patients were included in this study. Out of those, 160 were included in group 1 (guttata 0), 72 were included in group 2 (guttata +), and 30 were included in group 3 (guttata ++, +++, or ++++). A total number of 1582 preoperative donor cornea endothelial pictures had been taken for these 262 patients. Out of those pictures, 995 (62.9%) corresponded to group 1, 411 (26.0%) corresponded to group 2, and 176 (11.1%) corresponded to group 3.

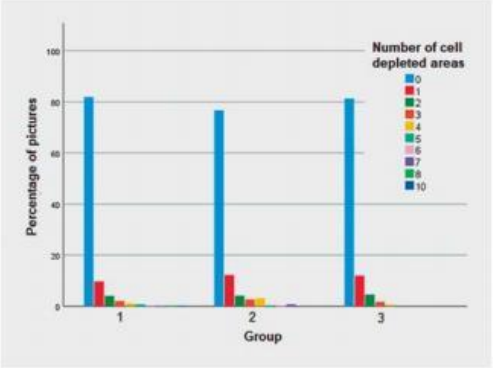
The results of each criterion applied to these pictures were compared between the three studied groups in order to determine whether the respective criterion was a predictive factor for cornea guttata detection or not.

Criterion 1

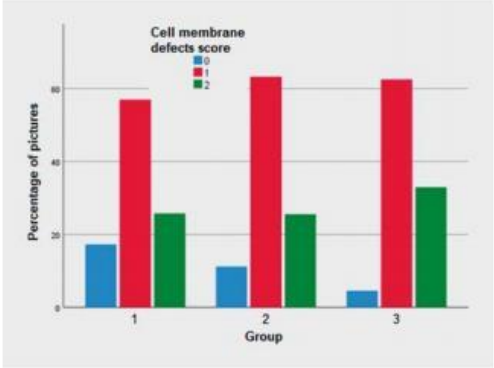
Concerning the first criterion, the number of pictures with one or more cell-depleted surfaces in groups 1, 2, and 3 was 184 (18.4%), 96 (23.4%), and 33 (18.8%), respectively. There was no statistically significant correlation between the number of cell-depleted surfaces and cornea guttata ($p = 0.181$). ▶ **Fig. 7** illustrates the number of cell-depleted surfaces in each group. The average surface area of the pictures showing at least one cell-depleted surface in groups 1, 2, and 3 was $2031 \pm 2\,011\,\mu m^2$, $2252 \pm 2324\,\mu m^2$, and $1696 \pm 1419\,\mu m^2$, respectively. The total surface area of the cell-depleted surfaces was also not a predictive factor for cornea guttata ($p = 0.669$).



▶ **Fig. 6** Endothelial picture of a donor cornea clarifying criterion 5. The rectangle shows a colony of whitish colored cells, in contrast to the other normal cells present in the picture where the cytoplasm appears black. This picture has a score of 1 on this criterion.



▶ **Fig. 7** The distribution of the number of cell-depleted surfaces in the endothelial pictures in each studied group of postoperative cornea guttata (group 1: patients with guttata 0, group 2: patients with guttata +, and group 3: patients with guttata ++, +++, and ++++).



▶ **Fig. 8** The distribution of the cell membrane defect scores in the endothelial pictures of each studied group of postoperative cornea guttata (group 1: patients with guttata 0, group 2: patients with guttata +, and group 3: patients with guttata ++, +++, and ++++).

Criterion 2

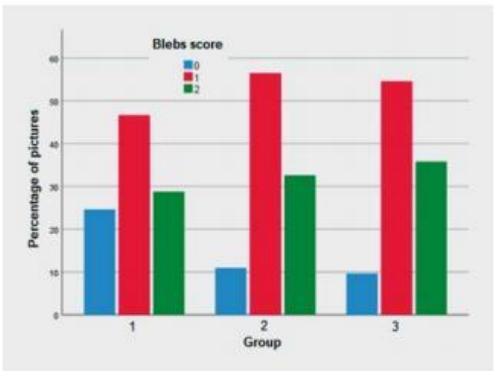
The number of preoperative endothelial pictures with a score of 1 on criterion 2, in other words, with less than 50% of the cells having a hexagonal or a circular shape, in groups 1, 2, and 3 was 640 (64.3%), 302 (73.5%), and 152 (86.4%), respectively. This criterion was found to be predictive of cornea guttata with a highly significant p value of < 0.001.

Criterion 3

Cell membrane defects and interruptions were also associated with cornea guttata with a highly statistically significant p value of < 0.001. ▶ **Fig. 8** illustrates the distribution of the results of the scoring system for this criterion among the three groups.

Criterion 4

The presence of blebs was indeed found to be correlated with the presence of cornea guttata with a highly significant p value of < 0.001. ▶ **Fig. 9** shows the results of the application of this criterion for the different studied groups.



▶ **Fig. 9** The distribution of the Blebs scores in the endothelial pictures of each studied group of postoperative cornea guttata (group 1: patients with guttata 0, group 2: patients with guttata +, and group 3: patients with guttata ++, +++, and ++++).

Criterion 5

For the last criterion, the presence of a group of cells having a distinct whitish color, we found just a trend for the prediction of cornea guttata, with a statistically insignificant p value of 0.069. The number of the pictures showing such a group of cells and thus having a score of 1 in groups 1, 2, and 3 was 237 (23.8%), 114 (27.7%), and 34 (19.3%), respectively.

Discussion

Cornea guttata can be classified into two groups according to the etiology; corneal guttata associated mainly with aging and may be an early stage of Fuchs endothelial dystrophy [7], and secondary guttata, which may result from an inflammatory process or surgical damage in the endothelial layer of the cornea [11]. The etiology of post-keratoplasty cornea guttata can be attributed to either undetected guttae in the eye bank or presumably to the surgical stress and the interactions with the recipient environment [10]. However, the latter has not been proven up to now. Due to the relatively high prevalence of cornea guttata in the general population, reaching around 15–23% [9], we assume that most of post-keratoplasty cornea guttata cases are the result of the transplantation of donor corneas with undetected guttae in the eye bank.

