CUTANEOUS AND OCULAR TOXICOLOGY
https://doi.org/10.1080/15569527.2021.1937206

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Corneal endothelial cell density in patients receiving chemotherapy

Shawn P. Gallagher[a], Barton L. Halpern[b] and Shanthi Sivendran[c,d]

[a]Department of Psychology, Millersville University of Pennsylvania, Millersville, PA, USA; [b]Eye Doctors of Lancaster, Lancaster, PA, USA; [c]Penn Medicine Lancaster General Health, Lancaster, PA, USA; [d]Hematology/Oncology Medical Specialists, Lancaster, PA, USA

ABSTRACT

Purpose: This study aimed to determine if the corneal endothelium was affected by chemotherapy.
Methods: Chemotherapy patients were recruited to undergo specular microscopy before treatment and again at 1- and 2-year follow-up visits. One eye per patient, per follow-up, was selected for comparison to baseline.
Results: Forty-six volunteers completed baseline and at least one follow-up assessment. From 51 eyes, there was no significant change in endothelial cell density for 41 eyes assessed at one year (M_D = 0.73%, 95% CI −1.33 to 2.78%) and 18 eyes at two years (M_D = 0.31%, 95% CI −3.53 to 4.15%).
Conclusion: Although other studies have shown that chemotherapy can adversely affect the corneal epithelium, this study showed no measurable change in endothelial cell density.

ARTICLE HISTORY
Received 21 February 2021
Revised 20 May 2021
Accepted 25 May 2021

KEYWORDS
Chemotherapy; ocular toxicology; corneal epithelium; corneal endothelium

Introduction

Corneal transplant surgery (keratoplasty) is the world's most common transplant procedure. In 2019, the Eye Bank Association of America (EBAA) supplied tissue for 51 336 corneal transplants in the United States[1]. In 2005, nearly 95% of this tissue was used for full-thickness penetrating keratoplasty (PK) but that number has dropped with advances in lamellar keratoplasty that allow surgeons to, when appropriate, selectively replace the affected deep lamellae[2,3]. The increase in disease-specific surgery calls for an assessment of events in a donor's medical history that could differentially affect the corneal layers. To this end, the following study assessed the impact of systemic chemotherapy on the corneal endothelium.

Cancer is the second leading cause of death in the United States[4], and cancer victims accounted for 19% of the grafts in two large-scale studies exploring factors that may affect cornea transplant success[5–7]. Although the investigations did not identify cancer as a complication risk for PK, the disease had one of the highest hazard ratios of all donor characteristics in a broad, and therefore low-powered ($\alpha = 0.01$), analysis[6].

Most cancers are epithelial cell cancers (carcinomas), and anti-neoplastic chemotherapies can have unintended effects on a range of noncancerous mitotic cells, including those in the corneal epithelium. For example, when intraocular methotrexate is used to treat ocular pathology, the corneal epithelium can show signs of inflammation while the non-mitotic endothelium appears unaffected[8–13].

When the pathology is not ocular, ophthalmic complications from systemic chemotherapy are probably underreported due to the priority given to the primary diagnosis, but many reports describe keratopathy from other antineoplastics that is similar to that associated with intraocular methotrexate[14–22]. One study describing 120 PK cases found that 5 of 29 corneas (17%) from cancer victims who received recent systemic chemotherapy developed significant subepithelial opacification, possibly due to antimetabolites in donor tear film that led to surface disease in the recipients[23]. Histopathology of the failed grafts suggested that the non-mitotic endothelial cells were unaffected. None of the other 91 cases developed corneal opacities.

Corneal endothelial cells can be quickly photographed with a non-contact specular microscope. The shape and density of the cells can then be quantified and used as an indicator of endothelial function[24]. The objective of this study was to assess endothelial cell density with specular microscopy before and after patients received systemic chemotherapy. This study complements a 6-month follow-up study on the effects of intraocular methotrexate on endothelial cell density by reporting the effects of systemic chemotherapy at a longer follow-up interval[13]. Findings could inform corneal transplant surgeons and oncologists who wish to know more about the ocular effects of treatment.

