• Clinical Research •

# Efficacy of mycophenolate mofetil combined with topical 0.05% tacrolimus in high-risk keratoplasty: 1-year cohort study

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Received: 2024-03-17 Accepted: 2024-08-02

## Abstract

● **AIM:** To investigate the efficacy of systemic mycophenolate mofetil (MMF) as an adjunct in combination with topical tacrolimus (FK506) and corticosteroid eyedrops for preventing corneal graft rejection after high-risk keratoplasty (HRK).

● **METHODS:** In this cohort study, 55 consecutive patients (55 eyes) from an eye center who met the criteria of HRK were included. The definition for HRK includes large grafts of no less than 9 mm diameter, vascularized cornea of two or more quadrants, regrafting, or eccentric grafts. After penetrating keratoplasty, 25 patients treated with systemic MMF in combination with 0.05% FK506 and tapering corticosteroid eyedrops were enrolled in Group 1 from October 2019. Thirty patients receiving postoperative treatment with 0.05% FK506 and tapering corticosteroid eyedrops alone were enrolled in Group 2 from January 2018 to September 2019. All participants were closely monitored after surgery, and episodes of graft rejection and relevant clinical data were collected and assessed over a one-year follow-up period.

● **RESULTS:** After a follow-up of 9.6±3.2mo, graft rejection episodes occurred in 4 cases (16%) in Group 1 and 18 cases (60%) in Group 2. One reversible and 3 irreversible graft rejections occurred in Group 1, while 3 reversible and 15 irreversible rejections occurred in Group 2. Kaplan-Meier analysis revealed that 82.5% of grafts in Group 1 and 37.1% in Group 2 did not experience corneal graft rejection ($P$<0.01, log-rank test). The clear graft survival rate was 83.6% in Group 1 and 36.7% in Group 2 ($P$<0.01, log-rank test) within one year of follow-up. No severe systemic side effects were observed in either group during the follow-up period.

● **CONCLUSION:** The triple treatment regimen consisting of MMF, topical FK506, and corticosteroid eyedrops represents a promising strategy for effectively preventing graft rejection and improving graft survival in patients with HRK.

● **KEYWORDS:** immunosuppressants; high-risk keratoplasty; mycophenolate mofetil

**DOI:10.18240/ijo.2024.12.10**

**Citation:** Zhang JY, Yu K, Jiang XY, Liang JX, Zhou SY. Efficacy of mycophenolate mofetil combined with topical 0.05% tacrolimus in high-risk keratoplasty: 1-year cohort study. *Int J Ophthalmol* 2024;17(12):2229-2235

## INTRODUCTION

Penetrating keratoplasty (PKP) is a vital and irreplaceable surgical approach when keratopathy involves the full thickness of the cornea[1]. After surgery, immunologic rejection remains the main cause of graft failure, especially for eyes undergoing high-risk keratoplasty (HRK), although the survival rate of corneal graft is greater than 90% in low-risk situation due to its immune-privilege[2-3]. At present, HRK is regarded as cases with no less than two quadrants of corneal neovascularization, re-grafts[4-6], large diameter graft[7], or eccentric graft[6,8]. In high-risk settings, rejection rates ranged from 50% to 70%, even with a maximal immunosuppression[9], although some modified surgeries have been found to reduce rejection rates in small sample studies[10-11].

Previously, systemic cyclosporine A (CsA) combined with corticosteroids was commonly used for patients who had undergone HRK[12], and this showed limited efficacy in suppressing graft rejection. In recent studies[13-16], the combine use of corticosteroids with systemic mycophenolate mofetil (MMF) or tacrolimus (FK506) eyedrops has demonstrated an advantage in preventing corneal graft rejection. MMF, a prodrug of mycophenolic acid, selectively inhibits the proliferation of T- and B-lymphocytes, which are highly dependent on de novo purine synthesis[17]. FK506 works as a macrolide immunosuppressant and inhibits the activity of calcineurin, thereby downregulating the expression of cytokines such as interleukin-2, and tumor necrosis factor, inhibiting the activation of T cells[18] and demonstrating an advantage in preventing corneal graft rejection[19]. However, there has been no consensus on pharmacological immunomodulation for HRK to date.

Previous research has reported that the corneal graft rejection rate is 39% to 60% in HRK with an application of topical FK506 and systemic corticosteroid[16,20]. However, the rates of corneal graft rejection with systemic MMF and topical corticosteroid eyedrops for HRK have been reported at 16% in a three-year follow-up[15], and 8% in a two-year follow-up[14]. Notably, a triple treatment with MMF, FK506, and corticosteroid is a common regimen for preventing graft rejection *via* multi-targets in the field of kidney transplantation[21]. This triple treatment has never been used for herpes simplex keratitis (HSK) patients. Whether the triple strategy for preventing graft rejection will work well in HRK has not been verified. This study aims to explore a comprehensive immunosuppression regimen for long-term corneal graft survival by evaluating the efficacy of a triple combination of systemic MMF, topical FK506, and corticosteroid eyedrops in HRK.