Up to now, cornea guttata detection in the donor cornea using inverted light microscopy is mentioned only in a few articles in the literature. However, in these articles, the methods used to detect

guttae were neither clarified nor validated, and no clear illustration of guttae-indicating features has been provided [10, 13, 18–21].

To the best of our knowledge, the effect of the presence of cell-depleted surfaces in the endothelial pictures of the donor cornea has not been discussed in the literature, except for one study showing its association with the exclusion of corneas from culture [22]. The present research article showed that this criterion is not correlated with cornea guttata. Nevertheless, the small number of pictures having cell-depleted surfaces presents a clear limitation at this time.

Pleomorphismus is a widely studied factor in corneal endothelial health. Although little is known about the exact influence of pleomorphismus on endothelial cells, the presence of high pleomorphismus is regarded as a negative sign and is associated with decreased corneal endothelial functional quality [21, 23]. Our second semiquantitative criterion showed that the presence of endothelial pictures with less than 50% of the cells having a hexagonal or circular shape was a predictive factor for cornea guttata.

Cell membrane morphology in the donor endothelial pictures has, to our knowledge, been rarely studied in the literature, and it was proven to be associated with the exclusion of corneas from culture [22]. The results of the present study demonstrated a correlation between cell membrane defects and postoperative cornea guttata.

According to our study, the presence of blebs (criterion 4) was significantly correlated with cornea guttata according to our study. Large blebs extending into the cytoplasm, as illustrated in ▸ **Fig. 5c**, can be a representation of vacuoles. Vacuoles are described in the literature as postmortem degenerative changes affecting the corneal endothelium [22, 24–26].

Our last criterion studied, the presence of a group of cells with a distinct whitish color, was not correlated with cornea guttata.

Being able to screen for preoperative cornea guttata in donor corneas would be a milestone in the quality management (QM) of our LIONS eye bank. The QM system was introduced in 2010 and aimed to increase both the quality and quantity of available donor corneas in our eye bank as demonstrated by Laun et al. (2021) and Kramp et al. (2020) [27, 28]. As part of the QM system of the donor cornea procurement, the eye bank also applies "sterile donor tomography" to avoid refractive surprises after keratoplasty [29–32].

Two main limitations were present in this study: firstly, the relatively small sample number, and secondly, the nature of the semiquantitative criteria that leaves a space for inaccuracy and imprecision of the results. Therefore, we are currently performing further studies together with researchers from the DFKI (German Research Centre for Artificial Intelligence) comprising a larger sample size, more objective criteria, and incorporating artificial intelligence (AI) as a more accurate and precise method of analyzing endothelial pictures in order to predict the presence of guttae in donor corneal endothelial cells. Creating an AI software that is able to precisely predict the risk of having guttae in the donor corneas would prevent the transplantation of such diseased corneas and may lead to a modest reduction in the rate of post-keratoplasty cornea guttata. The first step would be the segmentation of the data, i.e., determination of the relevant (pixel) zones and "region of interests" within an image. Afterwards, a hybrid classification algorithm would be created and evaluated that would be able to determine the quality of the corneas and to classify them according to the guttata grade. A so-called "deep learning" method would be used, i.e., a machine learning algorithm based on complex neural networks. For this purpose, neuronal models have to be created, which originate from the information of the input data, i.e., images and parameters of the preoperative endothelial cells. The accuracy of the guttata classifier is then reviewed, verified, and optimized in order to attain accurate results [33–35]. AI, and in particular deep learning, has currently already been in use for the calculation of the endothelial cell density in donor corneas [36, 37].

In conclusion, this retrospective study correlates three detailed light microscopic features with the presence of postoperative cornea guttata that can be detected in the eye bank. These features are the presence of blebs, cell membrane defects and interruptions, as well as endothelial pictures with less than 50% of the cells having a hexagonal or circular shape. Cell-depleted surfaces and the presence of groups of cells with a distinct whitish color do not seem to correlate with postoperative cornea guttata. Studies with larger sample sizes and incorporating AI are needed for a more accurate prediction of cornea guttata in donor corneas to further improve the quality of the donor corneas in the eye bank.

Acknowledgements

We acknowledge the Klaus Faber Center for Corneal Diseases incl. LIONS-Corneal Bank Saar-Lor-Lux, Trier/Westpfalz for their tremendous help in preserving and measuring the donor corneas, especially Ms. Katja Schulz and Mr. Marvin Schwarz. We acknowledge the Dr. Rolf M. Schwiete Foundation for their generous support with this project.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Flockerzi E, Maier P, Böhringer D et al. Trends in Corneal Transplantation from 2001 to 2016 in Germany: A Report of the DOG-Section Cornea and its Keratoplasty Registry. Am J Ophthalmol 2018; 188: 91–98. doi:10.1016/j.ajo.2018.01.018

[2] Rajesh S, Noopur G, Namrata S et al. Advances in keratoplasty procedures: A review. Indian J Ophthalmol 2010; 58: 457–463

[3] Seitz B, El-Husseiny M, Langenbucher A et al. Prophylaxe und Management von Komplikationen bei perforierender Keratoplastik. Ophthalmologe 2013; 110: 605–613. doi:10.1007/s00347-012-2678-9

[4] Giasson CJ, Solomon LD, Polse KA. Morphometry of corneal endothelium in patients with corneal guttata. Ophthalmology 2007; 114: 1469–1475. doi:10.1016/j.ophtha.2006.11.022

[5] Waring GO 3rd, Rodrigues MM, Laibson PR. Corneal dystrophies. II. Endothelial dystrophies. Surv Ophthalmol 1978; 23: 147–168. doi:10.1016/0039-6257(78)90151-0

