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Donor age predicts corneal endothelial cell density: a four-tier grading framework from 485 Chinese eye-bank corneas

Lutian Zhang
First Affiliated Hospital of Zhengzhou University
Guanyu Su
Henan Provincial People's Hospital
Haiyan Cai
Henan Provincial People's Hospital
Zhengyang Jin
Henan Provincial People's Hospital
Yitong He
Nanchang University
Jianjian Cheng
Henan Provincial People's Hospital
Guangying Zheng
zzzgy@zzu.edu.cn

First Affiliated Hospital of Zhengzhou University

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Abstract

Eye banks commonly judge a donor cornea from a single endothelial cell density (ECD) measurement and a binary accept-or-reject decision, although donor age is often treated as a proxy for endothelial quality. We analysed 485 donor corneas recovered at the Henan Eye Bank, China, between January 2019 and March 2026, all with complete ex vivo specular microscopy records. ECD, the coefficient of variation of cell area (CV) and hexagonality (HEX) were described, and their associations with donor age, sex, cause of death and cold ischaemia time were examined with non-parametric tests and multivariable linear regression. Median ECD was 2489 cells/mm² (interquartile range 2022–2895; range 812–4310); mean CV was 51.67% and mean HEX 45.14%, so polymegathism and pleomorphism were common. ECD did not differ by sex, and no cause-of-death category remained significant after adjustment for multiple comparisons. Donor age was the only variable associated with ECD, but the effect was small (− 5.01 cells/mm² per year) and the model explained 2.5% of the variance. We propose a four-tier framework (excellent > 2800, qualified 2000–2800, marginal 1500–2000, unacceptable ≤ 1500 cells/mm²) that makes donor assessment explicit and comparable between banks. The thresholds are preliminary and require validation against transplant outcomes.

Introduction

The corneal endothelium maintains transparency through a barrier–pump mechanism that keeps stromal hydration within narrow limits [1]. Human endothelial cells divide little after birth, so any substantial cell loss is irreversible; once the residual density falls below what is needed for dehydration, the cornea oedemates, scars, and vision is permanently reduced [2]. At that stage the only reliable option is surgical replacement of the endothelium [3], and how long a graft survives depends to a large extent on the endothelial reserve of the donor tissue [4,5].

Transplant-grade donor corneas are in short supply worldwide [6]. Shortage is not the whole problem, though. Even among recovered tissue a sizeable share is rejected before surgery, and discard rates as high as 41.4% have been reported in a single series [7]. Surgeons repeatedly identify donor screening and tissue procurement as the main bottlenecks of their programmes [8]. Part of this waste reflects the absence of agreed quantitative criteria for donor endothelial quality: where criteria are vague, assessment drifts towards subjective judgement, and functionally viable tissue gets discarded alongside genuinely unusable tissue.

Ex vivo specular microscopy yields three routine measurements: ECD, the coefficient of variation of cell area (CV) and the percentage of hexagonal cells (HEX). In daily practice ECD carries the most weight, and donor age is often used as a shorthand for it. The evidence behind this habit is thinner than it looks. Studies linking age, cause of death and cold ischaemia time to endothelial parameters have produced inconsistent results [9], and published series from Chinese eye banks remain scarce even though donor demographics and procurement practice in China differ considerably from Western settings [7].

We therefore set out to describe the real-world distribution of ECD, CV and HEX in a large single-centre Chinese eye-bank cohort; to quantify, in an explicitly exploratory analysis, how much of the variation in ECD the readily available donor variables account for; and to derive a graded assessment framework that could replace a binary accept-or-reject decision with reproducible thresholds.

Results

Study cohort

Of 748 donor cornea records recovered during the study period, 485 corneas had complete and quality-verified endothelial data and formed the analytical sample (Table 1). The median donor age was 48 years (interquartile range 38–57; range 6–74), and 385 of 485 donors (79.4%) were male. Cold ischaemia time was short: median 0.4 h (interquartile range 0.2–0.8 h), with 85.4% of corneas procured within 1 h and 91.3% within 2 h of death.