## PARTICIPANTS AND METHODS

**Ethical Approval** This study received approval from the institutional review board of Zhongshan Ophthalmic Center (No.2023KYPJ329) and was conducted following the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

**Participants** Consecutive patients were included in this retrospective cohort study at Zhongshan Ophthalmic Center, Guangzhou, China. The patients were divided into two groups based on the type of immunosuppression treatment they received. Group 1 patient received oral MMF (Sai Keping®, Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd., Hangzhou, Zhejiang Province, China), topical FK506 (Zhongshan Ophthalmic Center Pharmaceuticals, Guangzhou, China), and tapering corticosteroid eyedrops (TobraDex®, Alcon Inc., Texas, USA) starting in October 2019. Group 2 patient only received topical FK506 and tapering corticosteroid eyedrops from January 2018 to September 2019.

HSK was defined when one or more of the following criteria were met: having two or more quadrants of corneal neovascularization (CNV), repeat transplantation, large graft (diameter ≥9.0 mm), or eccentric graft (the distance between the edge of graft and limbus ≤1.0 mm). Exclusion criteria included a history of malignant tumor, systemic infection, gastrointestinal ulcers, age less than 18y, or pregnancy. The outcome of patients who dropped out were based on their last examination visit.

**Surgical Techniques** All surgeries were performed by a veteran surgeon (Zhou SY). Donor corneoscleral buttons were preserved in EUSOL-C storage medium (Alchimia, Padova, Italy) at 4°C and used within seven days of the donor's death. The enrolled patients underwent combined PKP and cataract surgery, or only PKP, including partial and entire PKP. In the partial PKP, a diameter of 7.5-8.5 mm vacuum trephine (Katena Instrument Co., Parsippany, NJ, USA) was used to cut the diseased cornea, and then a donor corneal button 0.25 mm larger than the host bed was replaced and sutured. In entire PKP, a whole donor cornea was sutured. Interrupted suturing was performed with 10-0 nylon.

**Postoperative Treatment and Follow-up** Postoperative oral MMF treatment was as follows: for the first six months, the dosage of MMF was determined based on the patients' body weight, calculated as: 0.25 g (weight 40-50 kg), 0.5 g (weight 51-60 kg), 0.75 g (weight 61-70 kg), and 1.0 g (weight greater than 70 kg), and all dosages were administered twice daily. For the next six months, MMF was cut to half of its initial dose. Patients without MMF treatment did not receive any oral immunosuppressants.

All patients received topical 0.05% FK506 eye drops four times daily for one week and then twice daily. Meanwhile, they all received oral prednisone of 1 mg/kg for five days, 0.1% dexamethasone eye drops four times per day for three weeks, 0.1% fluorometholone eye drops twice daily for six months, and then 0.05% fluorometholone eye drops for another six months. The dexamethasone ointment (TobraDex®, Alcon) was administered at night for the first two months.

Patients were instructed to schedule a follow-up appointment one week after surgery, followed by monthly visits for the first three months, then once every three months. The patients were advised to promptly return for examination if they experienced any decline in vision. During each follow-up visit, tests including visual acuity, intraocular pressure, slit-lamp examination, and fundus examination were performed. Drug-related systemic complications were monitored with full blood

**Table 1 Characteristics of included patients with high-risk corneal grafts**

| Parameters | Group 1 | Group 2 | *P* |
|---|---|---|---|
| Individuals, *n* | 25 | 30 | |
| Gender, male (%) | 19 (76.0) | 21 (70.0) | |
| Age, y (mean±SD) | 52.7±16.9 | 52.8±15.7 | 0.99[a] |
| Follow-up (mo), median (IQR) | 12.0 (8.5, 12) | 12.0 (5.8, 12) | 0.09[b] |
| Number of grafts before entry in this study, *n* (%) | | | 0.75[c] |
| 0 | 16 (64.0) | 21 (70.0) | |
| 1 | 8 (32.0) | 8 (26.7) | |
| 2 | 1 (4.0) | 0 | |
| 3 | 0 | 1 (3.3) | |
| Mean quadrants of CNV, median (IQR) | 2 (2, 4) | 2 (0, 3) | 0.15[b] |
| Graft diameter, (mm), median (IQR) | 8.00 (7.50, 8.50) | 7.75 (7.50, 9.75) | 0.74[b] |
| Surgical procedures | | | 0.72[d] |
| PKP | 19 | 24 | |
| PKP+ECCE | 6 | 6 | |
| Endothelium count of donor cornea, ($n$/mm$^2$) | 2792.08±308.27 | 2837.23±388.79 | 0.64[a] |
| Donor age, y (mean±SD) | 42.8±15.0 | 42.9±16.8 | 1.00[a] |

[a]Student's *t*-test; [b]Mann-Whitney *U* test; [c]Fisher's exact test; [d]$\chi^2$ test. CNV: Corneal neovascular; PKP: Penetrating keratoplasty; ECCE: Extracapsular cataract extraction; SD: Standard deviation; IQR: Interquartile range.

counts, blood pressure, blood urea nitrogen and creatinine, liver function, and electrolytes.