[6] Adamis AP, Filatov V, Tripathi BJ et al. Fuchs' endothelial dystrophy of the cornea. Surv Ophthalmol 1993; 38: 149–168. doi:10.1016/0039-6257(93)90099-s

[7] Weiss JS, Møller HU, Aldave AJ et al. IC3D classification of corneal dystrophies – edition 2. Cornea 2015; 34: 117–159. doi:10.1097/ICO.0000000000000307

[8] Zoega GM, Fujisawa A, Sasaki H et al. Prevalence and risk factors for cornea guttata in the Reykjavik Eye Study. Ophthalmology 2006; 113: 565–569. doi:10.1016/j.ophtha.2005.12.014

[9] Zoega GM, Arnarsson A, Sasaki H et al. The 7-year cumulative incidence of cornea guttata and morphological changes in the corneal endothelium in the Reykjavik Eye Study. Acta Ophthalmol 2013; 91: 212–218. doi:10.1111/j.1755-3768.2011.02360.x

[10] Nahum Y, Canton V, Ponzin D et al. Prevalence of guttae in the graft following corneal transplantation. Br J Ophthalmol 2015; 99: 1660–1663. doi:10.1136/bjophthalmol-2014-306569

[11] Kitagawa K, Fujisawa A, Mizuno T et al. Twenty-three cases of primary cornea guttata. Jpn J Ophthalmol 2001; 45: 93–98. doi:10.1016/s0021-5155(00)00295-1

[12] Eghrari AO, Gottsch JD. Fuchs' corneal dystrophy. Expert Rev Ophthalmol 2010; 5: 147–159. doi:10.1586/eop.10.8

[13] Borderie VM, Sabolic V, Touzeau O et al. Screening human donor corneas during organ culture for the presence of guttae. Br J Ophthalmol 2001; 85: 272–276. doi:10.1136/bjo.85.3.272

[14] Seitz B, Müller EE, Langenbucher A et al. [Reproducibility and validity of a new automatic method of specular microscopy analysis of corneal endothelium]. Ophthalmologe 1997; 94: 127–135

[15] Seitz B, Müller EE, Langenbucher A et al. [Endothelial keratopathy in pseudoexfoliation syndrome: quantitative and qualitative morphometry using automated video image analysis]. Klin Monbl Augenheilkd 1995; 207: 167–175. doi:10.1055/s-2008-1035363

[16] Blüthner K, Seitz B, Müller EE et al. Reliability of automated endothelial cell analysis in cornea guttata. Invest Ophthalmol Vis Sci 1996; 37 (Suppl. 4): S703

[17] Huang J, Tepelus TC, Baghdasaryan E et al. Correlation between guttata severity and thickness of Descemet's membrane and the central cornea. Curr Eye Res 2019; 44: 849–855. doi:10.1080/02713683.2019.1600194

[18] Safi T, Daas L, Seitz B. Preoperative guttae screening of the donor corneas. eLetter. Br J Ophthalmol 2020. URL: https://bjo.bmj.com/content/99/12/1660.responses

[19] Brooks AM, Grant G, Gillies WE. The preoperative assessment of the corneal endothelium. Aust N Z J Ophthalmol 1988; 16: 309–316. doi:10.1111/j.1442-9071.1988.tb01233.x

[20] Bigar F, Schimmelpfennig B, Hürzeler R. Cornea guttata in donor material. Arch Ophthalmol 1978; 96: 653–655. doi:10.1001/archopht.1978.03910050349010

[21] Schroeter J, Rieck P. Endothelial evaluation in the cornea bank. Dev Ophthalmol 2009; 43: 47–62. doi:10.1159/000223838

[22] Hermel M, Salla S, Fuest M et al. The role of corneal endothelial morphology in graft assessment and prediction of endothelial cell loss during organ culture of human donor corneas. Acta Ophthalmol 2017; 95: 205–210. doi:10.1111/aos.13108

[23] Sheng H, Bullimore MA. Factors affecting corneal endothelial morphology. Cornea 2007; 26: 520–525. doi:10.1097/ICO.0b013e318033a6da

[24] Kanavi MR, Javadi MA, Chamani T. Specular microscopic features of corneal endothelial vacuolation. J Ophthalmic Vis Res 2011; 6: 5–7

[25] Menzel-Severing J, Walter P, Plum WJ et al. Assessment of corneal endothelium during continued organ culture of pre-stripped human donor tissue for DMEK surgery. Curr Eye Res 2018; 43: 1439–1444. doi:10.1080/02713683.2018.1501805

[26] Safi T, Seitz B, Berg K et al. Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage. Am J Ophthalmol 2021; 221: 17–26. doi:10.1016/j.ajo.2020.08.001

[27] Laun D, Suffo S, Kramp K et al. [Impact of the introduction of the Quality Management System (according to DIN EN ISO 9001: 2008) on the rate and reasons for discarding human organ-cultured corneas at the LIONS eye bank Saar-Lor-Lux, Trier/Westpfalz from 2006 to 2016]. Klin Monbl Augenheilkd 2021. doi:10.1055/a-1327-3835

[28] Kramp K, Suffo S, Laun D et al. [Analysis of factors influencing the suitability of donor corneas in the LIONS cornea bank Saar-Lor-Lux, Trier/Westpfalz from 2006 to 2016]. Klin Monbl Augenheilkd 2020; 237: 1334–1342. doi:10.1055/a-1141-3703

[29] Quintin A, Hamon L, Mäurer S et al. [Comparison of sterile donor tomography in the eye bank and graft tomography after penetrating keratoplasty]. Ophthalmologe 2020. doi:10.1007/s00347-020-01256-6

[30] Mäurer S, Asi F, Rawer A et al. [Concept for 3D measurement of corneal donor tissue using a clinical OCT]. Ophthalmologe 2019; 116: 640–646. doi:10.1007/s00347-018-0801-2