Materials and methods

This prospective study was approved by the Lancaster General Health Institutional Review Board (Protocol: 2013–71). Cancer patient volunteers scheduled to begin systemic chemotherapy were enrolled at the Ann B. Barshinger Cancer Institute between October 2014 and September 2016. They completed a brief ocular history survey and agreed to undergo specular microscopy before beginning

CONTACT Shawn P. Gallagher sgallagher@millersville.edu Department of Psychology, Millersville University of Pennsylvania, PO Box 1002, Millersville, PA 17551, USA

chemotherapy and again at 1- and 2-year follow-up intervals. Patients were excluded if they had a history of eye disease or eye surgery, if they underwent eye surgery during the follow-up period, or if they underwent radiation therapy to the head or neck prior to or during treatment. Demographic information collected for this study included date of birth, sex, and race/ethnicity drawn from medical records.

During regular treatment visits, the central corneal endothelium of each eye was photographed by a trained staff member using a Konan Noncon Robo Pachy SP-9000 Specular Microscope (Konan Medical, Irvine, California) calibrated according to manufacturer specifications. Except for the fact that not all images were obtained by the same staff member, the methods were modelled according to the recommendations for using a specular microscope in clinical trials[24]. During image acquisition, an examiner oriented a seated patient towards the microscope and instructed them to fixate on an LED target. A button press activated the autofocus function which placed the focal plane approximately 500 μm behind the epithelial surface before an image was automatically captured. Digital images of the central endothelium were stored on a computer and a single investigator (S.G.), masked to all patient information except the identification code, date of examination, and eye, used the Konan KSS-300 software to evaluate the best quality image of each eye from each patient, for each exam. Image quality was classified according to criteria established for the Cornea Donor Study[25]. If the image displayed a clear and complete array of cells, it was scored *Excellent*. If the image did not show a full array but showed at least one group of 50 contiguous cells in the centre of the field, it was scored *Good*. If the image showed more than 50 cells, but no groups of at least 50 contiguous cells, it was scored *Fair*. If the image showed fewer than 50 cells, it was deemed ungradable and scored *Poor*. Eyes with images graded *Fair* or *Poor* were excluded from the study to minimize the effect that image quality would have on reliability[24].

The centre-dot method, which requires an operator to mark the centre of visible cells, was used to generate a single central endothelial density measurement (cells/mm^2) for the remaining images. The software also calculated a coefficient of variation, the degree of variation in the sizes of the cells (polymegethism), and hexagonality, a measure of what percentage of the cells fit an "ideal" shape. Hypothesis tests were performed using Microsoft Excel and Student's *t*-tables to calculate the 95% confidence intervals for mean percent change from baseline to 1-year and baseline to 2-year measurements. Reliability was assessed with Pearson's correlation ($\alpha = 0.05$). Calculations used only one randomly selected eye from each patient at each follow-up interval for comparison to that eye's baseline. If, for a given follow-up interval, the image from one eye was graded *Poor* or *Fair* while its fellow was graded *Good* or *Excellent*, the eye with the better image was selected nonrandomly.

Results

Forty-six volunteer patients (30 female and 16 male) underwent baseline imaging and at least one follow-up assessment. The ethnic demography of the sample reflected that of the local population[26]: White, non-Hispanic 91.3% (42/46); African American 2.2% (1/46); White, Hispanic/Latino 2.2% (1/46), Unidentified 4.4% (2/46), [χ^2 (3, $n = 46$) = 2.55, $p = 0.47$]. The primary diagnoses are summarized in Table 1. At baseline, this group had a mean (*SD*) age of 58.15 (9.52) and a range of 35–80 years and the 51 eyes under consideration had a mean endothelial cell density of 2577 (370) and a range of 1855–3300 cells/mm^2. The coefficient of variation had a mean of 0.32 (0.05) and hexagonality was 58.55% (7.64%). All three of these means are typical for a sample of this age range[24,27,28]. Although the visits did not include eye examinations, the patients were surveyed at each visit and reported no ocular changes or complaints when asked.

Table 1. Primary diagnoses of patients evaluated at each follow-up interval.

Primary diagnosis	Number of patients in 1-year sample, *n* (%) $n = 41$	Number of patients in 2-year sample *n* (%) $n = 18$
Bladder	1 (2)	0 (0)
Breast	15 (37)	6 (33)
Cholangiocarcinoma	1 (2)	1 (6)
Colon	2 (5)	0 (0)
Endometrial	0 (0)	1 (6)
Gastric	1 (2)	0 (0)
Gastroesophageal junction	1 (2)	1 (6)
Head and neck	2 (5)	0 (0)
Lung	3 (7)	2 (11)
Lymphoma	2 (5)	1 (6)
Multiple myeloma	1 (2)	0 (0)
Ovarian	3 (7)	2 (11)
Pancreatic	1 (2)	1 (6)
Peritoneal	1 (2)	1 (6)
Prostate	1 (2)	0 (0)
Rectal	3 (7)	1 (6)
Testicular	1 (2)	0 (0)
Urothelial	2 (5)	1 (6)

Rounding errors prevent percentages from adding up to 100%.

