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Risk factors and survival analysis in high-risk penetrating keratoplasty: a retrospective cohort study in 220 patients

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Abstract

Background High-risk penetrating keratoplasty (HRPK) is associated with significantly reduced graft survival compared to low-risk cases, with 5-year survival below 35% in developed countries. However, risk factors and outcomes may differ in developing regions where infectious and traumatic indications are more common. This study evaluates the 1- to 5-year graft survival rates and identified independent predictors of graft failure in HRPK from a tertiary center in China.

Methods We performed a retrospective cohort study of 220 HRPK adult patients between December 2019 and December 2023, meeting standardized high-risk criteria including corneal neovascularization in two or more quadrants, re-transplantation, active inflammation, or combined procedures. Preoperative, intraoperative, and postoperative data were collected. Graft failure was defined as central corneal opacity of ≥ 4 mm or persistent corneal edema. Multivariable Cox regression analysis identified independent risk factors, and a prediction nomogram was created and validated via bootstrapping.

Results Graft survival rates at 1, 3, and 5 years were 82.7%, 46.3%, and 34.7%, respectively. Graft failure occurred in 113 cases (51.36%) over the total follow-up period. Compared to non-failure cases, the failure group had higher rates of re-transplantation (53.1% vs. 38.3%, $p=0.039$), peripheral anterior synechiae (63.7% vs. 47.7%, $p=0.024$), and 4-quadrant neovascularization (34.5% vs. 17.8%, $p=0.036$). Cox proportional hazards regression identified advancing age (HR 1.02 per year, 95% CI 1.01–1.04, $p=0.023$), graft diameter ≥ 9 mm (HR 1.74, 95% CI 1.12–2.71, $p=0.02$), and 4 quadrants of neovascularization (HR = 2.53, $P < 0.01$) as independent predictors of failure. Peripheral anterior synechia and re-transplantation were also associated with increased risk. The five-variable predictive nomogram showed moderate predictive performance for 3-year (AUC = 0.715) and 5-year (AUC = 0.770) survival rate, with strong calibration. Subgroup analysis indicated that mycophenolate mofetil was mostly administered to higher-risk patients;

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however, propensity-matched analysis demonstrated no significant survival advantage (50.0% vs. 50.0% failure rate, $p=0.856$).

Conclusion This study identified advanced age, a large graft diameter (≥ 9 mm), and 4 quadrants of neovascularization as significant predictors of HRPK failure, with an especially elevated risk during the initial three postoperative years. Risk-based immunosuppressive strategies and close monitoring are essential to improving long-term outcomes in this high-risk population.

Keywords High-risk penetrating keratoplasty, Graft failure, Risk factors, Corneal neovascularization, Re-transplantation, Immunosuppressive therapy

Background

Corneal transplantation is a critical intervention for restoring vision in patients with advanced corneal pathology, and remains one of the most commonly performed allogeneic transplantation worldwide [1, 2]. Although lamellar keratoplasty, particularly endothelial and anterior lamellar techniques, has become the preferred surgical approach for many corneal pathologies [3], penetrating keratoplasty (PK) remains the most commonly performed corneal transplantation globally for conditions associated with significant stromal opacity or vascularization, such as bacterial, fungal, or viral infections, ocular trauma, and previous graft failure [4]. According to previous studies, primary PK performed in avascular and clear host corneas achieves a 1-year graft survival rate of over 90%, a 5-year survival rate exceeding 80%, and a cumulative rejection rate of less than 15% [5, 6]. However, in high-risk recipients, PK is associated with a significantly increased risk of postoperative graft rejection and failure [7, 8].

High-risk penetrating keratoplasty (HRPK) cases are defined as preexisting conditions that significantly increase postoperative failure risk. The Collaborative Cornea Transplantation Study has described high-risk conditions as presence of more than two quadrants of corneal neovascularization and sensitization due to a previous graft [9, 10]. Other conditions that may place the cornea at a higher risk of rejection are position of the graft close to limbus [11], severe atopic dermatitis [12] and herpes simplex keratitis (HSV) [13]. Some of the commonly-reported risk factors for rejection and/or graft failure may be inter linked and the extent to which these factors represent independent risks for graft failure is not well understood [14].

Current research on the risk factors for rejection and failure after PK is derived predominantly from developed countries. However, multiple studies have shown that the indications for PK vary significantly across region. In developed countries, the main indications for PK include pseudophakic bullous keratopathy, keratoconus, and re-transplantation [15, 16]. In contrast, in developing countries, including China, the leading indications are corneal leukoma secondary to infection or trauma,

re-transplantation, and acute infectious corneal ulcers [17–19]. The underlying indication for PK can significantly influence surgical outcomes.

Despite the high prevalence of vision-threatening corneal diseases in China, comprehensive studies on HRPK outcomes remain limited, partly due to severe donor shortages. Our study addresses this gap by analyzing one of the largest Chinese HRPK cohorts to date and implementing uniform perioperative management across all cases. We report 3-year and 5-year graft survival rates, identify failure-associated risk factors, and establish a risk-prediction model designed to guide individualized treatment strategies and improve outcomes in high-risk populations.