[31] Damian A, Seitz B, Langenbucher A et al. Optical coherence tomography-based topography determination of corneal grafts in eye bank cultivation. J Biomed Opt 2017; 22: 16001. doi:10.1117/1.JBO.22.1.016001

[32] Janunts E, Langenbucher A, Seitz B. In Vitro Corneal Tomography of Donor Cornea Using Anterior Segment OCT. Cornea 2016; 35: 647–653. doi:10.1097/ICO.0000000000000761

[33] Deru M, Ndiaye A. Deep Learning mit Tensorflow, Keras und Tensorflow. Js. 2nd ed. Bonn: Rheinwerk Computing; 2020

[34] Bishop C. Pattern Recognition and Machine Learning. Berlin: Springer; 2008. doi:10.5555/1162264

[35] LeCun Y, Bengio Y, Hinton G. Deep learning. Nature 2015; 521: 436–444. doi:10.1038/nature14539

[36] Heinzelmann S, Daniel MC, Maier PC et al. [Automated cell counting using "Deep Learning" in donor corneas from organ culture achieves high precision and accuracy]. Klin Monbl Augenheilkd 2019; 236: 1407–1412. doi:10.1055/a-1023-4339

[37] Daniel MC, Atzrodt L, Bucher F et al. Automated segmentation of the corneal endothelium in a large set of 'real-world' specular microscopy images using the U-Net architecture. Sci Rep 2019; 9: 4752. doi:10.1038/s41598-019-41034-2

6. References:

1. Abdin A, Daas L, Pattmöller M, Suffo S, Langenbucher A, Seitz B (2018) Negative impact of dextran in organ culture media for pre-stripped tissue preservation on DMEK (Descemet membrane endothelial keratoplasty) outcome. Graefes Arch Clin Exp Ophthalmol 256:2135-2142

2. Adamis A, Filatov V, Tripathi B, Tripathi RC (1993) Fuchs' endothelial dystrophy of the cornea. Surv Ophthalmol 38:149-168

3. Altaan S, Gupta A, Sidney L, Elalfy M, Agarwal A, Dua H (2015) Endothelial cell loss following tissue harvesting by pneumodissection for endothelial keratoplasty: an ex vivo study. Br J Ophthalmol 99:710-713

4. Amann J, Holley G, Lee S, Edelhauser H (2003) Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. Am J Ophthalmol 135:584-590

5. Baydoun L, Ham L, Borderie V, Dapena I, Hou J, Frank LE, Oellerich S, Melles GR (2015) Endothelial survival after Descemet membrane endothelial keratoplasty: Effect of surgical indication and graft adherence status. JAMA ophthalmology 133:1277-1285

6. Bayyoud T, Röck D, Hofmann J, Bartz-Schmidt K, Yoeruek E (2012) [Precut technique for Descemet's membrane endothelial keratoplasty, preparation and storage in organ culture]. Klin Monbl Augenheilkd 229:621-623

7. Bigar F, Schimmelpfennig B, Hürzeler R (1978) Cornea guttata in donor material Arch Ophthalmol 96:653-655

8. Birbal R, Sikder S, Lie J, Groeneveld-van Beek E, Oellerich S, Melles GR (2018) Donor tissue preparation for Descemet membrane endothelial keratoplasty. Cornea 37:128-135

9. Bishop C (2008) Pattern recognition and machine learning. Springer, Berlin

10. Blüthner K, Seitz B, Müller EE, Langenbucher A, Naumann GO (1996) Reliability of automated endothelial cell analysis in Cornea guttata. Invest Ophthalmol Vis Sci 37(Suppl. 4):S703

11. Bonanno J (2012) Molecular mechanisms underlying the corneal endothelial pump. Exp Eye Res 95:2-7

12. Borboli S, Colby K (2002) Mechanisms of disease: Fuchs' endothelial dystrophy. Ophthalmol Clin North Am 15:17-25

13. Borderie VM, Sabolic V, Touzeau O, Scheer S, Carvajal-Gonzalez S, Laroche L (2001) Screening human donor corneas during organ culture for the presence of guttae. Br J Ophthalmol 85:272-276

14. Brooks AM, Grant G, Gillies WE (1988) The preoperative assessment of the corneal endothelium. Aust N Z J Ophthalmol 16:309-316

15. Chiou AG, Kaufman SC, Beuerman RW, Ohta T, Soliman H, Kaufman HE (1999) Confocal microscopy in Cornea guttata and Fuchs' endothelial dystrophy. Br J Ophthalmol 83:185-189

16. Damian A, Seitz B, Langenbucher A, Eppig T (2017) Optical coherence tomography-based topography determination of corneal grafts in eye bank cultivation. J Biomed 22:16001

17. Daniel MC, Atzrodt L, Bucher F, Wacker K, Böhringer S, Reinhard T, Böhringer D (2019) Automated segmentation of the corneal endothelium in a large set of 'real-world' specular microscopy images using the U-Net architecture. Sci Rep 9:4752

18. Deru M, Ndiaye A (2020) Deep learning mit tensorflow, keras und tensorflow. Js. 2nd ed. Rheinwerk Computing, Bonn

19. Downes K, Tran K, Stoeger C, Chamberlain W (2018) Cumulative endothelial cell loss in Descemet membrane endothelial keratoplasty grafts from preparation through insertion with glass injectors. Cornea 37:698-704

20. Droutsas K, Alexopoulos P, Giachos I, Giallouros E, Sekundo W, Lazaridis A (2021) Secondary DMEK following failed primary DMEK. Int Ophthalmol 41:3287-3293

21. Eagle, RC Jr (2017) Eye pathology: An atlas and text, 3rd ed. Wolters Kluwer, Philadelphia (Permission to use images from the book was obtained)

