BMJ Open Ophthalmology

Preoperative surgeon evaluation of corneal endothelial status: the Viability Control of Human Endothelial Cells before Keratoplasty (V-CHECK) study protocol

Matteo Airaldi ,[1,2] Yalin Zheng ,[3] Francesco Aiello ,[4] Björn Bachmann ,[5] Lamis Baydoun ,[6,7] Sorcha Ní Dhubhghaill ,[8,9] Mor M Dickman ,[10,11] Stephen B Kaye ,[2,3] Luigi Fontana ,[12] Kunal A Gadhvi ,[2] Antonello Moramarco ,[12] Marina Rodriguez Calvo de Mora ,[13,14,15] Carlos Rocha de Lossada ,[13,14,15,16] Vincenzo Scorcia ,[17] Pietro Viola ,[18] Stefano Calza ,[1] Hannah J Levis ,[3] Mohit Parekh ,[19] Alessandro Ruzza ,[20] Stefano Ferrari ,[20] Diego Ponzin ,[20] Francesco Semeraro ,[21] Vito Romano [3,21]

To cite: Airaldi M, Zheng Y, Aiello F, *et al*. Preoperative surgeon evaluation of corneal endothelial status: the Viability Control of Human Endothelial Cells before Keratoplasty (V-CHECK) study protocol. *BMJ Open Ophthalmology* 2023;**8**:e001361. doi:10.1136/bmjophth-2023-001361

This work was presented at the 2023 ESCRS Annual Meeting, held in Vienna, Austria, September 8-12, 2023

Received 21 June 2023
Accepted 31 August 2023

For numbered affiliations see end of article.

Correspondence to
Professor Vito Romano; vito.romano@gmail.com

ABSTRACT

Introduction The success of keratoplasty strongly depends on the health status of the transplanted endothelial cells. Donor corneal tissues are routinely screened for endothelial damage before shipment; however, surgical teams have currently no means of assessing the overall viability of corneal endothelium immediately prior to transplantation. The aim of this study is to validate a preoperative method of evaluating the endothelial health of donor corneal tissues, to assess the proportion of tissues deemed suitable for transplantation by the surgeons and to prospectively record the clinical outcomes of a cohort of patients undergoing keratoplasty in relation to preoperatively defined endothelial viability.

Methods and analysis In this multicentre cohort study, consecutive patients undergoing keratoplasty (perforating keratoplasty, Descemet stripping automated endothelial keratoplasty (DSAEK), ultra-thin DSAEK (UT-DSAEK) or Descemet membrane endothelial keratoplasty) will be enrolled and followed-up for 1 year. Before transplantation, the endothelial viability of the donor corneal tissue will be evaluated preoperatively through trypan blue staining and custom image analysis to estimate the overall percentage of trypan blue-positive areas (TBPAs), a proxy of endothelial damage. Functional and structural outcomes at the end of the follow-up will be correlated with preoperatively assessed TBPA values.

Ethics and dissemination The protocol will be reviewed by the ethical committees of participating centres, with the sponsor centre issuing the final definitive approval. The results will be disseminated on ClinicalTrials.gov, at national and international conferences, by partner patient groups and in open access, peer-reviewed journals.

Trial registration number NCT05847387.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Corneal endothelial cell viability is the main predictor for corneal graft survival. Currently, surgeons have no means to assess the endothelial status independently before transplantation.

WHAT THIS STUDY ADDS

⇒ We developed a simple and widely applicable preoperative method to assess the viability of corneal endothelial cells before keratoplasty.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The proposed method might contribute to further standardise keratoplasty outcomes, improve graft survival and provide eye banks and surgical teams with an accessible tool to validate tissues before transplantation, reinforcing the quality of their services.