Table 1
Baseline donor characteristics and endothelial morphological parameters in 485 donor corneas

Variable	n	Mean ± SD	Median (IQR)	Range
Donor age (years)	485	46.5 ± 14.7	48 (38–57)	6–74
Cold ischaemia time (h)	485	1.02 ± 3.13	0.4 (0.2–0.8)	0–41.5
Endothelial cell density (cells/mm²)	485	2441 ± 677	2489 (2022–2895)	812–4310
Coefficient of variation of cell area (%)	485	51.67 ± 17.96	46 (39–62)	28–119
Hexagonal cells (%)	485	45.14 ± 13.81	47 (37–55)	13–74

Donor sex: male 385 (79.4%), female 100 (20.6%). SD, standard deviation; IQR, interquartile range. ECD, CV and HEX were measured by ex vivo specular microscopy on one central field per cornea.

Distribution of endothelial parameters

ECD was non-normally distributed, with a median of 2489 cells/mm² (interquartile range 2022–2895) and a range of 812–4310 cells/mm² (Table 1, Fig. 1b,c). The distribution was left-skewed and peaked at approximately 2600 cells/mm². Mean CV was 51.67 ± 17.96% and mean HEX 45.14 ± 13.81%, so polymegathism and pleomorphism were common even in this donor population. CV and HEX varied inversely across corneas (Spearman $r = -0.244$, $p < 0.001$), and corneas in the lower ECD grades tended to combine both unfavourable features (Fig. 1d).

A four-tier grading framework

The 25th and 75th percentiles of ECD were 2022 and 2895 cells/mm². To obtain thresholds that are easy to remember and comparable across eye banks, these percentiles were rounded to 2000 and 2800 cells/mm² (within 22 and 95 cells/mm² of the observed values). Combining these boundaries with conventional clinical cut-offs used in corneal banking practice [10,11] yielded four morphological grades: excellent (ECD > 2800 cells/mm²; n = 145), qualified (2000–2800; n = 223), marginal (1500–2000; n = 67) and unacceptable (≤ 1500; n = 50). Figure 3 places these tiers in the context of the assessment pathway, from donor characteristics through specular microscopy to the proposed allocation. Because recipient follow-up data were not available, no direct association between grades and graft survival could be established here.

Donor factors and ECD

Univariable results are summarised in Table 2 and Fig. 2. ECD did not differ between male and female donors (median 2489 [interquartile range 2032–2900] vs 2490 [1931–2858] cells/mm²; Mann–Whitney U = 19 758, p = 0.684; Fig. 2a). Donor age was weakly and negatively correlated with ECD (Spearman r = − 0.116, p = 0.010; Fig. 2c). Cold ischaemia time showed a similar weak negative rank correlation (Spearman r = − 0.134, p = 0.003; Fig. 2d). For cause of death, the omnibus Kruskal–Wallis test across the ten categories was statistically significant (H = 19.62, df = 9, p = 0.020; Fig. 2b), but after accounting for multiple comparisons no post-hoc pairwise comparison reached significance, so we found no robust evidence of systematic baseline differences in ECD across cause-of-death groups.

Table 2
Univariable associations between donor characteristics and ECD (n = 485)

Variable	Category / unit	ECD (cells/mm²)	Test statistic	p
Donor age	Per year	Spearman r = − 0.116	—	0.010
Donor sex	Male vs female	2489 (2032–2900) vs 2490 (1931–2858)	Mann–Whitney U = 19 758	0.684
Cause of death	Ten categories (1–10)	See Fig. 2b	Kruskal–Wallis H = 19.62 (df = 9)	0.020
Cold ischaemia time	Per hour	Spearman r = − 0.134	—	0.003

Values are median (interquartile range) unless otherwise stated. The omnibus Kruskal–Wallis test for cause of death was significant, but no post-hoc pairwise comparison survived adjustment for multiple comparisons.