22. Eghrari AO, Gottsch JD (2010) Fuchs' corneal dystrophy. Expert Rev Ophthalmol 5:147-159

23. Eye Bank Association of America (2017) 2016 Eye banking statistical report. Available at: http://restoresight.org/wpcontent/uploads/2017/04/2016_Statistical_Report-Final-040717.pdf. Accessed November 15, 2019

24. Feizi S (2018) Corneal endothelial cell dysfunction: etiologies and management. Ther Adv Ophthalmol 10:2515841418815802

25. Flockerzi E, Maier P, Böhringer D, Reinshagen H, Kruse F, Cursiefen C, Reinhard T, Geerling G, Torun N, Seitz B. All German keratoplasty registry contributors (2018) Trends in corneal transplantation from 2001 to 2016 in Germany: A report of the DOG-section cornea and its keratoplasty registry. Am J Ophthalmol 188:91-98

26. Formisano N, Sahin G, Català P, Truckenmüller R, Nuijts RMMA, Dickman MM, LaPointe VLS, Giselbrecht S (2021) Nanoscale topographies for corneal endothelial regeneration. Appl Sci 11:827

27. Fuchs E. Dystrophia epithelialis corneae (1910) Graefes Arch Clin Exp Ophthalmol 76:478-508

28. Geroski DH, Matsuda M, Yee RW, Edelhauser HF (1985) Pump function of the human corneal endothelium. Effects of age and cornea guttata. Ophthalmology 92:759-763

29. Giasson C, Solomon L, Polse K (2007) Morphometry of corneal endothelium in patients with Corneal guttata. Ophthalmology 114:1469-1475

30. Guerra FP, Anshu A, Price MO, Giebel AW, Price FW (2011) Descemet's membrane endothelial keratoplasty: prospective study of 1-year visual outcomes, graft survival, and endothelial cell loss. Ophthalmology 118:2368-2373

31. Heinzelmann S, Daniel MC, Maier PC, Reinhard T, Böhringer D (2019) [Automated cell counting using “Deep Learning” in donor corneas from organ culture achieves high precision and accuracy]. Klin Monbl Augenheilkd 236:1407-1412

32. Hermel M, Salla S, Fuest M, Walter P (2017) The role of corneal endothelial morphology in graft assessment and prediction of endothelial cell loss during organ culture of human donor corneas. Acta Ophthalmol 95:205-210

33. Higa A, Sakai H, Sawaguchi S, Iwase A, Tomidokoro A, Amano S, Araie M (2011) Prevalence of and risk factors for Cornea guttata in a population-based study in a southwestern island of Japan: The Kumejima study. Arch Ophthalmol 129:332-336

34. Huang J, Tepelus TC, Baghdasaryan E, Huang P, Shi Y, Hsu HY, Sadda SR, Lee OL (2019) Correlation between guttata severity and thickness of Descemet’s membrane and the central cornea. Curr Eye Res 44:849-855

35. Jackson AJ, Robinson FO, Frazer DG, Archer DB (1999) Corneal guttata: A comparative clinical and specular micrographic study. Eye 13:737-743

36. Janunts E, Langenbucher A, Seitz B (2016) In Vitro Corneal Tomography of Donor Cornea Using Anterior Segment OCT. Cornea 5:647-653

37. Jardine G, Holiman J, Stoeger C, Chamberlain W (2014) Imaging and quantification of endothelial cell loss in eye bank prepared DMEK grafts using trainable segmentation software. Curr Eye Res 39:894-901

38. Kanavi MR, Javadi MA, Chamani T (2011) Specular microscopic features of corneal endothelial vacuolation. J Ophthalmic Vis Res 6:5-7

39. Kitagawa K, Fujisawa A, Mizuno T, Sasaki K (2001) Twenty-three cases of primary Cornea guttata. Jpn J Ophthalmol 45:93-98

40. Kitagawa K, Kojima M, Sasaki H, Shui YB, Chew SJ, Cheng HM, Ono M, Morikawa Y, Sasaki K (2002) Prevalence of primary Cornea guttata and morphology of corneal endothelium in aging Japanese and Singaporean subjects. Ophthalmic Res 34:135-138

41. Krabcova I, Studeny P, Jirsova K (2011) Endothelial cell density before and after the preparation of corneal lamellae for Descemet membrane endothelial keratoplasty with a stromal rim. Cornea 30:1436-1441

42. Krachmer JH, Purcell JJ Jr, Young CW, Bucher KD (1978) Corneal endothelial dystrophy. A study of 64 families. Arch Ophthalmol 96:2036-9

43. Kramp K, Suffo S, Laun D, Bischoff-Jung M, Huber M, Langenbucher A, Seitz B (2020) [Analysis of factors influencing the suitability of donor corneas in the LIONS cornea bank Saar-Lor-Lux, Trier/Westpfalz from 2006 to 2016]. Klin Monbl Augenheilkd 237:1334-1342

44. Laun D, Suffo S, Kramp K, Bischoff M, Huber M, Langenbucher A, Seitz B (2021) [How implementing a quality management system at the LIONS eye bank Saar-Lor-Lux, Trier/Western Palatinate from 2006 to 2016 impacted the rate and reasons for discarding human organ-cultured corneas.] Klin Monbl Augenheilkd [Epub ahead of print]

45. LeCun Y, Bengio Y, Hinton G (2015) Deep learning. Nature 521:436-444

46. Lie J, Birbal R, Ham L, van der Wees J, Melles GR (2008) Donor tissue preparation for Descemet membrane endothelial keratoplasty. J Cataract Refract Surg 34:1578-1583

47. Lisch W, Seitz B (2012) [Endothelial Corneal Dystrophies (CD) – Diagnosis and Therapy]. Klin Monbl Augenheilkd 229:594-602

48. Lorenzetti DW, Uotila MH, Parikh N, Kaufman HE (1967) Central Cornea guttata: Incidence in the general population. Am J Ophthalmol 64:1155-1158