INTRODUCTION

Endothelial cell viability is one of the most important determinants of long-term graft survival in corneal transplantation.[1 2] Higher endothelial cell density (ECD) values, both before transplantation and in the early postoperative period, have been associated with better long-term survival rates of lamellar and perforating keratoplasties (PK).[3 4] However, ECD represents only an estimation of total corneal endothelial health and, although ECD loss after keratoplasty has been accurately reported and modelled,[5 6] it is a less than ideal proxy for future endothelial failure.[2 6 7]

In the current chain of supply of corneal tissues for keratoplasty, the surgical team performing the transplantation has no means to independently evaluate the global endothelial cell viability of the graft. This task is in fact relied entirely on the eye bank providing the tissue. Pan-corneal evaluations of corneal endothelium viability using triple staining (Hoechst/ethidium homodimer/calcein-AM combination) suggest that central ECD as measured by eye banks might overestimate the true cell density.[8–10] However, triple staining methods are not reproducible routinely in the operating theatre, as they require the endothelium-Descemet membrane complex to be peeled, incubated with appropriate stains and mounted in imaging chambers or microscope slides for examination, making the tissue no longer fit for transplantation.

The Viability Control of Human Endothelial Cells before Keratoplasty (V-CHECK) is a cohort study that aims to validate a preoperative method to assess the global corneal endothelial viability of donor tissue. The V-CHECK method only employs trypan blue 0.05%, a vital dye widely available in operating theatres which is capable of staining areas of denuded Descemet membrane and areas of endothelial cell damage or mortality.[11 12] Stained donor corneas will be preoperatively assessed with a custom image analysis tool to check for areas of endothelial damage, and the cohort of transplanted patients will be followed over 1 year postoperatively to evaluate the correlation of the V-CHECK method with long-term ECD measurements and graft survival.

By validating this new preoperative assessment of global corneal endothelial viability, we aim to provide eye banks and surgical teams with an accessible tool to perform a final quality assurance before transplantation of the donor tissue and to identify potential issues in their chain of supply.

METHODS AND ANALYSIS

The V-CHECK study protocol has been established according to the Strengthening the Reporting of Observational studies in Epidemiology checklist for cohort studies.

Study design and setting

The V-CHECK study is a multicentre, prospective, single-arm cohort study of patients affected by corneal disease requiring keratoplasty. The purposes of this study are to validate a simple and accessible method of image analysis designed to preoperatively evaluate the viability of the donor corneal graft endothelium in the operating theatre, to report the proportion of tissues that are deemed suitable for transplantation by the surgical teams, and to investigate the relationship of preoperatively measured endothelial viability with the long-term ECD and overall survival of transplanted corneas. The study described adheres to the tenets of the Declaration of Helsinki.

Consecutive patients undergoing keratoplasty at participating sites will be evaluated for inclusion in the cohort study starting from 1 September 2023. After institutional review board (IRB) approval, patient recruitment will last until 30 September 2024, date of primary completion of the study. The start of the follow-up will correspond to the day of surgery. The follow-up of recruited patients will last 1 year, and secondary outcomes will be evaluated at 1, 3, 6 and 12 months after keratoplasty. The study completion date will be 30 September 2025.

Treatment decisions, such as the indication for corneal transplantation, the choice of corneal graft, the timing of surgical treatment and concurrent systemic and/or topical medications administered, will be at the discretion of the treating practitioner in consultation with the patient with no intervention by the investigators, reflecting routine clinical practice.

Participants

Patients undergoing either full thickness corneal transplantation (ie, PK) or endothelial keratoplasty (ie, Descemet stripping automated endothelial keratoplasty [DSAEK], ultra-thin [UT] DSAEK or Descemet membrane endothelial keratoplasty [DMEK) satisfying eligibility criteria will be asked to participate in the study. To avoid the enrolment of patients at higher risk of endothelial cell loss, only patients undergoing procedures considered to be at low risk for graft rejection (ie, keratoconus, Fuchs endothelial dystrophy) will be included, as indications for low-risk surgeries account in fact for the majority of keratoplasties.

The investigators will confirm the patients' ability to understand the study and their willingness to participate. Only one eye per patient will be included. In cases where both eyes of a patient are scheduled for corneal transplantation, only the first eye undergoing the procedure will be considered.