Multivariable model

In the multivariable linear regression model (Table 3), donor age was the only variable statistically associated with ECD (β = −5.01 cells/mm² per year, 95% confidence interval − 9.21 to − 0.81; p = 0.020), corresponding to a decline of roughly 5 cells/mm² for each additional year of donor age. Donor sex (β =

−51.73, 95% confidence interval − 203.59 to 100.13; p = 0.504), cold ischaemia time (β = −15.54, − 35.25 to 4.17; p = 0.122) and all cause-of-death categories relative to central nervous system (CNS)-related death were not associated with ECD (all p > 0.05). The model explained only a small fraction of the variability in ECD (R^2 = 0.025; adjusted R^2 = 0.013) and did not reach statistical significance as a whole (F = 1.77, p = 0.091); no multicollinearity was detected (all variance inflation factors < 1.2). The age association should therefore be read as weak and exploratory rather than as evidence of a clinically useful predictor.

Table 3
Multivariable linear regression of variables associated with ECD (n = 485)

Variable	β coefficient	95% CI	p
Donor age (per year increase)	−5.01	−9.21 to − 0.81	0.020
Donor sex (female vs male)	−51.73	−203.59 to 100.13	0.504
Cold ischaemia time (per hour increase)	−15.54	−35.25 to 4.17	0.122
Cause of death (reference: CNS-related)			
Traumatic vs CNS-related	12.27	−210.32 to 234.86	0.914
Cardiogenic vs CNS-related	86.39	−170.94 to 343.72	0.510
Other vs CNS-related	−89.82	−232.83 to 53.19	0.218

Dependent variable: ECD (cells/mm²). Model: n = 485; R^2 = 0.025; adjusted R^2 = 0.013; F = 1.77, p = 0.091. No multicollinearity (all variance inflation factors < 1.2). CI, confidence interval; CNS, central nervous system.

Discussion

In this cohort of 485 donor corneas we report a left-skewed ECD distribution peaking near 2600 cells/mm², an age effect so small that it is of little use at the level of the individual donor, and a graded assessment framework that eye banks can implement with the equipment they already own. Mean CV and HEX were consistently less favourable than ideal textbook references, which is worth bearing in mind when local thresholds are set.

Endothelial cell loss remains a leading cause of corneal decompensation, and graft outcomes are constrained both by limited donor tissue and by variable quality-assessment standards [12,13]. The annual decline of approximately 5 cells/mm² observed here is consistent with eye-bank series [7,9] and somewhat smaller than in some clinical cohorts. The biological explanation is well rehearsed: ageing endothelial cells accumulate oxidative damage, lose mitotic capacity and become more susceptible to apoptosis [14,15]. What our data add is a quantitative caveat. Although the age–ECD association was statistically significant, the pre-specified model explained only 2.5% of the variance and was not significant as a whole. Age is a poor surrogate for endothelial health in the individual donor. Unmeasured factors — pre-

mortem medication exposure, prolonged hypoxia, terminal systemic burden, pre-existing ocular disease and genetic background — probably account for most of the remaining variation. Diabetes mellitus, for instance, has been associated with modestly lower ECD in donor eyes [16], but donor medical histories were incomplete in this database. The practical implication is that donor age should not be used as a stand-alone exclusion criterion.

We found no robust difference in ECD across causes of death, in contrast with earlier reports that corneas from donors dying of accidental causes are of higher quality. Two mechanisms probably contribute. Donors with sepsis, prolonged intensive-care admission or severe metabolic disease are largely removed during pre-banking screening, which homogenises the sample before any analysis begins. In addition, broad clinical categories may simply lack the resolution to detect small differences, although specific terminal states such as severe systemic inflammation may still injure the endothelium [17].