49. Martin C, Tschernig T, Loic H, Daas L, Seitz B (2020) Corneae from body donors in anatomy department: valuable use for clinical transplantation and experimental research. BMC Ophthalmol 20:284

50. Mäurer S, Asi F, Rawer A, Damian A, Seitz B, Langenbucher A, Eppig T (2019) [Concept for 3D measurement of corneal donor tissue using a clinical OCT]. Ophthalmologe 116:640-646

51. Mayko ZM, Benetz BA, Menegay H, Donovan CP, Stoeger CG, Terry MA, Lass JH (2016) Donor endothelial cell density measurements do not change immediately after DMEK preparation. Cornea 35:1556-1561

52. McCarey B, Edelhauser H, Lynn M (2000) Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices and new intraocular drugs and solutions. Cornea 27:1-16

53. Melles GR, Eggink FA, Lander F, Pels E, Rietveld FJ, Beekhuis WH, Binder PS (1998) A surgical technique for posterior lamellar keratoplasty. Cornea 17:618-626

54. Melles GR, Ong TS, Ververs B, van der Wees J (2006) Descemet membrane endothelial keratoplasty (DMEK). Cornea 25:987-990

55. Melles GR, Ong TS, Ververs B, van der Wees J (2008) Preliminary clinical results of Descemet membrane endothelial keratoplasty. Am J Ophthalmol 145:222-227

56. Menzel-Severing J, Walter P, Plum W, Kruse F, Salla S (2018) Assessment of corneal endothelium during continued organ culture of pre-stripped human donor tissue for DMEK surgery. Curr Eye Res 43:1439-1444

57. Monnereau C, Bruinsma M, Ham L, Baydoun L, Oellerich S, Melles GR (2014) Endothelial cell changes as an indicator for upcoming allograft rejection following Descemet membrane endothelial keratoplasty. Am J Ophthalmol 158:485-95

58. Muraine M, Gueudry J, He Z, Piselli S, Lefevre S, Toubeau D (2013) Novel technique for the preparation of corneal grafts for Descemet membrane endothelial keratoplasty. Am J Ophthalmol 156:851-859

59. Nahum Y, Canton V, Ponzin D, Busin M (2015) Prevalence of guttae in the graft following corneal transplantation. Br J Ophthalmol 99:1660-1663

60. Nakashima Y, Yoshitomi F, Oshika T (2007) Clinical evaluation of cornea pseudoguttata. Br J Ophthalmol 91:22-5

61. Naumann GO, Schlötzer-Schrehardt U (2000) Keratopathy in pseudoexfoliation syndrome as a cause of corneal endothelial decompensation: a clinicopathologic study. Ophthalmology 107:1111-24

62. Oie Y, Watanabe S, Nishida K (2016) Evaluation of visual quality in patients with Fuchs endothelial corneal dystrophy. Cornea 35 Suppl 1:S55-S58

63. Parekh M, Ruzza A, Ferrari S, Busin M, Ponzin D (2016) Preloaded tissues for Descemet membrane endothelial keratoplasty. Am J Ophthalmol 166:120-125

64. Price MO, Feng MT, Price FW Jr (2021) Endothelial Keratoplasty Update 2020. Cornea 40:541-547

65. Quintin A, Hamon L, Mäurer S, Langenbucher A, Seitz B (2021) [Comparison of sterile donor tomography in the eye bank and graft tomography after penetrating keratoplasty]. Ophthalmologe 118:1038-1044

66. Quintin A, Hamon L, Mäurer S, Langenbucher A, Seitz B (2021) OCT application for sterile corneal graft screening in the eye bank. Klin Monbl Augenheilkd 238:688-692

67. Rajesh S, Noopur G, Namrata S, Jeewan T, Rasik V (2010) Advances in keratoplasty procedures: A review. Indian J Ophthalmol 58:457-463

68. Röck T, Landenberger J, Bramkamp M, Bartz-Schmidt KU, Röck D (2017) The evolution of corneal transplantation. Ann Transplant 22:749-754

69. Safi T, Daas L, Seitz (2020) Preoperative guttae screening of the donor corneas. eLetter Br J Ophthalmol. URL: https://bjo.bmj.com/content/99/12/1660.responses

70. Safi T, Seitz B, Berg K, Schulz K, Langenbucher A, Daas L (2021) Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage. Am J Ophthalmol 221:17-26

71. Safi T, Daas L, Kiefer GL, Sharma M, Ndiaye A, Deru M, Alexandersson J, Seitz B (2021) Semi-quantitative criteria in the eye bank that correlate with Cornea guttata in donor corneas. Klin Monbl Augenheilkd 238:680-687

72. Schallhorn J, Holiman J, Stoeger C, Chamberlain W (2016) Quantification and patterns of endothelial cell loss due to eye bank preparation and injector method in Descemet membrane endothelial keratoplasty tissues. Cornea 35:377-382

73. Schmidt I, Schlötzer-Schrehardt U, Langenbucher A, Eppig T, Hager T, Zimpfer A, Seitz B (2019) Ultrastructural findings in graft failure after Descemet membrane endothelial keratoplasty (DMEK) and new triple procedure. Medicine 98:e15493

74. Schmitz LM, Safi T, Munteanu C, Seitz B, Daas L (2022) Prevalence and severity of cornea guttata in the graft following Descemet Membrane Endothelial Keratoplasty (DMEK). Acta Ophthalmol. Doi: 10.1111/aos.15195. [Epub ahead of print]

75. Schönit S, Maamri A, Zemova E, Munteanu C, Safi T, Daas L, Seitz B (2022) Prevalence and impact of Cornea guttata in the graft after penetrating keratoplasty in Germany. Cornea. Doi:10.1097/ico.0000000000002971. [Epub ahead of print]

76. Schroeter J, Maier P, Bednarz J, Blüthner K, Quenzel M, Pruss A, Reinhard T (2009) [Procedural guidelines. Good tissue practice for cornea banks]. Ophthalmologe 106:265-276