Methods

Enrollment and inclusion criteria

Patients meeting the criteria for HRPK were consecutively enrolled at Beijing Tongren Eye Center between December 2019 and December 2023. Eligibility for HRPK was determined by the presence of at least one of the following clinical features in adult patients (> 18 years old) [20–25]:

1. Corneal opacity with corneal neovascularization (in at least two quadrant) or peripheral anterior synechia (PAS).
2. Re-transplantation after a previous failed corneal transplant, including failed PK (Penetrating Keratoplasty), ALK (Anterior Lamellar Keratoplasty), or EK (Endothelial Keratoplasty).
3. Corneal graft diameter ≥ 9 mm.
4. Active ocular inflammation or infectious keratitis.
5. Combined surgical procedures, including pars plana vitrectomy, cataract surgery, intraocular lens implantation/repositioning.

The study was approved by the Ethics Committee of Beijing Tongren Hospital (Approval No. TREC2019-03), and written informed consent was obtained from all participants prior to enrollment.

Data collection

Preoperative data included demographic characteristics, ocular/systemic history, medication use, and relevant imaging findings. Comprehensive clinical information specific to HRPK was collected, including both preoperative and intraoperative ocular conditions. The following variables were recorded: history of previous corneal transplantation, acute infection-induced corneal melt/perforation, viral keratitis status, corneal neovascularization extent (1–4 quadrants), lens status, coexisting glaucoma, prior glaucoma surgeries, other ocular surgical interventions, presence of PAS, and any combined procedures (e.g., cataract extraction, intraocular lens implantation/repositioning, vitrectomy). The diameter of the corneal graft was documented for all cases.

Graft preparation and surgical procedure

Corneal tissues were voluntarily donated by local citizens and processed by the Beijing Tongren Eye Bank following donor death. Fresh donor corneas were preserved in Optisol-GS storage medium and used within 10 days of preservation. Preoperatively, all grafts underwent specular microscopy to assess endothelial cell density. Only tissues with an endothelial cell density ≥ 2000 cells/mm² were selected for penetrating keratoplasty. In addition, all donor tissues were subjected to microbiological culture, and only those with negative culture results were used for surgery. All surgeries were performed by a single experienced surgeon (Dr. Pan). Routine postoperative medication protocol included: (1) glucocorticoid eye drops (initiated on postoperative day 1 with 0.1% tobramycin-dexamethasone eye drops 4 times daily for 15 days, then switched to 1% prednisolone acetate eye drops 4 times daily; tapered to 3 times daily at 2 months, then to twice daily at 4 months, maintained until 2 year postoperatively); (2) 0.1% tobramycin-dexamethasone ointment once nightly (discontinued after 1 week in routine cases); (3) 0.1% tacrolimus eye drops (initially 4 times daily, reduced to 3 times daily at 2 months, then to twice daily at 6 months, continued for at least 2 year); and (4) ocular surface lubricants (artificial tears, administered as needed without time restrictions). In cases with bacterial, fungal, or amoebic infections, targeted anti-infective treatments were administered based on microbiological culture and susceptibility results. For patients identified as very high-risk, defined as those with a history of multiple graft failures, neovascularization in 4 quadrants, very large grafts (≥ 10 mm) or alkali burns, systemic immunosuppression was initiated when not contraindicated. These patients received oral mycophenolate mofetil (MMF) 500 mg twice daily for 6 months postoperatively, followed by a reduced dose of 250 mg twice daily for the next 6 months. Continuation beyond one year was determined at the discretion of the treating physician.

Postoperative follow-up

Patients were followed up until December 2024 to assess graft survival and rejection events. Follow-up visits were scheduled at postoperative day 14, monthly from months 1 to 6 months, and annually thereafter for up to 5 years. At each follow-up, visual acuity, intraocular pressure (IOP), graft clarity, and complication were documented. Graft rejection was defined as new-onset graft edema accompanied by a rejection line or anterior chamber inflammation. A standardized stepwise protocol was followed for all cases of acute graft rejection. The treatment strategy was tailored to the severity of the episode and patient response.

The protocol consists of [26]: (1) Intensified Topical Therapy: At diagnosis, patients were started on or escalated to 1% prednisolone acetate eye drops (4–6 times daily) and 0.1% tobramycin-dexamethasone eye ointment (nightly). (2) Subconjunctival/Periocular Injections: For moderate to severe cases, or when response to topical therapy was inadequate, subconjunctival or periocular dexamethasone sodium phosphate or triamcinolone acetonide injections were administered either daily or every other day, as clinically indicated. (3) Systemic Corticosteroids: In severe cases (e.g., extensive endothelial rejection), oral prednisone was initiated at a high dose and tapered gradually. (4) Adjunctive Immunosuppression: Topical 0.1% tacrolimus (4 times daily) was used as an adjunctive agent at discretion of treating physician.