There was no significant change in endothelial cell density for 41 patient eyes at one year (M_D = 0.73%, 95% CI −1.33 to 2.78%) and 18 eyes at two years (M_D = 0.31%, 95% CI −3.53 to 4.15%) after beginning chemotherapy (Figure 1). The mean endothelial cell density for the 41 eyes measured at one year was 2584 (367) with a range of 1862–3472 cells/mm^2 and the mean endothelial cell density for the 18 eyes measured at two years was 2618 (390) with a range of 1828–3468 cells/mm^2. Consistent with these findings, the baseline and follow-up measurements were significantly correlated at 1 year, [$r(39) = 0.90$, $p < 0.05$], and 2 years, [$r(16) = 0.89$, $p < 0.05$].

Administered chemotherapies were classified *post hoc* into five categories by an oncologist (S.S.) and percent differences were evaluated for subsets of patients who received each type of treatment. There was no significant change in cell density associated with Alkylating Agents, Antimetabolites, Monoclonal Antibodies, Taxane/Antimicrotubulars, or Topoisomerase I and Topoisomerase II Inhibitors (Table 2). It should be noted that these analyses are not independent because most patients received more than one type of chemotherapy and are therefore represented in multiple groups. Two groups had a sample size of only two at 2 years. Once initiated, treatment regimens were not changed for patients in this sample.

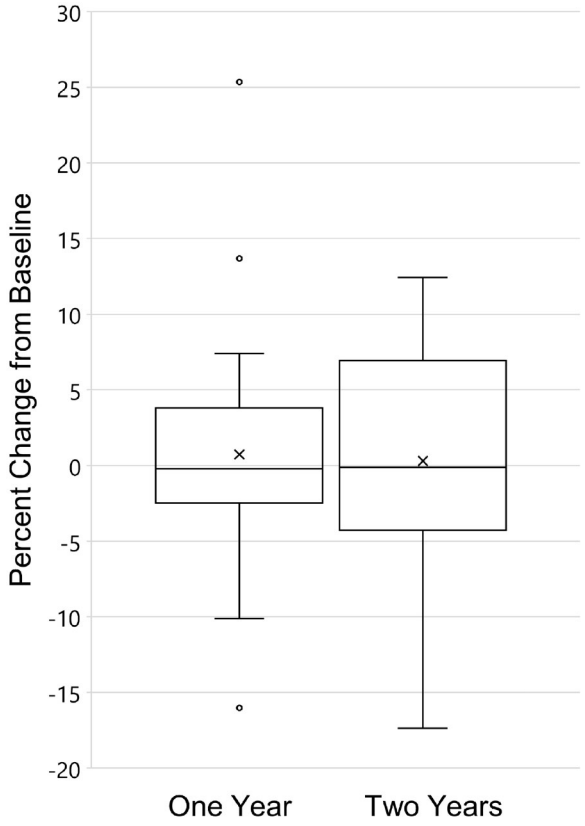

Figure 1. Box and Whisker plots of the distribution of percent change in endothelial cell density for 41 patient eyes at one year and 18 eyes at two years after beginning chemotherapy. Lower and upper box boundaries show 25th and 75th percentiles, respectively, the lines inside boxes show medians, whiskers show minimum and maximum values excluding 3 outliers denoted by circles and defined as values more than 1.5 times the IQR beyond the proximate quartile. There was no significant change in mean (x) endothelial cell density at 1 year (M_D = 0.73%, 95% CI −1.33 to 2.78%) or 2 years (M_D = 0.31%, 95% CI −3.53 to 4.15%).

Table 2. Percent change in endothelial cell density by chemotherapy type.