Cold ischaemia time, defined here as the death-to-procurement interval, correlated weakly and negatively with ECD in univariable analysis ($r = -0.134$, $p = 0.003$), but the finding did not survive adjustment and needs careful interpretation. More than 85% of corneas in this cohort were procured within one hour of death, so the variable mostly captures procurement logistics rather than a meaningful ischaemic exposure, and the few corneas with long intervals came from unusual circumstances. Comparable series with short, uninterrupted storage intervals have made similar observations [18]. Our data say little about the situations that actually concern eye banks — delayed procurement, unstable cold chains or prolonged transport — and should not be read as reassurance in those settings.

The main methodological contribution is a data-driven four-tier framework classifying donor corneas as excellent, qualified, marginal or unacceptable on the basis of ECD (Fig. 3). It addresses known weaknesses of binary pass/fail assessment, which is vulnerable to arbitrary cut-offs and inter-observer variation. The framework remains a preoperative morphological classification, not a validated prognostic model: without post-transplant outcomes the thresholds cannot be linked to graft survival, and external validation with clinical endpoints is required. The qualified stratum (2000–2800 cells/mm²) is broad and does not imply uniform suitability, because graft eligibility also depends on donor characteristics, recipient risk and surgical strategy.

Thresholds of this kind are already used informally. Many banks apply a floor somewhere between 2000 and 2300 cells/mm², sometimes with a separate requirement for hexagonality, but these local rules are rarely published or compared between centres [10,11]. A graded scheme does not remove the underlying judgement. It does make the trade-off visible: allocating a marginal cornea to a low-risk recipient with a clear media and a favourable prognosis is a different decision from using the same cornea in a high-risk eye, and a four-tier label lets a bank record which choice it made. It also gives a shared language for audit, so that discard rates and graft outcomes can be compared between banks with different case mixes.

This study has limitations. It is retrospective and single-centre, and pre-banking screening removes corneas with severe pre-existing endothelial damage, which compresses the age–ECD gradient and limits generalisability to eye banks with different donor criteria or preservation systems. The regression model had low explanatory power because relevant covariates — comorbidity, medication exposure, preservation details and genetic background — were unavailable. Latent cytomegalovirus or herpes simplex virus infection can cause subclinical endotheliitis and endothelial loss [19,20] but was not screened. The thresholds are sample-derived, and only one specular field per cornea was measured, so sampling variability contributed to measurement error.

Methods

Study design and setting

This was a retrospective cohort study reported in accordance with the STROBE statement for observational studies. Donor cornea data were retrieved from the electronic eye-bank management database of the Henan Eye Bank (Henan Provincial People's Hospital / Henan Eye Hospital), which supplies corneal tissue to a regional transplant network in central China. All corneas recovered between January 2019 and March 2026 with a complete endothelial morphological record were eligible. Because all eligible records were included, no a priori sample-size calculation was performed.

Participants and data quality control

Corneas were included if a complete specular microscopy record (ECD, CV and HEX) was available. Records missing critical administrative fields (time of death, procurement time or tissue-use time) were excluded. To safeguard data quality, ECD values outside 1000–4000 cells/mm² were not treated as automatic exclusions; instead they were flagged and the original images re-inspected for measurement artefacts caused by staining debris, bubbles or defocusing. Only measurements confirmed to be technical artefacts were discarded; physiologically plausible values outside this window were retained. The final analytical sample comprised 485 donor corneas.

Endothelial assessment

All corneas were examined by certified eye-bank technicians using an ex vivo specular microscope (Konan, Hyogo, Japan) following the manufacturer's standard workflow. For each cornea one representative central field was selected for formal ECD, CV and HEX measurement, and all numerical outputs were independently verified by a second experienced technician. In-house quality-control records indicated acceptable inter-observer reproducibility for ECD (intraclass correlation coefficient 0.91). Images compromised by focusing artefacts, staining debris or bubble interference were excluded before data extraction.