Treatment success was defined as the resolution of epithelial or endothelial rejection lines, reduction in graft edema, and reduction in keratic precipitates [26]. Graft failure was defined as the presence of central corneal opacity with a diameter ≥ 4 mm, irreversible corneal edema, persistent corneal epithelial defect, or progressive graft melt/ulceration lasting more than 2 months [26]. The study endpoint was set as December 31, 2024. The final follow-up date was determined as: (1) the date of first confirmed corneal graft failure diagnosis for patients with failure events, and (2) the most recent follow-up visit prior to December 31, 2024, for patients without graft failure.

Statistical analysis

The study used R (Version 4.4.0) program, with 'pROC', 'rms', 'ggplot2', 'survminer' and 'survival' packages. Clinical and demographic variables were compared between rejection and non-rejection groups, as well as between failure and non-failure groups. Survival outcomes were measured from time of intervention. Graft survival rates were estimated using the Kaplan-Meier method, with comparisons between groups performed by the log-rank test. Categorical variables were analyzed using Pearson's Chi-Square test ($n \geq 5$) or Fisher's exact test ($n < 5$). Continuous variable was described as mean \pm standard

deviation (SD) and compared by independent *t* tests. Univariate binary logistic regression models were performed to test risk factors for graft failure. The Receiver Operating Characteristic (ROC) curve was used to evaluate the predictive effect of the nomogram for graft failure, and the Area Under the Curve (AUC) value was used for quantification. Univariable and multivariable Cox analysis was performed on risk factors associated with graft failure, and variables with statistically significant differences were used to construct a nomogram. The calibration plots were utilized to assess the predictive performance of the nomogram. $P < 0.05$ indicated statistical significance.

Results

Cohort characteristics and overall prognosis

A total of 220 patients (220 eyes) who underwent HRPK between December 2019 and December 2023 were included in the final analysis. The median postoperative follow-up duration was 27.27 ± 18.02 months. The most common primary indications were re-transplantation (46%), followed by corneal leucoma (23.6%), corneal ulceration/perforation (21.4%), and bullous keratopathy (combined with other surgeries, 7.73%) (Table S1). During the observation period, graft rejection occurred in 40% cases, including 39% of rejection cases within 1 year, 34% between 1 and 2 years, 18% between 2 and 3 years, and 8% occurred beyond 3 years. Among the 34 patients with rejection within the first year, 18% showed recovery after treatment while 82% progressed to graft failure. Among the patients with rejection beyond 1 year, 25% recovered whereas 75% did not. Graft failure occurred in 51% cases during the follow-up period. The cumulative graft survival rates at 1-year, 2-year, 3-year, 4-year, and 5-year were 82.7%, 58.3%, 46.3%, 37.6%, and 34.7% respectively.

Baseline demographic and clinical characteristics are summarized in Table 1. Patients in the graft failure group had a significantly higher mean age (56.1 ± 14.1 years) compared to the non-failure group (51.9 ± 16.1 years, $P = 0.041$). Additionally, the failure group showed significantly higher proportions of re-transplantation (53.1% vs. 38.3%, $P = 0.039$), PAS (63.7% vs. 47.7%, $P = 0.024$), and four-quadrant neovascularization (34.5% vs. 17.8%, $P = 0.036$). Among re-transplantation cases, the leading cause of previous graft failure was graft rejection (63.4%) followed by viral/microbial keratitis (27.7%), traumatic injuries (21.8%), post-surgical endothelial decompensation (19.8%), non-infectious keratitis (5.0%), corneal dystrophies/degenerations (6.9%), corneal ectasia (4.0%), and congenital/unspecified causes (14.9%). In total, 71.3% patients undergoing re-transplantation had only one prior corneal transplant, while 28.7% had two or more previous transplants. No significant differences in

baseline characteristics were observed between the rejection and non-rejection groups.

Among the 113 cases of graft failures, 74 cases (65.5%) were attributed to graft rejection. The remaining 39 cases experienced graft failure without documented rejection during the follow-up period. The underlying causes in these non-rejection cases were as follows: 19 persistent epithelial non-healing with stromal melt, 10 graft endothelial decompensation, 5 graft conjunctivalization with central opacification, 4 postoperative graft re-infection or uncontrolled primary infection, and 1 had primary graft failure.

Univariate analysis of graft failure related factors

Univariate Cox regression analysis identified age (HR = 1.02, $P = 0.01$), graft diameter ≥ 9 mm (HR = 1.82, $P = 0.01$), re-transplantation (HR = 1.59, $P = 0.01$), and neovascularization involving 3–4 quadrants (3 quadrants: HR = 2.17, $P = 0.03$; 4 quadrants: HR = 2.66, $P < 0.01$) as significant risk factors for graft failure (Table 2). Kaplan-Meier curves demonstrated significantly lower 5-year graft survival rates in patients with re-transplantation (Fig. 1A), graft diameter ≥ 9 mm (Fig. 1B), ≥ 3 quadrants of neovascularization (Fig. 1C–D), and PAS (Fig. 1E).

