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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

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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).

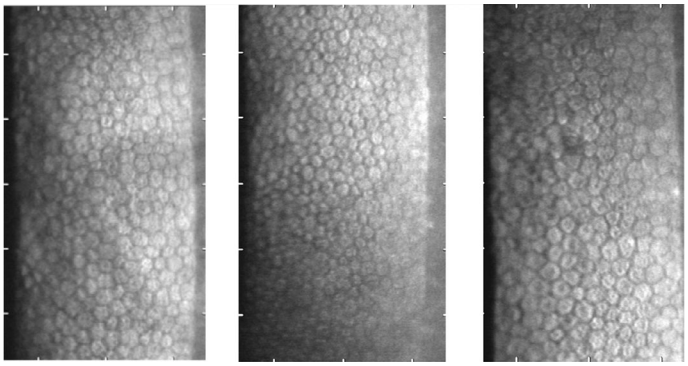
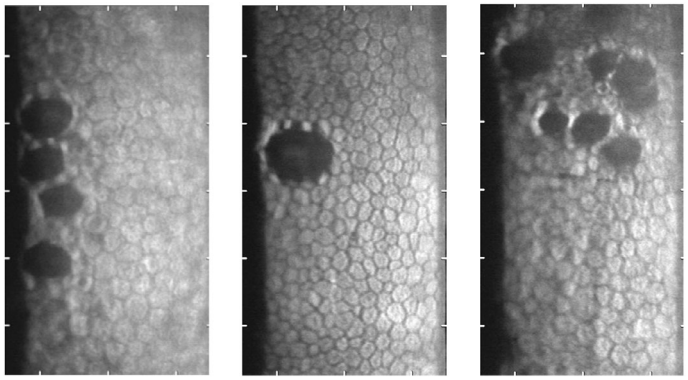
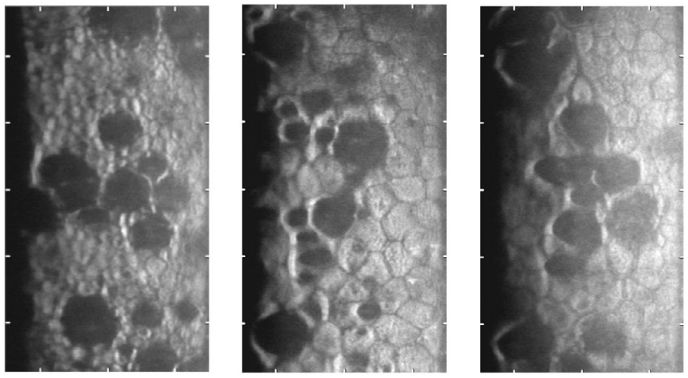
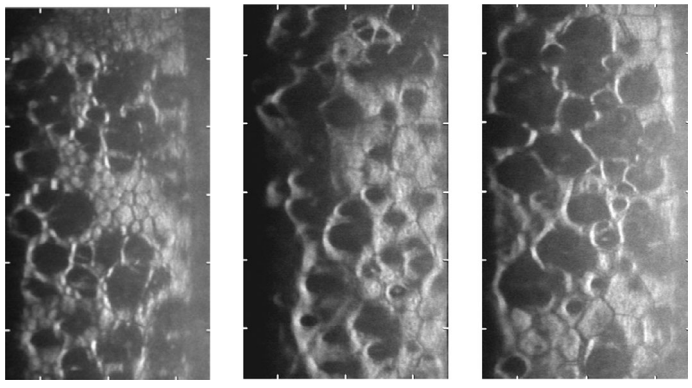
Overall, 56 (8.5%) cases showed a progression either from healthy corneas with G0 to corneas with any grade of CG or from a low-grade to a high-grade CG. At the end of the follow-up period, 513 (77.3%) of the population remained classified as a G0, 113 (17.0%) eyes showed G1, 18 (2.7%) eyes G2 and 20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