Inclusion criteria

- Age older than 18 years old.
- Planned corneal transplantation: PK, DSAEK, UT-DSAEK or DMEK.
- Indication for corneal transplantation: keratoconus, Fuchs endothelial dystrophy.
- Ability to provide written informed consent.

Exclusion criteria

- Prior corneal transplantation.
- Planned preloaded DSAEK or preloaded DMEK.
- Planned keratoplasty not involving corneal endothelium transplantation (ie, superficial/deep anterior lamellar keratoplasty).
- Evidence of concurrent microbial keratitis.
- Evidence of non-infectious or autoimmune keratitis.
- Impending or frank corneal perforation.
- Uncontrolled, elevated intraocular pressure (IOP).
- Previous glaucoma surgery.

- Acute or chronic inflammatory/infectious anterior segment uveitis.
- Presence of an anterior chamber intraocular lens for phakic or aphakic purposes.

Data measurements and variables

Preoperative endothelial viability evaluation

The ECD, overall mortality[13] and mortality on corneal folds of corneal tissues destined for transplantation as declared by the eye bank providing the tissue will be recorded at baseline (ie, the day of surgery). Corneal tissues will then be assessed preoperatively for endothelial viability before transplantation following the method hereby described.

In the operating room, donor corneas will be rinsed in balanced salt solution (BSS) to remove excess transport medium. The corneal endothelium will be stained for 30–45 s with 0.05% trypan blue, then the dye will be gently washed away with BSS. Trypan blue is a vital dye commonly used in anterior segment surgery, which stains the nuclei of severely damaged and dead endothelial cells of donor corneas, as well as areas of Descemet membrane denuded of endothelial cells.[11]

The corneal tissue will then be placed face-up on a custom trephine base designed with no suction holes (e.janach, Como, Italy) in order to provide a smooth, concave surface to rest the cornea on, with no background optical disturbance that could prevent optimal image analysis of the central endothelium. The corneoscleral rim will be filled with BSS to avoid the formation of reflexes of the microscope lights on the central endothelium.

Images of the stained corneal endothelium will then be acquired and exported. In order to improve reproducibility of the acquisition process and limit variability across different sites, magnification levels of images obtained through a surgical microscope will be adjusted to make sure that the entirety of the Schwalbe line is visible just inside the image frame. Illumination levels will be adjusted to avoid areas of hypoexposure/hyperexposure of the image and to provide a uniform, diffuse illumination of the corneal tissue. An example image can be seen in figure 1.

A custom algorithm will be developed to analyse the images in real time. All the images will be automatically enhanced with contrast limited adaptive histogram equalisation. A weakly supervised segmentation model will be used to segment the cornea from the enhanced images.[14] An unsupervised clustering method will be applied to the pixels within the cornea so to group pixels into different regions according to the colour and detect the trypan blue-positive pixels. The percentage of trypan blue-positive areas (TBPAs) over the whole area of the corneal endothelium will be calculated based on the segmentation results.

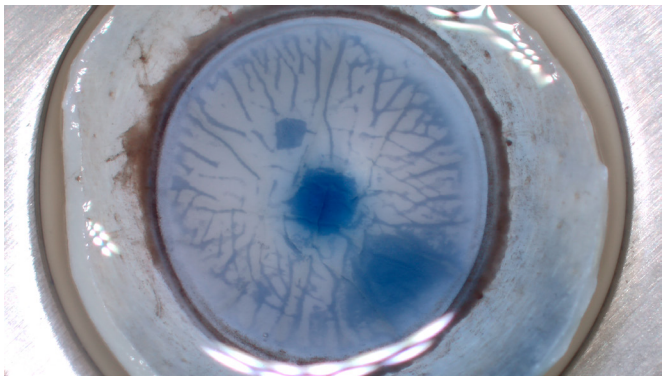

Figure 1 Example image acquired with surgical microscope showing correct positioning of the tissue and lighting. The corneoscleral rim is filled with BSS to avoid the formation of central reflexes. Several linear streaks of TBPAs are visible on corneal folds, along with circular areas of TBPA corresponding to mechanically induced endothelial damage. The cornea is resting on a custom trephine base with no suction holes to improve the visibility of the central endothelium (e.janach, Como, Italy). BSS, balanced salt solution; TBPA, trypan blue-positive area.