Initial univariable analysis revealed a paradoxical association between MMF therapy and increased graft failure risk (HR = 1.59, $P = 0.02$) (Fig. 1F). However, significant baseline imbalances were observed prior to adjustment (Table S2): MMF-treated patients exhibited lower re-transplantation rates (23.8% vs. 66.2%, $P < 0.001$) but higher preoperative risks, including 4-quadrant neovascularization (47.6% vs. 17.8%, $P < 0.001$) and PAS (73.0% vs. 49.0%, $P = 0.002$). To adjust for these confounders, 1:1 propensity score matching (caliper = 1) was performed, resulting in balanced distribution of all prognostic covariates between MMF-treated and untreated groups. After matching, MMF therapy was not significantly associated with graft failure (50.0% vs. 50.0%, $\chi^2 = 0.32$, $P = 0.856$) (Table S3).

Prediction model construction and validation for graft failure

Variables with $P < 0.05$ from univariate analysis were included in a multivariate model. The multivariate Cox regression identified age (HR: 1.02 per year, $P = 0.023$), graft diameter ≥ 9 mm (HR: 1.74, $P = 0.02$) and 4 quadrants of neovascularization (HR = 2.53, $P < 0.01$) as independent predictors of graft failure (Table 3). A predictive nomogram was developed to estimate 3-year and 5-year graft survival probabilities (Fig. 2A). The model demonstrated moderate predictive performance, with an AUC of 0.715 for 3-year and 0.770 for 5-year survival (Fig. 2B–C). Bootstrap calibration plots showed strong agreement between predicted and observed probabilities (Fig. 2D).

Table 1 Comparison of clinical characteristics between rejection vs. non-rejection groups and failure vs. non-failure groups following HRPK

	Total (N=220)	Non-rejection (N=133)	Rejection (N=87)	P value	Non-failure (N=107)	Failure (N=113)	P value
Age (Year)				0.230			0.041
Mean ± SD	54.1 ± 15.2	53.1 ± 15.4	55.6 ± 14.9		51.9 ± 16.1	56.1 ± 14.1	
Sex (%)				0.892			1.000
Female	91 (41.4)	56 (42.1)	35 (40.2)		44 (41.1)	47 (41.6)	
Male	129 (58.6)	77 (57.9)	52 (59.8)		63 (58.9)	66 (58.4)	
Laterality (%)				0.188			0.666
Left	117 (53.2)	76 (57.1)	41 (47.1)		59 (55.1)	58 (51.3)	
Right	103 (46.8)	57 (42.9)	46 (52.9)		48 (44.9)	55 (48.7)	
Re-transplantation	101 (45.9)	57 (42.9)	44 (50.6)	0.325	41 (38.3)	60 (53.1)	0.039
Acute Infection-Induced Melt/Perforation (%)	72 (32.7)	46 (34.6)	26 (29.9)	0.562	33 (30.8)	39 (34.5)	0.663
Glaucoma (%)	45 (20.5)	29 (21.8)	16 (18.4)	0.658	20 (18.7)	25 (22.1)	0.643
Peripheral Anterior Synechiae (%)	123 (55.9)	72 (54.1)	51 (58.6)	0.606	51 (47.7)	72 (63.7)	0.024
Neovascularization Quadrants (%)				0.386			0.036
0	39 (17.7)	26 (19.5)	13 (14.9)		24 (22.4)	15 (13.3)	
1	26 (11.8)	17 (12.8)	9 (10.3)		15 (14.0)	11 (9.7)	
2	63 (28.6)	41 (30.8)	22 (25.3)		34 (31.8)	29 (25.7)	
3	34 (15.5)	20 (15.0)	14 (16.1)		15 (14.0)	19 (16.8)	
4	58 (26.4)	29 (21.8)	29 (33.3)		19 (17.8)	39 (34.5)	
Preoperative Surgical History	118 (53.6)	70 (52.6)	48 (55.2)	0.817	54 (50.5)	64 (56.6)	0.434
Post-cataract Surgery (%)	94 (42.7)	54 (40.6)	40 (46.0)	0.517	40 (37.4)	54 (47.8)	0.155
Post Glaucoma Surgery (%)	29 (13.2)	18 (13.5)	11 (12.6)	1.000	12 (11.2)	17 (15.0)	0.522
Post Other Ocular Surgeries (%)	49 (22.3)	27 (20.3)	22 (25.3)	0.482	22 (20.6)	27 (23.9)	0.666
Graft Diameter (%)				0.244			0.060
< 9 mm	186 (84.5)	116 (87.2)	70 (80.5)		96 (89.7)	90 (79.6)	
≥ 9 mm	34 (15.5)	17 (12.8)	17 (19.5)		11 (10.3)	23 (20.4)	
Combined Surgery (%)	127 (57.7)	71 (53.4)	56 (64.4)	0.141	55 (51.4)	72 (63.7)	0.087
Combined Cataract Surgery (%)	70 (31.8)	40 (30.1)	30 (34.5)	0.590	31 (29.0)	39 (34.5)	0.461
Combined Intraocular Lens Implantation/Repositioning (%)	78 (35.5)	45 (33.8)	33 (37.9)	0.633	34 (31.8)	44 (38.9)	0.333
Combined vitrectomy (%)	40 (18.2)	23 (17.3)	17 (19.5)	0.807	17 (15.9)	23 (20.4)	0.494
At Least Two Combined Other Surgeries (%)	54 (24.5)	34 (25.6)	20 (23.0)	0.784	27 (25.2)	27 (23.9)	0.941
Postoperative High IOP*							
Early phase (≤ 2 weeks) (%)	59 (26.8)	37 (27.8)	22 (25.3)	0.796	30 (28.0)	29 (25.7)	0.806
Late phase (> 2 weeks) (%)	74 (33.6)	44 (33.1)	30 (34.5)	0.945	37 (34.6)	37 (32.7)	0.884