Variable definitions

The primary outcome was ECD (cells/mm²). CV (%) and HEX (%) were recorded as secondary descriptive parameters and were not modelled. The donor variables examined were age (years, continuous), sex, cause of death and cold ischaemia time. Cause of death was recorded in ten clinical categories (1 = cerebral haemorrhage, 2 = brain injury, 3 = traumatic death, 4 = cerebral infarction, 5 = cardiogenic death, 6 = cancer, 7 = accidental trauma, 8 = respiratory failure, 9 = circulatory failure, 10 = other) for the univariable analysis, and consolidated into four groups (CNS-related, traumatic, cardiogenic and other) for regression modelling. Cold ischaemia time was defined as the interval between death and tissue procurement (hours), the window in which endothelial ischaemic stress occurs.

All donor specimens underwent the standard serological screening protocol for hepatitis B and C, human immunodeficiency virus and syphilis. Universal serological screening for latent cytomegalovirus and herpes simplex virus was not performed, so subclinical or latent viral endotheliitis could remain undetected.

Statistical analysis

Analyses were performed with GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA) and SPSS 26.0 (IBM Corp., Armonk, NY, USA). Normality was assessed with the Shapiro–Wilk test. Continuous variables are presented as mean ± standard deviation or median with interquartile range according to their distribution. Between-group comparisons used the Mann–Whitney U test (binary variables) and the Kruskal–Wallis H test (multi-category variables); where the omnibus test was significant, post-hoc pairwise comparisons were adjusted for multiple testing. Spearman rank correlations were used for bivariate associations involving continuous variables. Donor age, sex, cold ischaemia time and cause of death were entered simultaneously into a multivariable linear regression model irrespective of their univariable results; cause of death was coded as dummy variables with CNS-related death as the reference category, and multicollinearity was assessed with variance inflation factors. Because the model was exploratory, the overall fit (F test, R^2 and adjusted R^2) is reported alongside the individual coefficients: a statistically significant coefficient does not by itself make a clinically useful predictive model. All tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Ethics approval

The study was approved by the Ethics Committee of Henan Eye Hospital (Approval No. 2026-KY-16) and conducted in accordance with the Declaration of Helsinki. Given the retrospective design and the use of fully anonymised donor data, the requirement for written informed consent was waived by the committee.

Declarations

Additional Information

Competing interests

The authors declare no competing interests.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by L.Z., G.S., H.C. and Z.J. The first draft of the manuscript was written by L.Z., and all authors commented on previous versions. G.Z. and J.C. supervised the project. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. Because the data contain sensitive donor medical information, they are not publicly deposited but are stored securely by the Henan Eye Bank