77. Schroeter J, Rieck P (2009) Endothelial evaluation in the cornea bank. Dev Ophthalmol 43:47-62

78. Seitz B, Müller EE, Langenbucher A, Kus MM, Naumann GO (1995) [Endothelial keratopathy in pseudoexfoliation syndrome: quantitative and qualitative morphometry using automated video image analysis]. Klin Monbl Augenheilkd 207:167-175

79. Seitz B, Müller EE, Langenbucher A, Kus MM, Naumann GO (1997) [Reproducibility and validity of a new automatic method of specular microscopy analysis of corneal endothelium]. Ophthalmologe 94:127-135

80. Seitz B, El-Husseiny M, Langenbucher A, Szentmáry N (2013) [Prophylaxis and management of complications in penetrating keratoplasty]. Ophthalmologe 110:605-613

81. Seitz B, Daas L, Bischoff-Jung M, Szentmáry N, Suffo S, El-Husseiny M, Viestenz A, Milioti G (2017) Anatomy-based DMEK Wetlab in Homburg/Saar: novel aspects of donor preparation and host maneuvers to teach Descemet membrane endothelial keratoplasty. Clin Anat 31:16-27

82. Seitz B, Daas L, Flockerzi E, Suffo S (2020) [Descemet membrane endothelial keratoplasty DMEK - Donor and recipient step by step]. Ophthalmologe 117:811-828

83. Sheng H, Bullimore MA (2007) Factors affecting corneal endothelial morphology. Cornea 26:520-525

84. Son H-S, Villarreal G, Meng H, Eberhart CG, Jun AS (2014) On the origin of 'guttae'. Br J Ophthalmol 98:1308.1-1310

85. Tan DT, Dart JK, Holland EJ, Kinoshita S (2012) Corneal transplantation. Lancet 379:1749-1761

86. Tavakol M, Dennick R (2011) Making sense of Cronbach's alpha. Int J Med Educ 2:53-55

87. Terry M (2012) Endothelial keratoplasty. Cornea 31:469-471

88. Tran K, Dye P, Odell K, Galloway J, Stoeger C, Straiko M, Terry M (2017) Evaluation and quality assessment of pre-stripped, preloaded Descemet membrane endothelial keratoplasty grafts. Cornea 36:484-490

89. Vogt A (1921) Weitere Ergebnisse der Spaltlampenmikroskopie des vorderen Bulbusabschnittes: (Cornea, Vorderkammer, Iris, Linse, vorderer Glaskörper, Conjunctiva, Lidränder.) I. Abschnitt: Hornhaut Graefes Arch Clin Exp Ophthalmol 106:63-103

90. Wacker K, Grewing V, Fritz M, Böhringer D, Reinhard T (2020) Morphological and optical determinants of visual disability in Fuchs endothelial corneal dystrophy. Cornea 39:726-731

91. Wacker K, McLaren JW, Amin SR, Baratz KH, Patel SV (2015) Corneal high-order aberrations and backscatter in Fuchs endothelial corneal dystrophy. Ophthalmology 122:1645-1652

92. Wacker K, Reinhard T, Maier P (2019) [Pathogenesis and diagnostic evaluation of Fuchs' endothelial corneal dystrophy]. Ophthalmologe 116:221-227

93. Waring G 3rd, Rodrigues M, Laibson P (1978) Corneal dystrophies. II. Endothelial dystrophies Surv Ophthalmol 23:147-168

94. Watanabe S, Oie Y, Fujimoto H, Soma T, Koh S, Tsujikawa M, Maeda N, Nishida K (2015) Relationship between corneal guttae and quality of vision in patients with mild Fuchs' endothelial corneal dystrophy. Ophthalmology 122:2103-2109

95. Weiss J, Møller H, Aldave A, Seitz B, Bredrup C, Kivelä T, Munier F, Rapuano C, Nischal K, Kim E, Sutphin J, Busin M, Labbé A, Kenyon K, Kinoshita S, Lisch W (2015) IC3D classification of corneal dystrophies – edition 2. Cornea 34:117-159

96. Wilson SE, Bourne WM (1988) Fuchs' dystrophy. Cornea 7:2-18

97. Yee RW, Matsuda M, Schultz RO, Edelhauser HF (1985) Changes in the normal corneal endothelial cellular pattern as a function of age. Curr Eye Res 4:671-678

98. Yoeruek E, Bayyoud T, Hofmann J, Bartz-Schmidt KU (2013) Novel maneuver facilitating Descemet membrane unfolding in the anterior chamber. Cornea 32:370-373

99. Zantos SG, Holden BA (1981) Guttate endothelial changes with anterior eye inflammation. Br J Ophthalmol 65:101-3

100. Zoega GM, Fujisawa A, Sasaki H, Kubota A, Sasaki K, Kitagawa K, Jonasson F (2006) Prevalence and risk factors for Cornea guttata in the Reykjavik Eye Study. Ophthalmology 113:565-569

101. Zoega GM, Arnarsson A, Sasaki H, Söderberg PG, Jonasson F (2013) The 7-year cumulative incidence of Cornea guttata and morphological changes in the corneal endothelium in the Reykjavik Eye Study. Acta Ophthalmol 91:212-218

7. Author´s Publication List:

Publications:

1. Chalhoub K, Abou Zahr R, **Safi T**, Zgheib J, Nohra J (2019) Rare renal vascular anomaly: a challenge in laparoscopic nephrectomy. Videoscopy 29

2. **Safi T**, Salameh P, Aoude L, Waked M, Kobrossy B (2019) EPR19-072: Breast cancer laterality and immunohistochemical characteristics. J Natl Compr Cancer Netw 17:EPR19-072

3. Ghosn Y, Hussein Kamareddine M, Abdessamad H, **Safi T**, Adem C, Jabbour R (2019) Fulminant Guillain-Barré syndrome (GBS) with a constellation of uncommon clinical manifestations: differential diagnosis and treatment challenges. J Case Rep 157-161