Table 2 Univariable cox regression of risk factors for graft failure following HRPK

Variable	HR	95% CI	P value
Age	1.02	1.00 ~ 1.03	0.01
Re-transplantation	1.59	1.10 ~ 2.30	0.01
Graft Diameter ≥ 9 mm	1.82	1.15 ~ 2.89	0.01
Peripheral Anterior Synechia	1.57	1.07 ~ 2.31	0.02
Neovascularization	1.82	1.07 ~ 3.09	0.03
1 Quadrant	1.37	0.63 ~ 2.99	0.43
2 Quadrants	1.43	0.77 ~ 2.68	0.26
3 Quadrants	2.17	1.10 ~ 4.29	0.03
4 Quadrants	2.66	1.46 ~ 4.83	< 0.01
Postoperative MMF	1.59	1.08 ~ 2.36	0.02

Discussion

PK remains the definitive treatment for full-thickness corneal pathologies [27]. In low-risk scenarios, PK achieves excellent graft survival with reported 2-, 5-, and 10-year survival rates of approximately 90%, 90%, and 82%, respectively [6, 28–30]. However, immunological rejection and graft failure remain major challenges in HRPK. Our predictive nomogram provides a clinically valuable tool for preoperative counseling in high-risk PK. By quantifying individualized failure risks based on age, graft size, and neovascularization extent, surgeons can visually demonstrate to patients their projected 3-year (AUC=0.715) and 5-year (AUC=0.770) survival probabilities using Fig. 2A. For example, a 65-year-old patient requiring a 9.5 mm graft with 4-quadrant neovascularization would have a >90% probability of 5-year failure, facilitating informed decision-making about surgical