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Figures

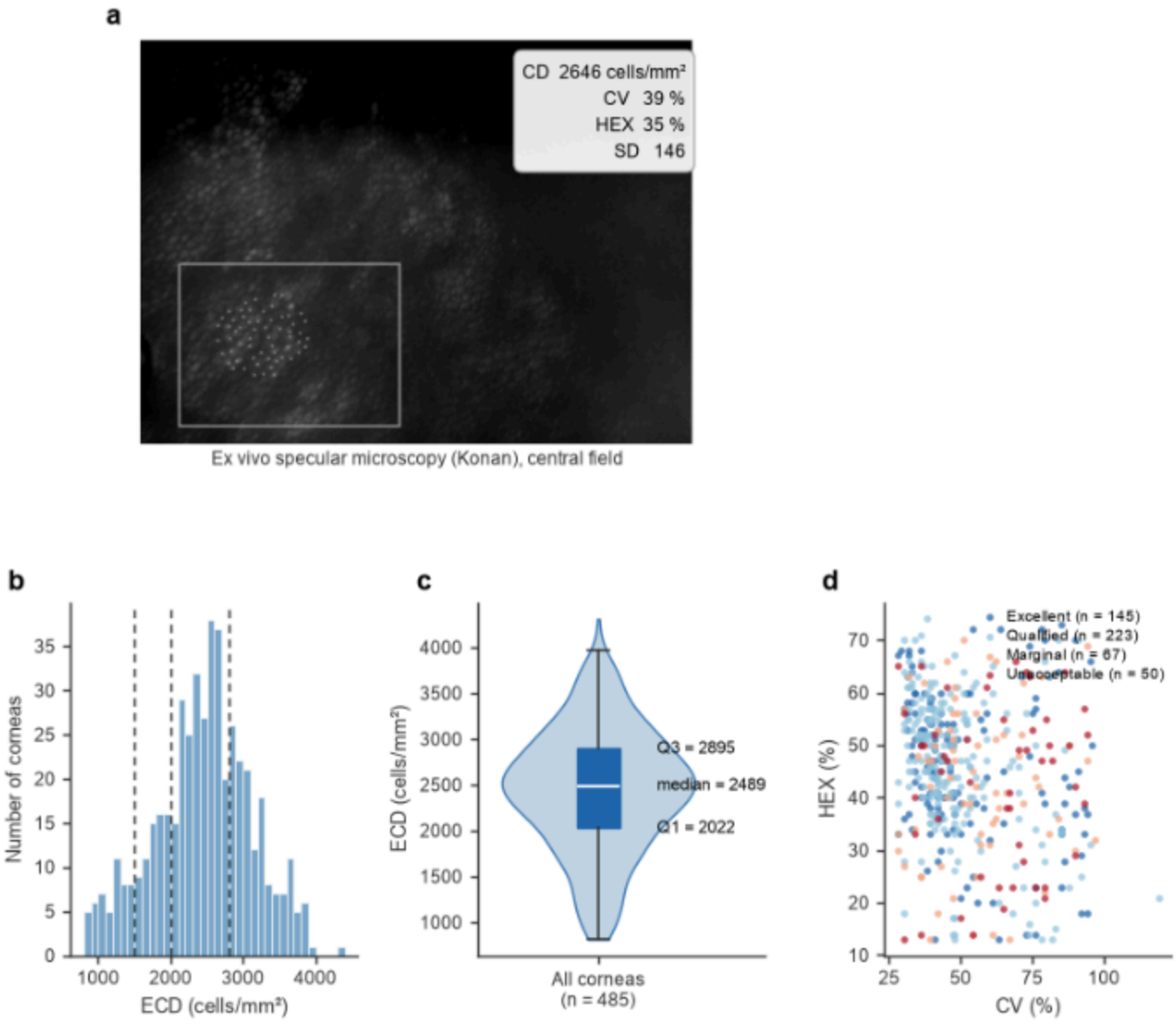


Figure 1

Distribution of endothelial parameters in 485 donor corneas. **a**, Representative ex vivo specular microscopy image (Konan); the box marks the analysed central region of interest and the inset shows the quantitative readout of this field. **b**, Frequency histogram of ECD (bin width 100 cells/mm²); dashed lines mark the grading thresholds at 1500, 2000 and 2800 cells/mm². **c**, Violin and box plots of ECD; median 2489 cells/mm² (interquartile range 2022–2895). **d**, CV versus HEX for individual corneas, coloured by proposed grade (Spearman r = −0.244, p < 0.001).