4. **Safi T**, Daas L, Seitz (2020) Preoperative guttae screening of the donor corneas. eLetter Br J Ophthalmol. URL: https://bjo.bmj.com/content/99/12/1660.responses

5. **Safi T**, Seitz B, Berg K, Schulz K, Langenbucher A, Daas L (2021) Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage. Am J Ophthalmol 221:17-26

6. Daas L, Hamon L, Ardjomand N, **Safi T**, Seitz B (2021) Excimer laser-assisted DALK: a case report from the homburg keratoconus center (HKC). Ophthalmologe 118:1245-1248

7. **Safi T**, Daas L, Kiefer GL, Sharma M, Ndiaye A, Deru M, Alexandersson J, Seitz B (2021) Semi-quantitative criteria in the eye bank that correlate with Cornea guttata in donor corneas. Klin Monbl Augenheilkd 238:680-687

8. Abou Zahr R, Chalhoub K, Akl B, **Safi T**, El Khoury F (2021) Flexible ureteroscopy in crossed fused renal ectopia. Urology Video Journal. 11:100093

9. El Tom J, Serhan A, **Safi T**, Kabbara W (2021) Summary of the clinical pharmacist's role in the management of acute pancreatitis: a clinical review. Int J Hosp Pharm 6:41

10. **Safi T**, Seitz B, Flockerzi E, Flockerzi F, Daas L (Published online 2021) Haze after PRK (without use of mitomycin C) on a pretreated cornea with DALK and

LASIK - clinical and histopathological findings. Klin Monbl Augenheilkd. Doi:10.1055/a-1675-2696

11. Schönit S, Maamri A, Zemova E, Munteanu C, **Safi T**, Daas L, Seitz B (2022) Prevalence and impact of Cornea guttata in the graft after penetrating keratoplasty in Germany. Cornea. Doi:10.1097/ico.0000000000002971. [Epub ahead of print]

12. Schmitz LM, **Safi T**, Munteanu C, Seitz B, Daas L (2022) Prevalence and severity of cornea guttata in the graft following Descemet Membrane Endothelial Keratoplasty (DMEK). Acta Ophthalmol. Doi: 10.1111/aos.15195. [Epub ahead of print]

Presentations, Posters

1. **Safi T**, Salameh P, Aoude L, Waked M, Kobrossy B. EPR19-072: Breast cancer laterality and immunohistochemical characteristics. March 2019, National Comprehensive Cancer Network 2019 Annual Conference, Orlando, U.S.A.

2. **Safi T**, Seitz B, Berg K, Schulz K, Langenbucher A, Daas L. [Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage]. November 2019, 92th Meeting of Ophthalmologists from the Rhein-Main Region, Darmstadt, Germany – 1st Price.

3. **Safi T**, Seitz B, Berg K, Schulz K, Langenbucher A, Daas L. [Reproducibility of non-invasive endothelial cell loss assessment of the pre-stripped DMEK roll after preparation and storage]. September 2020, Annual Meeting of the German Ophthalmological Society, online congress, Germany.

4. **Safi T**, Daas L, Kiefer GL, Sharma M, Ndiaye A, Deru M, Alexandersson J, Seitz B. Semi-quantitative criteria in the eye bank that correlate with Cornea guttata in donor corneas. March 2021, Virtual 3D INTERREG CONGRESS interregional & interprofessional: Simulation – Virtuality – Digitalization, online congress, Germany.

5. **Safi T**, Daas L, Kiefer GL, Sharma M, Ndiaye A, Deru M, Alexandersson J, Seitz B. Semi-quantitative criteria in the eye bank that correlate with Cornea guttata in donor corneas. September 2021, Annual Meeting of the German Ophthalmological Society, online congress, Germany.

6. **Safi T**, Daas L, Kiefer GL, Nadig M, Sharma M, Sakha M, Ndiaye A, Deru M, Alexandersson J, Seitz B. An artificial-intelligence-based decision support tool for the detection of Cornea guttata and the assessment of the donor corneas in the eye bank. May 2022, Annual Meeting of the Association for Research in Vision and Ophthalmology, Denver, Colorado, U.S.A

8. Acknowledgment:

I would like to express my special gratitude to the following persons, without whom would have the preparation of this dissertation never been possible.

First of all, I would like to thank my Doctoral father and director of the Department of Ophthalmology at Saarland University Hospital, **Professor Dr. Berthold Seitz**. He gave me the topic, had great confidence in me and encouraged me to participate in many congresses and to publish several articles.

Special thanks go to my supervisor **Dr. Loay Daas**, senior consultant of the Department of Ophthalmology at Saarland University Hospital, for his helpful supervision. I had many intensive discussions with him about different subjects, from which I was able to extensively increase my knowledge about this topic.

A big thanks goes of course to the **Klaus Faber Center for Corneal Diseases, incl. LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz**, who provided us with all necessary materials, data and support. Furthermore, I would like to thank the German Research Center for Artificial Intelligence and especially **Dr.-Ing. Jan Alexandersson, Dr.-Ing. Matthieu Deru and Dr.-Ing. Alassane Ndiaye** for their cooperation, enthusiasm and willingness to take our project one step further by integrating artificial intelligence.

Of course, such a project was impossible to achieve without the support of my family. This support has been given to me in a special way by my parents, to whom I dedicate this work. Finally, I would like to thank everyone who has contributed in their own way to the successful completion of this thesis.

The curriculum vitae was removed from the electronic version of the doctoral thesis for reasons of data protection.

Tag der mündlichen Prüfung: 13.02.2023

Dekan: Univ.-Prof. Dr. Michael D. Menger

1. Berichterstatter: Prof. Dr. Berthold Seitz

2. Berichterstatter: Prof. Dr. Carola Meier