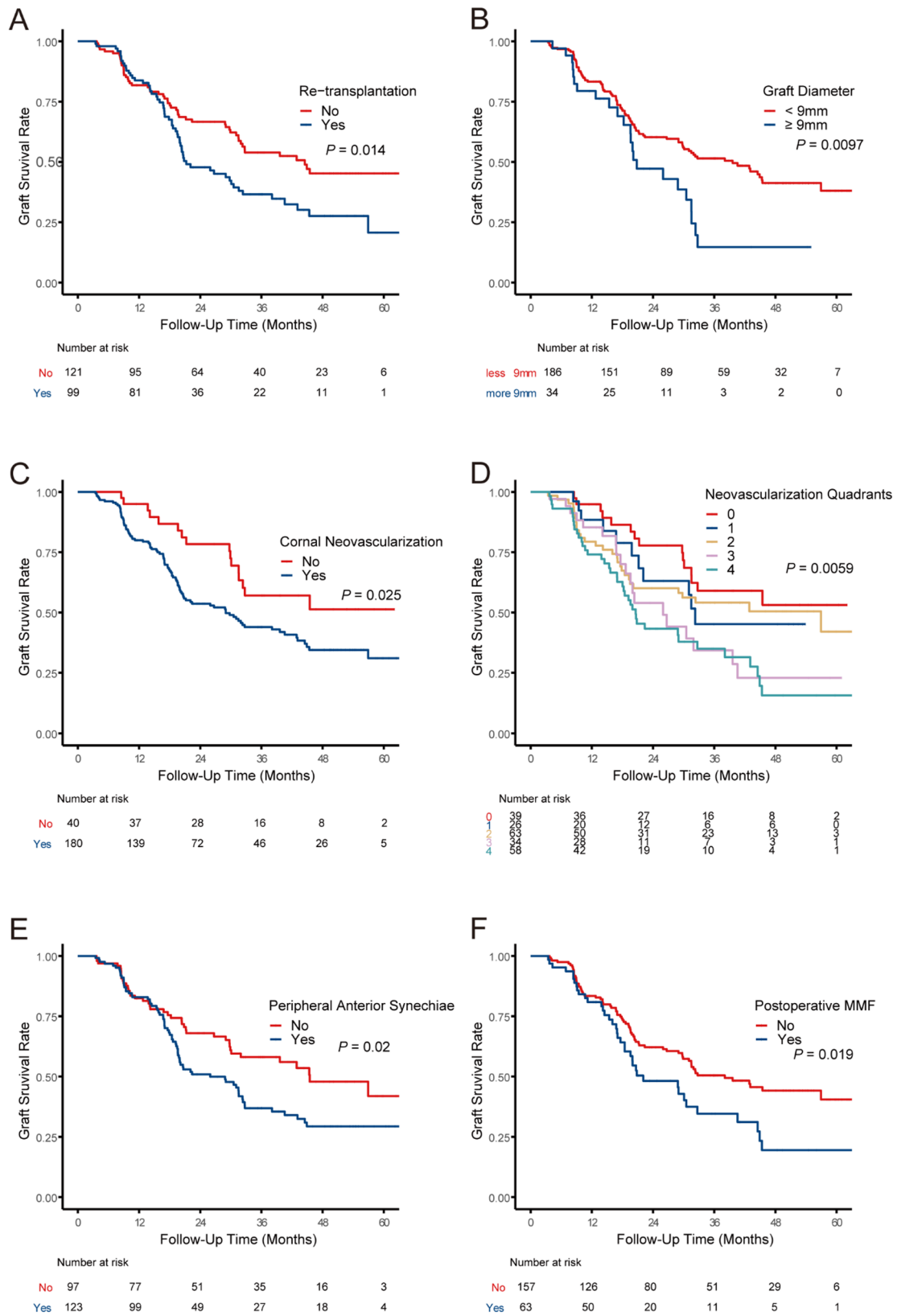


Fig. 1 Kaplan-Meier survival curves demonstrating probability of graft survival stratified by: **(A)** Re-transplantation (absent vs. present; log-rank $p=0.014$), **(B)** Graft diameter (<7.95 mm vs. ≥7.95 mm; log-rank $p < 0.01$), **(C)** Neovascularization (absent vs. present; log-rank $p=0.025$), **(D)** Neovascularization quadrants (1, 2, 3 vs. 4; log-rank $p < 0.01$), **(E)** Peripheral anterior synchia (PAS) (absent vs. present; log-rank $p=0.02$), **(F)** Postoperative MMF (absent vs. present; log-rank $p=0.019$)

Table 3 Multivariable cox regression of risk factors for graft failure following HRPK

Variable	HR	95% CI	P value
Age	1.02	1.00~1.03	0.023
Re-transplantation	0.79	0.53~1.19	0.260
Graft Diameter \geq 9 mm	1.74	1.08~2.80	0.023
Peripheral Anterior Synechia	1.38	0.93~2.04	0.11
Neovascularization Quadrants			
1	1.49	0.67~3.33	0.33
2	1.49	0.79~2.79	0.22
3	1.87	0.93~3.77	0.08
4	2.53	1.36~4.71	<0.01

timing, the need for systemic immunosuppression, and realistic expectations for visual rehabilitation.

Our study confirms that re-transplantation is a significant risk factor for graft failure. This is likely due to immune sensitization, memory T-cell activation, disruption of anterior chamber immune privilege, and coexisting corneal NV [6, 31]. In our cohort, prior graft rejection accounted for 63% of initial graft failures among re-transplantation cases, underscoring the need for intensified perioperative immunosuppression in these patients.

Although PAS was significantly associated with graft failure in univariate analysis, it was not an independent risk factor in the multivariate model, likely due to confounding or intercorrelation with other variables. PAS may increase failure risk through angle distortion, elevated IOP, and chronic endothelial damage. Alternatively, it may coexists with other high-risk features such as graft neovascularization but does not independently induce graft failure. Synechiolysis and meticulous prevention of adhesion during surgery remain essential to minimize these risks. The Collaborative Corneal Transplantation Studies (CCTS) reported that failure rates nearly doubled in eyes with PAS involving three to four quadrants [33]. Although postoperative IOP elevation was not an independent predictor of graft failure, it occurred in approximately 50% of failed grafts. Regardless of its direct association with graft failure, elevated IOP is linked to glaucomatous damage and should therefore be closely monitored and treated promptly.

Preoperative corneal NV is a well-established risk factor for HRPK failure, with survival rates inversely correlated to the extent of NV [32, 33]. NV disrupts immune privilege by promoting antigen presentation and local inflammation [34, 35]. In our cohort, 81.8% of HRPK cases had corneal NV, with >40% of NV cases involving 3–4 quadrants, reflecting the severity of the risk. For such patients, preoperative NV-targeted therapies such as subconjunctival [36, 37] or topical anti-VEGF agents [37], mitomycin intravascular chemoembolization (MICE) [38, 39], photodynamic therapy (PDT) [40]) warrant consideration to mitigate rejection and failure.

A graft diameter of \geq 9 mm was another independent risk factor for graft failure [41, 42], likely due to proximity to limbal vasculature [43], anatomical instability [44, 45], and elevated risk of secondary glaucoma. While a prior study reported a hazard ratio of 1.58 [1.11–2.28] for each 0.5 mm increase in diameter, this finding was not significant in multivariate analysis [46]. Additional research suggests that large grafts, frequently necessary in severe infections to avoid recurrence [44, 47] or in re-transplantation requiring excision of opaque host tissue [48], are associated with poor outcomes. We recommend limiting graft size (7.5–9.0 mm) where feasible, optimizing postoperative anti-inflammatory and IOP management.