Patient data

Demographic data, indication for corneal transplantation, type of graft transplanted and other surgery-related variables—including intraoperative times and complications—will be recorded at baseline.

Clinical data will be recorded by the treating practitioner at each follow-up visit at 1, 3, 6 and 12 months postoperatively. Recorded data will include the number of letters read on a logarithm of the minimum angle of resolution visual acuity chart, presence of clinically definite corneal oedema, measurements of IOP, central corneal thickness, corneal tomography, and ECD, and occurrence of clinically defined postoperative complications such as graft rejection, infective keratitis, endophthalmitis.

Outcomes and variables

Primary outcome

The primary outcome of this study will be the comparison of the endothelial mortality of included donor corneas measured preoperatively (TBPA) and the overall endothelial mortality as declared by the eye bank providing the tissue. TBPA will be estimated preoperatively with the above descripted novel method. TBPA and nominal overall endothelial mortality will be expressed as mean and 95% CI.

Secondary outcomes

Secondary outcomes will include the correlation of preoperatively measured TBPA and nominal ECD as declared by the eye bank providing the tissue, the correlation of TBPA with mortality on corneal folds as provided by the eye bank, the final ECD of transplanted corneas at 12 months, the rate of change of ECD over the follow-up, the incidence of endothelial graft failure (ie, the occurrence of clinically definite corneal oedema and/or need

for endothelial keratoplasty), and the risk of endothelial graft failure associated with TBPA.

Study size

Sample size was estimated based on the width of the CI for the LOA (limit of agreement) of a Bland-Altman plot.[15]

Anticipating a sample difference (TBPA vs overall mortality) with mean and SD of 0 and 1%, respectively, a sample of 108 subjects achieves 80% power to detect agreement when the confidence level of the LoA is 95% and the confidence level of the CIs about the LoAs is 95%, given a maximum allowable difference of 2.5%.

Accounting for a 25% drop-out rate for the estimation of 12-month secondary outcome, we calculated a final sample size of 135 subjects.

Statistical methods

Data will be summarised using mean (SD) or median (IQR) for quantitative variable, and counts (percentages) for qualitative variables.

Concordance between preoperative TBPA and nominal as ECD, as measured by the eye bank providing the tissue, will be estimated using Bland-Altman model on the log (or arcsine) transformed variables.

A Pearson correlation coefficient will be estimated for preoperatively measured TBPA and nominal ECD as measured by the eye bank providing the tissue. Pearson correlation coefficients will also be reported for the correlation of TBPA with mortality on folds and ECD of the donor cornea.

A generalised linear mixed model, with random intercepts (practice and patient) and suitable family distribution, will be used to evaluate the within patient trend of ECD over 12-month ECD. The model will include visits from baseline through 12 months from all eyes, regardless of whether 12 months of follow-up have been completed, as well as age and eye bank ECD values.

Time to endothelial graft failure as a function of preoperatively measured TBPA will be evaluated using Cox proportional hazards model. Cumulative incidence curve will be used to display the trend of endothelial graft failure over 12 months.

Raw, unadjusted estimates for the other secondary outcomes will be computed using the last observation carried forward in case of subjects lost to follow-up.

All models will be evaluated assuming a 5% significance level and two-sided tests. All analyses will be conducted using R Statistical Software (V.4.3.0 or higher, R Core Team 2023).

Patient and public involvement

International and national organ donors' associations will be involved in the assessment of the patients' burden of recruitment to and participation in the cohort study. Dissemination of the study results is expected to involve trial participants, patient groups and organ donors' associations in addition to the traditional routes of publications outlined in the 'Ethics and dissemination' section.