Systemic chemotherapy	Mean percent change from baseline at 1 year (95% CI, *n*)	Mean percent change from baseline at 2 years (95% CI, *n*)
Alkylating agents	0.81 (±2.24, 37)	−0.29 (±4.52, 15)
Antimetabolites	−0.35 (±4.46, 11)	−1.96 (±9.07, 6)
Monoclonal antibodies	0.42 (±9.68, 8)	1.73 (±102.37, 2)
Taxane/antimicrotubulars	0.93 (±3.24, 21)	−3.00 (±42.18, 2)
Topoisomerase I/II inhibitors	0.58 (±2.41, 16)	0.29 (±4.05, 13)

All confidence intervals encompass 0% change. Most patients received more than one type of therapy and are therefore represented in multiple therapy groups. Alkylating Agents: Bendamustine, Carboplatin, Cisplatin, Cyclophosphamide, Dacarbazine, Oxaliplatin. Antimetabolites: 5-floururacil, Capecitabine, Gemcitabine, Methotrexate, Pemetrexed. Monoclonal Antibodies: Pertuzumab, Rituximab, Trastuzumab. Taxane/Antimicrotubulars: Docetaxel, Paclitaxel, Vinblastine. Topoisomerase I/II Inhibitors: Doxorubicin, Etoposide, Irinotecan.

The types of cancer varied in this patient sample and the fifteen breast cancer patients (Table 1) represented the only diagnosis that constituted more than ten percent of those evaluated at one year. 13 of these patients received similar treatment regimens that included an alkylating agent and a topoisomerase inhibitor. This subset showed no significant change in endothelial density (M_D = 2.20%, 95% CI −2.50 to 6.90%).

Discussion

This prospective study assessed the corneal endothelium of cancer patients before and after systemic chemotherapy and revealed no significant change in cell density for up to two years after treatment. Our results are consistent with the normal density change of −0.5% per year that occurs through adulthood but is undetectable at the measured intervals[24]. Although many studies have described the impact that systemic chemotherapy can have on the corneal surface[14–23], the current findings are consistent with those from another study evaluating the effects of intravitreal methotrexate on the corneal endothelium[13]. They are also consistent with the paucity of data showing endothelial consequences and suggest that grafts are safer for EK when recent exposure to systemic chemotherapy raises concerns about epithelial toxicity[23,29]. Reports of cancerous cells successfully migrating from donor to recipient are vanishingly rare given that eye banks understand the risks that many diseases pose and they universally reject corneas from donors with haematologic malignancies, anterior segment tumours, and retinoblastoma[30,31]. Assuming that a donor cornea meets EBAA standards, mitigating concerns that systemic antimetabolite therapy might compromise the endothelium should promote the use of corneal tissue from cancer patients for EK.

The participants in this study were all cancer patients and we cannot comment on the ocular consequences of, for example, systemic antimetabolite treatment for autoimmune disease. Additionally, we had no control group, and our patients did not present all forms of cancer nor did they receive every possible type of chemotherapeutic agent administered through every possible route. Future studies addressing specific types of cancers might uncover mechanisms by which disease and treatment can interact to affect the corneal endothelium in unforeseen ways. Our result showing no change in the breast cancer patients receiving a common regimen of chemotherapy is an encouraging finding, at least for this subset of patients, but future studies controlling for cancer type and treatment type would be informative. Notably, most patients in this study were treated for common epithelial cell cancers (carcinomas) and the corneal effects of treatments targeting endothelial cancers warrant separate consideration.

Patients who received other types of anticancer treatment such as targeted therapy or immunotherapy were excluded from the study. Additionally, patients who receive direct treatment to the head or neck, like intraarterial chemotherapy to the carotid, can suffer from unique ocular complications that should preclude them from serving as cornea donors[32–34].

Of course, mortality affected data collection and some patients chose to participate at irregular intervals after their baseline assessment. It is not known if these factors biased the results. A previous study of 946 donor eyes led investigators to suspect that cancer *and* advanced age (>75 years) could interact and accelerate the loss of endothelial cells[35]. Most of the current patients were under 75 and, although they showed no evidence of adverse effects within two years of starting chemotherapy, the impact that a prolonged bout

with cancer and the associated cachexia would have on the corneal endothelium remains unknown.

There have been recent changes in automated endothelial cell photography and newer fully automated algorithms for calculating cell density were not employed in this investigation. However, studies that have assessed consistency across different specular microscopes and reliability within devices have shown only marginal improvements in validity and reliability over the past two decades[24,36,37]. In fact, one study suggested that density counts determined as done here, using the centre-dot method, are more accurate than ones from newer, fully automated methods. The device used here demonstrated high reliability for quality images over one and 2-year periods as indicated by the narrow 95% confidence intervals for mean percent change from baseline at each interval and the high correlations between baseline and follow-up images. These results may reflect the fact that, unlike those pooled in a multicenter study, all images in this study were captured at the same site, by the same microscope, and analyzed by the same individual. These criteria have been identified as hallmarks of an ideal specular microscopy investigation[24].