Contrary to some earlier reports in pediatric population [49–51], our analysis found that older, not younger age independently predicted graft failure. This may reflect our exclusive adult cohort (>18 years) excluding pediatric cases, with 42% aged over 60 years. Additionally, re-transplantation made up 50% of the cohort, hence compounding the risk. High IOP, epithelial abnormalities, endothelium decompensation, as well as potentially inconsistent follow-up adherence may also contribute to age-related failure.

The impact of combination surgeries during PK such as cataract extraction, glaucoma and vitreoretinal surgery, remains controversial. Some studies suggest no added risk [52, 53], while others, such as Fasolo et al. report a 2.8-fold increased chance of failure when PK is combined with vitrectomy [54]. Our findings did not identify combined procedure as independent predictors of failure, but we recommend careful surgical planning, meticulous tissue handling, and robust postoperative inflammation and IOP control to minimize complications.

While topical corticosteroids and tacrolimus are the mainstays of postoperative care, emerging evidence suggests that HRPK demands more aggressive local and systemic immunosuppression [55–58]. In our protocol, high-risk patients maintained topical steroid therapy (1% prednisolone acetate) at a dose of twice daily for a minimum of 2 years postoperatively - reflecting our institutional practice of indefinite immunosuppression for HRPK cases unless contraindicated by steroid-induced complications. One of the few studies assessing steroid use beyond 1 year evaluated 3-year PKP outcomes for bullous keratopathy and, using a time-dependent covariate analysis, found that grafts not receiving corticosteroids were 1.5 times (95% CI, 1.0–2.2; $P < 0.03$) more likely to fail [59]. MMF has shown promise in reducing rejection [185, 181] with a more favorable safety profile compared with cyclosporine A [184, 186, 187]. However, optimal dosing strategies remains undefined. Triple therapy (MME, tacrolimus, steroids) [194] may offer advantages in extreme-risk patients [177], though the risk of