ETHICS AND DISSEMINATION

Local IRB approval for participation will be sought by all centres willing to participate to the study. The study protocol is currently undergoing registration as observational study at ClinicalTrial.gov, and the results from this study will be submitted and published on ClinicalTrials.gov.

In addition, every attempt will be made to publish results in open access, peer-reviewed journals and to present these data at national and international meetings. Consistent with the collaborative nature of the proposed research, the principal investigator anticipates sharing all data generated by the study with collaborators. Furthermore, data generated by the study will be made available on reasonable request.

Author affiliations

[1]Department of Molecular and Translational Medicine, Università degli Studi di Brescia, Brescia, Italy
[2]St. Paul's Eye Unit, Royal Liverpool University Hospital, Liverpool, UK
[3]Department of Eye and Vision Science, University of Liverpool, Liverpool, UK
[4]Department of Experimental Medicine, Università degli Studi di Roma Tor Vergata, Roma, Italy
[5]Department of Ophthalmology, Uniklinik Köln, Koln, Germany
[6]Department of Ophthalmology, Universitätsklinikum Münster, Munster, Germany
[7]ELZA Institute, Dietikon, Zurich, Switzerland
[8]Department of Translational Neurosciences, University Hospital Antwerp, Wilrijk, Belgium
[9]Department of Ophthalmology, University Hospital of Brussel, Jette, Belgium
[10]Eye Clinic, Maastricht UMC+, Maastricht, The Netherlands
[11]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands
[12]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[13]Hospital Regional Universitario de Málaga, Málaga, Spain
[14]Qvision, VITHAS Almería, Almería, Spain
[15]Department of Ophthalmology, VITHAS Málaga, Málaga, Spain
[16]Surgery Department, Ophthalmology Area, University of Seville, Sevilla, Spain
[17]Department of Medical and Surgical Sciences, Università degli Studi "Magna Græcia" di Catanzaro, Catanzaro, Italy
[18]Ophthalmology Unit, Ospedale San Bortolo di Vicenza, Vicenza, Italy
[19]Mass Eye and Ear, Harvard Medical School, Boston, Massachusetts, USA
[20]Fondazione Banca degli Occhi del Veneto, Venezia, Italy
[21]Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Università degli Studi di Brescia, Brescia, Italy

Contributors MA and VR have conceptualised the study, conducted exploratory analysis, defined the protocol design, and selected collaborators. HL, MP, AR, SF and DP have developed and evaluated the viability assessment. YZ has developed the image analysis algorithms. SC is the biostatistician that will have access to the final data set. FA, BB, LB, SND, MMD, SK, LF, KG, AM, MR-C-d-M, CRdL, VS, FS and PV are the principal investigators of their respective centres.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests YZ, SK and VR are editors of BMJ Open Ophthalmology.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and this study obtained approval from the Comitato Etico Territoriale Lombardia 6 with protocol number NP

6028. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available on reasonable request. Data generated by the study will be made available on reasonable request.

ORCID iDs
Matteo Airaldi http://orcid.org/0000-0001-9010-1208
Yalin Zheng http://orcid.org/0000-0002-7873-0922
Francesco Aiello http://orcid.org/0000-0001-6479-1807
Björn Bachmann http://orcid.org/0000-0002-3974-7609
Lamis Baydoun http://orcid.org/0000-0002-2324-5268
Sorcha Ní Dhubhghaill http://orcid.org/0000-0002-1115-7834
Mor M Dickman http://orcid.org/0000-0001-9545-343X
Stephen B Kaye http://orcid.org/0000-0003-0390-0592
Luigi Fontana http://orcid.org/0000-0003-3877-0417
Kunal A Gadhvi http://orcid.org/0000-0002-8706-3922
Antonello Moramarco http://orcid.org/0000-0003-2847-1691
Marina Rodriguez Calvo de Mora http://orcid.org/0000-0002-5513-8790
Carlos Rocha de Lossada http://orcid.org/0000-0001-7464-2493
Vincenzo Scorcia http://orcid.org/0000-0001-6826-7957
Pietro Viola http://orcid.org/0000-0001-8274-2662
Stefano Calza http://orcid.org/0000-0003-4996-7995
Hannah J Levis http://orcid.org/0000-0002-3923-1178
Mohit Parekh http://orcid.org/0000-0002-5186-068X
Alessandro Ruzza http://orcid.org/0000-0003-4582-1948
Stefano Ferrari http://orcid.org/0000-0003-3983-3328
Diego Ponzin http://orcid.org/0000-0002-6038-3977
Francesco Semeraro http://orcid.org/0000-0002-2275-4917
Vito Romano http://orcid.org/0000-0002-5148-7643