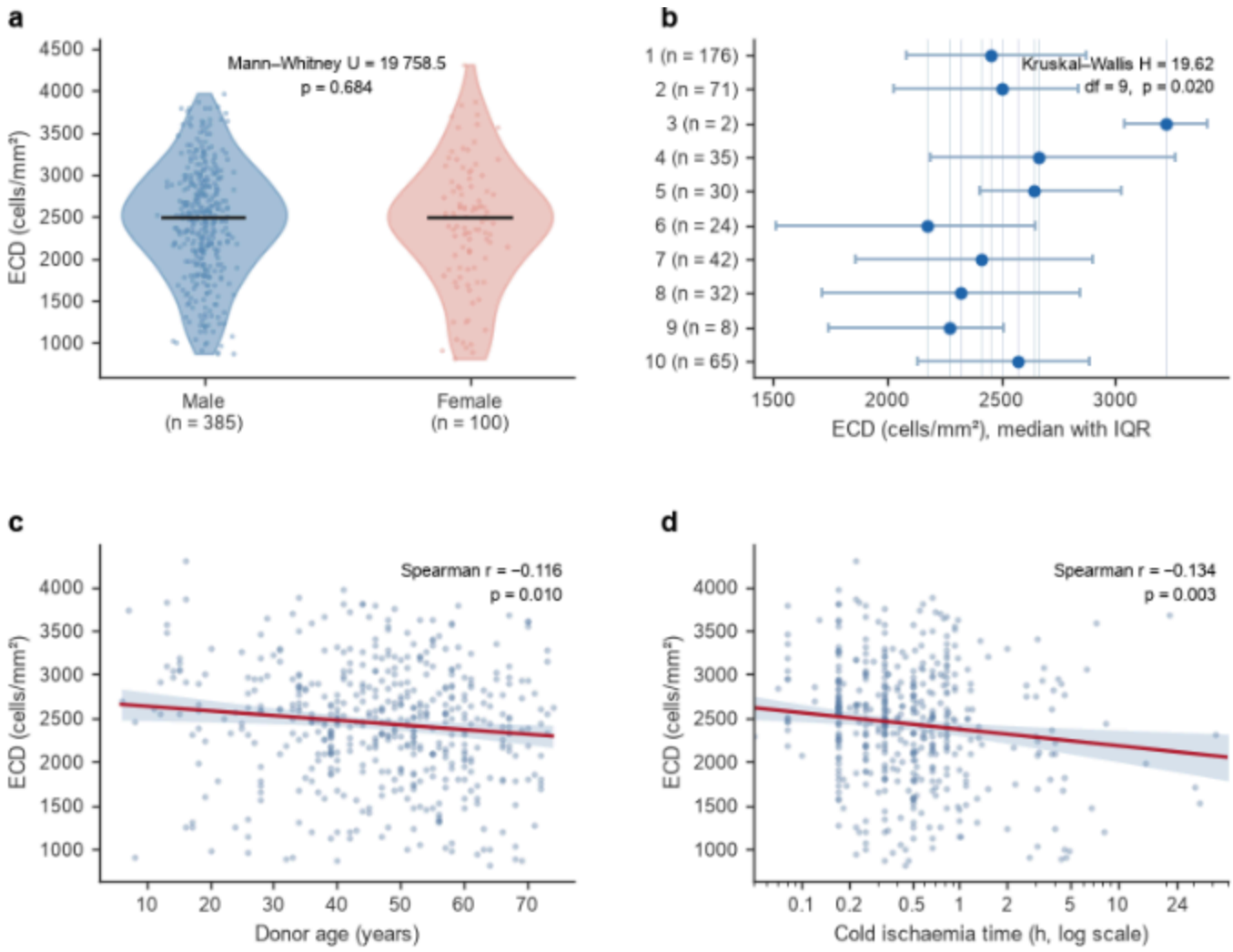


Figure 2

Univariable associations between donor factors and ECD. **a**, ECD in male (n = 385) and female (n = 100) donors; dots represent individual corneas and horizontal lines the medians (Mann–Whitney U = 19 758.5, p = 0.684). **b**, ECD across ten categories of cause of death (1 = cerebral haemorrhage, 2 = brain injury, 3 = traumatic death, 4 = cerebral infarction, 5 = cardiogenic death, 6 = cancer, 7 = accidental trauma, 8 = respiratory failure, 9 = circulatory failure, 10 = other); dots show medians and whiskers the interquartile ranges (Kruskal–Wallis H = 19.62, df = 9, p = 0.020, with no significant post-hoc comparison). **c**, Donor age versus ECD with linear fit and 95% confidence band (Spearman r = −0.116, p = 0.010). **d**, Cold ischaemia time versus ECD on a logarithmic scale with linear fit and 95% confidence band (Spearman r = −0.134, p = 0.003); values of 0 h are plotted at 0.03 h.