The current study prioritized cell density as an indicator of endothelial function. A *post-hoc* evaluation of changes in endothelial cell morphology showed a significant percent change for the mean coefficient of variation at one year (M_D = 7.26%, 95% CI 1.01 to 13.52%), but not at two years (M_D = −0.85%, 95% CI −8.60 to 6.91%). The percent change in hexagonality showed no change at one year (M_D = −0.94%, 95% CI −5.75 to 3.86%), but a significant *increase* (improvement) at two years (M_D = 8.72%, 95% CI 1.56 to 15.88%). Unlike endothelial cell density, which is calculated according to an investigator's ability to consistently *identify* a cell, these two measures depend upon the ability to consistently *mark the centre* of a cell, a task that is more difficult to replicate. These *post-hoc* analyses were done without Bonferroni correction and, although it is possible that morphology changes precede measurable changes in endothelial density, these measures are less conclusive than the measures of density.

We cannot comment on chemotherapy's effects on other ocular tissues and, although it would have been beneficial to perform full ophthalmological examinations at the time of these oncological visits, this study used specular microscopy to look for otherwise subclinical corneal changes that would be undetectable with a slit lamp microscope. Additionally, restrictions at the facility made full ocular examinations unfeasible and few patients were willing to undergo off-site eye examinations for the purpose of this investigation.

Cancer victims provide a significant portion of the tissue used for corneal transplant surgery and, although previous investigations have suggested that intraocular and systemic chemotherapy can adversely affect the corneal epithelium, this study adds to a growing body of evidence suggesting that the non-mitotic endothelium is less vulnerable to the effects of antimetabolites.

Acknowledgements

The Louise von Hess Medical Research Grant provided funding support for clinical staff who collected the data and performed specular microscopy for this study. Krista Budzik, BSN, RN, OCN assisted in data collection and supervised the staff collecting the specular microscopy images. Alistair Barber, PhD reviewed and provided valuable feedback on an early draft of this manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Shawn P. Gallagher http://orcid.org/0000-0002-1979-7473
Barton L. Halpern http://orcid.org/0000-0002-9871-5893
Shanthi Sivendran http://orcid.org/0000-0002-3603-4558

References

1. Eye Bank Association of America. EBAA eye banking statistical report. 2020. Available from: www.restoresight.org
2. Park CY, Lee JK, Gore PK, et al. Keratoplasty in the United States: a 10-year review from 2005 through 2014. Ophthalmology 2015;122:2432–2442.
3. Price FW, Jr, Feng MT, Price MO. Evolution of endothelial keratoplasty: where are we headed? Cornea 2015;34:S41–S47.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA A Cancer J Clin 2020;70:7–30.
5. Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. Cornea 2015;34:601–608.
6. Sugar J, Montoya M, Dontchev M, et al. Donor risk factors for graft failure in the cornea donor study. Cornea 2009;28:981–985.
7. Sugar A, Gal RL, Kollman C, et al. Factors associated with corneal graft survival in the cornea donor study. JAMA Ophthalmol 2015;133:246–254.
8. Samson CM, Waheed N, Baltatzis S, Foster CS. Methotrexate therapy for chronic noninfectious uveitis: analysis of a case series of 160 patients. Ophthalmology 2001;108:1134–1139.
9. Smith JR, Rosenbaum JT, Wilson DJ, et al. Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. Ophthalmology 2002;109:1709–1716.
10. Frenkel S, Hendler K, Siegal T, et al. Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. Br J Ophthalmol 2008;92:383–388.
11. Gorovoy I, Prechanond T, Abia M, et al. Toxic corneal epitheliopathy after intravitreal methotrexate and its treatment with oral folic acid. Cornea 2013;32:1171–1173.
12. Jeong Y, Ryu JS, Park UC, Oh JY. Corneal epithelial toxicity after intravitreal methotrexate injection for vitreoretinal lymphoma: clinical and in vitro studies. J Clin Med 2020;9(8): doi:10.3390/jcm9082672.
13. Ghasemi Falavarjani K, Golabi S, Hadavandkhani A. Effect of intravitreal injection of methotrexate on human corneal endothelial cells. Cornea 2016;35:217–219.
14. Schmid KE, Kornek GV, Scheithauer W, Binder S. Update on ocular complications of systemic cancer chemotherapy. Surv Ophthalmol 2006;51:19–40.
15. Harman LE. Ophthalmic complications related to chemotherapy in medically complex patients. Cancer Control 2016;23:150–156.
16. Zinchuk O, Watanabe M, Hayashi N, et al. A case of tamoxifen keratopathy. Arch Ophthalmol 2006;124:1046–1048.
17. Papathanassiou M, Nikita E, Theodossiadis P, et al. Exemestane-induced corneal epithelial changes. Cutan Ocul Toxicol 2010;29:209–211.
18. Karamitsos A, Kokkas V, Goulas A, et al. Ocular surface and tear film abnormalities in women under adjuvant chemotherapy for breast cancer with the 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC) regimen. Hippokratia 2013;17:120–125.