REFERENCES

1 Sugar A. The importance of corneal endothelial cell survival after endothelial keratoplasty. *JAMA Ophthalmol* 2015;133:1285–6.
2 Writing Committee for the Cornea donor study research group. Factors associated with corneal graft survival in the cornea donor study. *JAMA Ophthalmol* 2015;133:246–54.
3 Anshu A, Li L, Htoon HM, *et al*. Long-term review of penetrating keratoplasty: a 20-year review in Asian eyes. *Am J Ophthalmol* 2021;224:254–66.
4 Williams KA, Lowe M, Bartlett C, *et al*. Risk factors for human corneal graft failure within the Australian corneal graft registry. *Transplantation* 2008;86:1720–4.
5 Riddlesworth TD, Kollman C, Lass JH, *et al*. A mathematical model to predict endothelial cell density following penetrating keratoplasty with selective dropout from graft failure. *Invest Ophthalmol Vis Sci* 2014;55:8409–15.
6 Writing Committee for the Cornea Donor Study Research Group, Lass JH, Benetz BA, *et al*. Donor age and factors related to endothelial cell loss 10 years after penetrating keratoplasty: specular microscopy ancillary study. *Ophthalmology* 2013;120:2428–35.
7 Baydoun L, Ham L, Borderie V, *et al*. Endothelial survival after descemet membrane endothelial keratoplasty: effect of surgical indication and graft adherence status. *JAMA Ophthalmol* 2015;133:1277–85.
8 Pipparelli A, Thuret G, Toubeau D, *et al*. Pan-corneal endothelial viability assessment: application to endothelial grafts predissected by eye banks. *Invest Ophthalmol Vis Sci* 2011;52:6018–25.
9 Bhogal M, Balda MS, Matter K, *et al*. Global cell-by-cell evaluation of endothelial viability after two methods of graft preparation in descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2016;100:572–8.
10 Romano V, Kazaili A, Pagano L, *et al*. Eye bank versus surgeon prepared DMEK tissues: influence on adhesion and re-bubbling rate. *Br J Ophthalmol* 2022;106:177–83.
11 van Dooren BTH, Beekhuis WH, Pels E. Biocompatibility of trypan blue with human corneal cells. *Arch Ophthalmol* 2004;122:736–42.
12 Weber IP, Rana M, Thomas PBM, *et al*. Effect of vital dyes on human corneal endothelium and elasticity of Descemet's membrane. *PLoS One* 2017;12.
13 Groeneveld-van Beek EA, Lie JT, Mangundap K, *et al*. No-touch stripping donor tissue preparation for descemet membrane endothelial keratoplasty. In: Parekh M, Ferrari S, Ponzin D, eds. *Eye Banking: Changing Face of Corneal Transplantation*. Nova Science Publishers Incorporated, 2015: 141–50. Available: https://novapublishers.com/shop/eye-banking-changing-face-of-corneal-transplantation/
14 Zhang H, Burrows L, Meng Y, *et al*. Weakly supervised segmentation with point annotations for histopathology images via contrast-based variational model. 2023 IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR); Vancouver, BC, Canada.2023
15 Lu M-J, Zhong W-H, Liu Y-X, *et al*. Sample size for assessing agreement between two methods of measurement by bland-Altman method. *Int J Biostat* 2016;12.