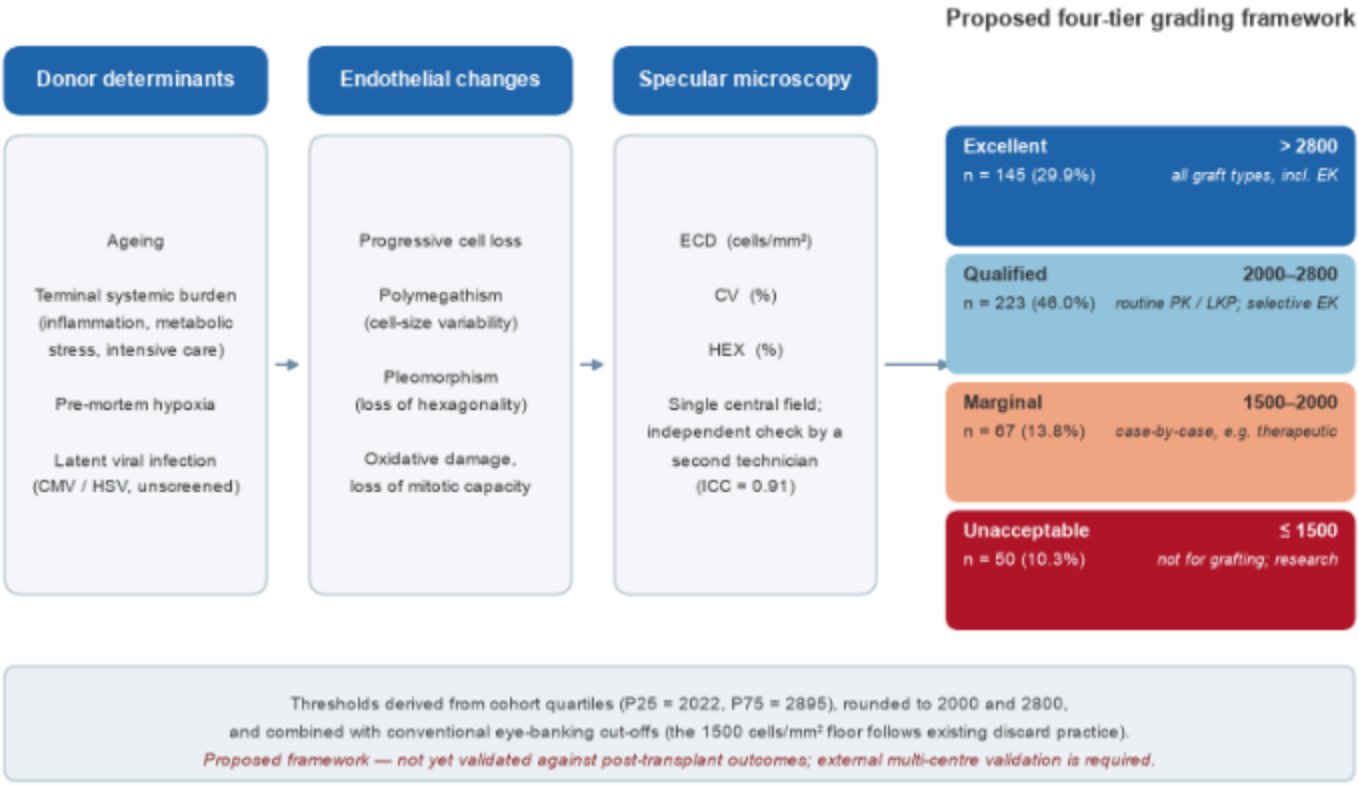


Figure 3

Proposed assessment and grading pathway for donor corneas. Donor determinants act on endothelial morphology, which is quantified by ex vivo specular microscopy (ECD, CV, HEX) and translated into a four-tier grading framework derived from cohort quartiles (25th percentile 2022, 75th percentile 2895 cells/mm²) rounded to 2000 and 2800 cells/mm², combined with conventional eye-banking cut-offs. The allocation suggested for each tier is indicative and requires validation against transplant outcomes.