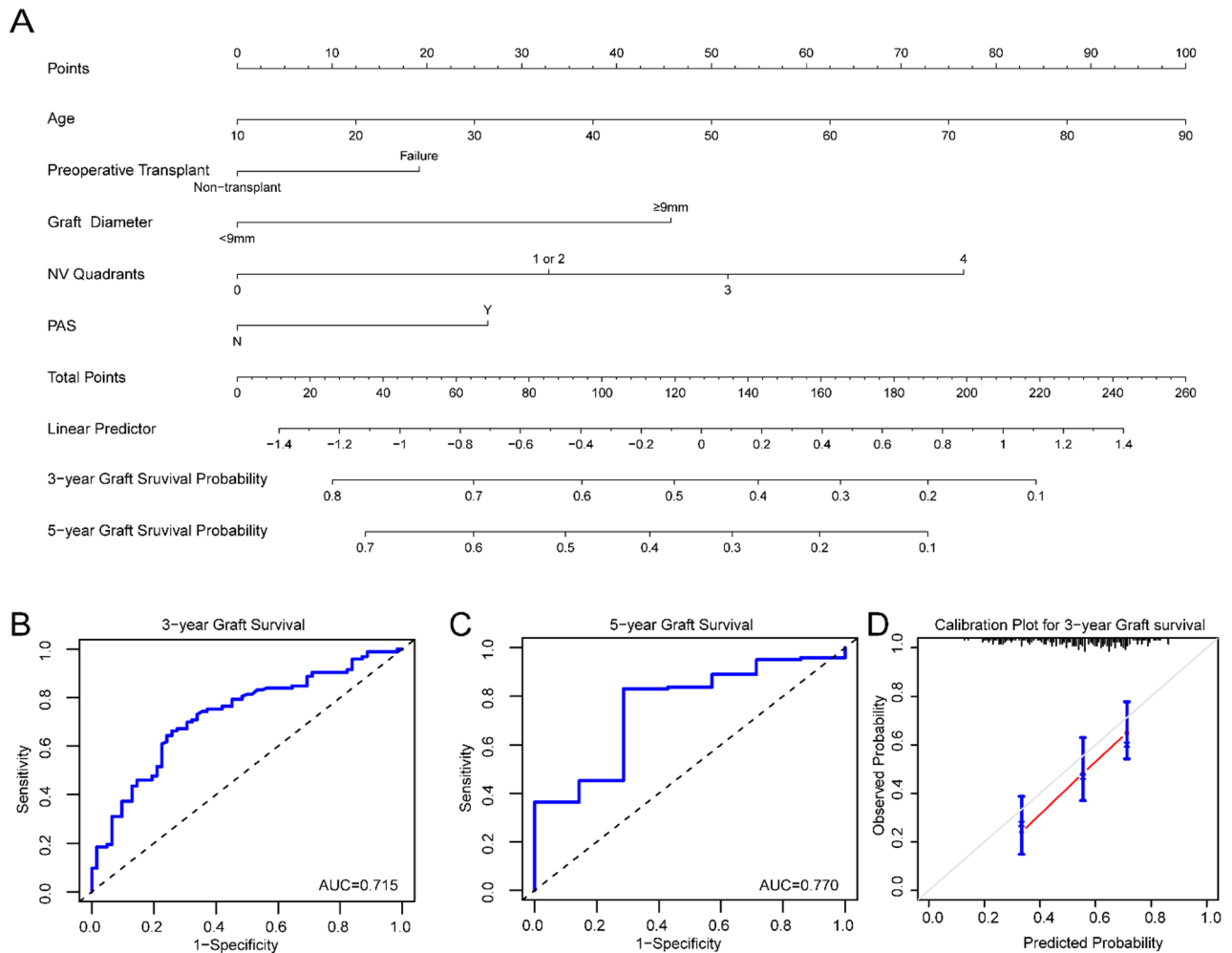


Fig. 2 (A) Nomogram incorporating five prognostic factors to estimate the probability of 3- and 5-year graft survival. In the nomogram, the ‘Linear Predictor’ (LP) is a score that combines the patient’s characteristics weighted by the regression coefficients from the Cox model. The LP itself is not a hazard ratio; rather, it represents the log of the relative hazard. The hazard ratio (HR) relative to the baseline is calculated by taking the exponential of the LP: $HR \text{ relative to baseline} = e^{LP}$. For example, an LP of 0 corresponds to $e^0 = 1$, meaning the patient has the baseline risk. Positive LP values indicate higher risk ($HR > 1$), while negative LP values indicate lower risk ($HR < 1$). In the nomogram, each patient’s characteristics are converted into points, summed to give the total points, which correspond to the LP. This LP is then used to estimate the predicted 3-year or 5-year graft survival probability. In short, the nomogram converts patient features into a linear score (LP), which is then exponentiated to give relative risk. Our study cohort included patients aged 18–89 years, with no participants under 18 or over 89. For practical use, we recommend interpreting the nomogram primarily within the 18–89 year range, as extrapolation beyond these ages may be unreliable without further validation. (B–C) Receiver operating characteristic (ROC) curve demonstrating the nomogram’s ability to discriminate 3- and 5-year graft survival. (D) Bootstrap-validated calibration plots (1,000 resamples) showing the agreement between predicted and observed graft failure rate at 3-year follow-up. The 45° reference line indicates perfect calibration. Abbreviation: NV, Neovascularization; PAS, Peripheral Anterior Synechia

systemic toxicity (renal or hepatic) and infection require careful individualized assessment.

In our cohort, MMF was administered at half the typical dose (500 mg twice daily vs. 1000 mg twice daily in other studies [191, 177]), and several patients demonstrated suboptimal compliance (<1 year in some cases). These factors may explain the lack of observed benefit. Future large-scale multicenter trials are needed to rigorously evaluate the efficacy of triple therapy and establish evidence-based long-term immunosuppression protocols to optimize graft survival in high-risk populations.

This study has several limitations. First, its single-center design may limit generalizability due to institutional-specific practices and a relatively homogeneous patient population. Second, the retrospective nature of this study is an inherent limitation. Despite the use of propensity score matching, unmeasured or residual confounders may remain, introducing potential selection bias. Third, the follow-up duration may be insufficient to fully evaluate long-term outcomes, as most cases monitored for ≤3 years and relatively few reached 5-years. To address these gaps, large-scale multicenter studies incorporating

a standardized high-risk stratification system—categorizing recipients into low-, medium-, and high-risk cohorts preoperatively—are needed to enhance risk prediction, refine donor/recipient matching, and optimize immunosuppressive strategies. Furthermore, mechanistic investigations into HRPK rejection pathogenesis should be prioritized. Collaborative efforts between clinicians and translational researchers could clarify the underlying drivers of neovascularization, immune-mediated endothelial injury, and chronic graft failure, thereby accelerating the development of targeted therapy.

Conclusion

This study identified advanced age, graft diameter ≥ 9 mm and 4 quadrants of neovascularization as independent risk factors for graft failure in HRPK. PAS and re-transplantation may also increase failure risk but did not reach statistical significance in this study. The 1-year, 3-year and 5-year graft survival rates were 82.7%, 46.3% and 34.7%, respectively, with the steepest decline occurring within years 1–3 after PK, highlighting a critical window requiring intensified follow-up and optimized immunosuppressive management. Based on these findings, we recommend enhanced preoperative adherence counseling and individualized immunosuppression (e.g., triple therapy) for patients with re-transplantation, multi-quadrant NV, PAS, or advanced age. Early identification and proactive management of high-risk features may help improve long-term graft survival in this vulnerable patient population.

Supplementary Information

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Supplementary Material 1

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Author contributions

Design of the study (ZQP); Conduct of the study (LL, JTL); Data collection (LL, YXZ); Analysis and interpretation (JTL, LL); Manuscript preparation and review (YH, ZQP).

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Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Beijing Tongren Hospital (Approval No. TREC2019-03), and adhered to the tenets of the declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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