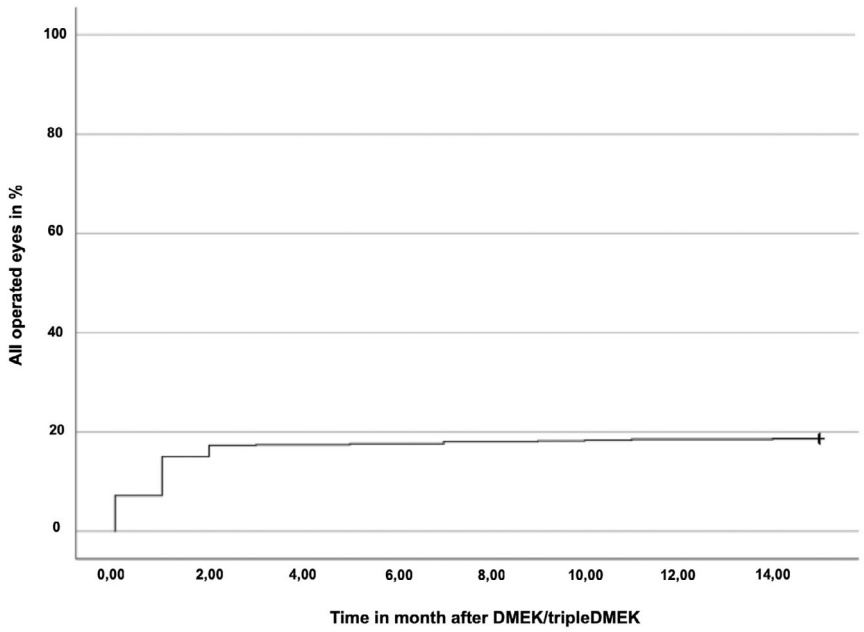


Fig. 1. Kaplan–Meier curve of the first cornea guttata (CG) detection immediately after DMEK/triple-DMEK.

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

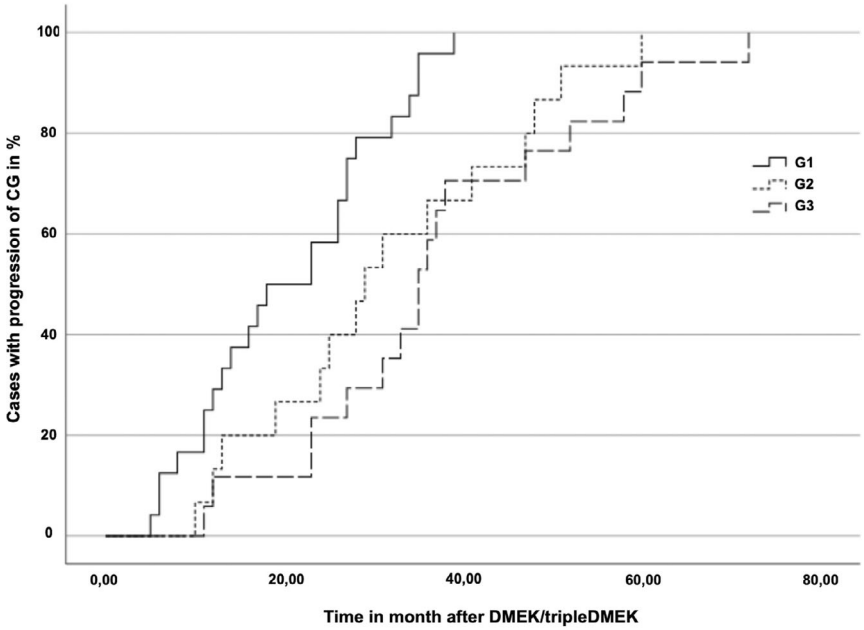


Fig. 2. Kaplan–Meier curves illustrating the progression of the cornea guttata (CG) during the follow-up to the maximum expression (continuous curve: Progression from healthy cornea (G0) to cornea guttata grade 1 (G1); dotted curve: Progression from healthy or low-grade cornea guttata (G0/G1) to cornea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

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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