19. Kau H-C, Tsai C-C. Erlotinib-related keratopathy in a patient underwent laser in situ keratomileusis. Cutan Ocul Toxicol 2016;35: 257–259.
20. Hsiao C-C, Yao M, Liu J-H, Chen W-L. Pembrolizumab induced acute corneal toxicity after allogeneic stem cell transplantation. Clin Exp Ophthalmol 2018;46:698–700.
21. Kiratli H, Aygün FB, Gedikoğlu G. Corneal relapse of peripheral T-cell lymphoma under systemic chemotherapy. Cornea 2018;37: 1593–1595.
22. Kuriki R, Hata T, Nakayama K, et al. Tegafur and 5-fluorouracil levels in tears and changes in tear volume in long-term users of the oral anticancer drug S-1. Nagoya J Med Sci 2019;81:415–425.
23. Van Meter WS. Central corneal opacification resulting from recent chemotherapy in corneal donors. T Am Ophthalmol Soc 2007;105: 207–212.
24. McCarey BE, Edelhauser HF, Lynn MJ. Review of corneal endothelial specular microscopy for FDA clinical trials of refractive procedures, surgical devices, and new intraocular drugs and solutions. Cornea 2008;27:1–16.
25. Benetz BA, Gal RL, Ruedy KJ, et al. Specular microscopy ancillary study methods for donor endothelial cell density determination of cornea donor study images. Curr Eye Res 2006;31:319–327.
26. QuickFacts, Lancaster County, Pennsylvania. United States Census Bureau. 2020. Available from: https://www.census.gov/quickfacts/fact/table/lancastercountypennsylvania/PST040219
27. Philips C, Laing R, Yee R. Specular Microscopy. In: Krachmer JH, Mannis MJ, Holland EJ, eds. Cornea, volume one: fundamentals, diagnosis, and management. 2nd ed. Amsterdam (The Netherlands): Elsevier Inc.; 2005.
28. Jones SS, Azar RG, Cristol SM, et al. Effects of laser in situ keratomileusis (LASIK) on the corneal endothelium. Am J Ophthalmol 1998;125:465–471.
29. Redbrake C, Sieben P, Salla S, Reim M. [Effect of donor illness on endothelial cell number of human corneas]. Klin Monatsbl Augenh 1995;206:46–48.
30. Deb-Joardar N, Thuret G, Dumollard J-M, et al. Experimental study of the survival of metastatic cancer cells in corneal organ culture. Invest Ophth Vis Sci 2006;47:1339–1347.
31. Yao X, Lee M, Ying F, et al. Transplanted corneal graft with metastatic cholangiocarcinoma to the donor eye. Eye Contact Lens 2008;34:340–342.
32. Margo CE, Murtagh FR. Ocular and orbital toxicity after intracarotid cisplatin therapy. Am J Ophthalmol 1993;116:508–509.
33. Grimson BS, Mahaley MS, Jr, Dubey HD, Dudka L. Ophthalmic and central nervous system complications following intracarotid BCNU (Carmustine). J Clin Neuro-Ophthal 1981;1:261–264.
34. Watanabe W, Kuwabara R, Nakahara T, et al. Severe ocular and orbital toxicity after intracarotid injection of carboplatin for recurrent glioblastomas. Graefes Arch Clin Exp Ophthalmol 2002;240: 1033–1035.
35. Krohn J, Høvding G. The influence of donor age and cause of death on corneal endothelial cell density. Acta Ophthalmol Scan 2005;83:746–750.
36. Maruoka S, Nakakura S, Matsuo N, et al. Comparison of semi-automated center-dot and fully automated endothelial cell analyses from specular microscopy images. Int Ophthalmol 2018;38:2495–2507.
37. Nichols JJ, Kosunick GM, Bullimore MA. Reliability of corneal thickness and endothelial cell density measures. J Refract Surg 2003;19: 344–352.