If corneal graft rejection was noted, the patient was treated with intense administration of dexamethasone (2.5 mg *via* subconjunctival injection, eye drops once an hour) and FK506 eye drops every two hours. The oral MMF was changed to its initial dosage. If the rejection was reversible and the corneal graft became clear, the above drugs were adjusted to the original protocol. If the graft rejection was irreversible, the treatment was discontinued.

**Main Outcome Measurement** The main outcome measurements were episodes of graft rejection and the survival of a clear graft. The graft rejection episodes were diagnosed by typical manifestations: endothelial rejection line, stromal infiltration and edema, keratic precipitates, and corneal opacity. A clear corneal graft was defined as no opacity or presenting a translucent zone without edema in the center 4-mm zone[14].

**Statistical Analysis** All statistical analyses were performed using SPSS Version 20.0 for Microsoft Windows 10 (IBM Corp, Armonk, New York, USA). A student's *t*-test was conducted to compare the differences between the two groups in patient and donor ages as well as endothelium cell density of donor corneas. The Mann-Whitney *U* test was used for comparison of the follow-up time, pre-existing CNV, and graft diameter. A Chi-square test and Fisher's exact test were used for the classification of high rejection risk and surgical procedures. The Kaplan-Meier method was used to evaluate rejection-free survival and clear graft survival. A *P*-value of less than 0.05 was considered statistically significant.

## RESULTS

**Subject Characteristics** Twenty-five eyes of 25 patients were recruited in Group 1, and 30 eyes of 30 patients were recruited in Group 2. In Group 1, 76% of recipients were male, and in Group 2, 70% were male. The median follow-up showed no significant differences between Group 1 and Group 2 ($P$=0.09). More than 60% of patients were receiving keratoplasty for the first time in both groups. For the surgical procedure, 19 patients in Group 1 and 24 patients in Group 2 received PKP, and 6 patients in each group received PKP combined with extracapsular cataract extraction. There were no statistically significant differences between the two groups in the follow-up time, patients' ages, donors' ages, diameter of corneal grafts, surgical procedures, endothelium cell density of donor corneas, the distribution of four high-risk factors for HRK, or the number of previous grafts per patient (all $P$>0.05). The extent of corneal vascularization in the transplant beds was similar in both groups ($P$=0.15; Table 1). The indications of keratoplasty included 9 types, as listed in Table 2.

**Systemic MMF Administration** In Group 1, 18 (72%) patients completed a one-year MMF administration regimen. MMF was discontinued in 3 patients at 6, 9, and 10mo after surgery due to their refusal to follow up. MMF treatment was discontinued from 2 to 9mo in the other 4 patients due to the COVID-19 pandemic.

Two patients in Group 1 (8%) had slight elevations in liver enzymes, including alanine aminotransferase and aspartate transaminase. One patient had a mild increase in blood pressure (up to 160/81 mm Hg) during systemic MMF treatment. One patient (an 18-year-old man) developed a

mild upper respiratory tract infection and then recovered after medical treatment in the respiratory department. Neither patient discontinued MMF treatment. In Group 2, no serious side effects were noted.

**Efficacy of Combination Treatment with or Without Systemic MMF** During the follow-up period, immunologic rejection occurred in 22 of 55 patients (40.0%). Other factors, such as persistent corneal epithelial defect, secondary glaucoma, and recurrence of infectious keratitis, also contributed to graft failure in 5 patients (9.1%; Figure 1 and Table 3).

In Group 1, graft rejection was noted in 4 of 25 patients (16.0%). The time interval between transplantation and rejection ranged from 3 to 12mo. Among them, one patient was non-compliant with the treatment protocol, and his immunosuppressive treatment was discontinued 2mo after transplantation. In the remaining 3 patients, the grafts recovered to transparent after a short course of intensive therapy for graft rejection once. Two of them eventually experienced irreversible rejection during a later follow-up. The other patient had a history of ocular alkali burn and experienced persistent epithelial defects, and the corneal graft ultimately lost its transparency 6mo after transplantation, even after being treated with amniotic membrane grafting. Together, 84.0% (21/25) had clear grafts in Group 1 at the one-year follow-up (Table 3).

In Group 2, graft rejection occurred in 18 of 30 patients (60.0%). Three patients' graft rejections were reversible, and their corneal grafts remained transparent until their last follow-up visit. In the remaining 15 patients, the grafts failed due to irreversible rejection. Four patients in Group 2 (13.3%) showed a loss of graft clarity, two of whom experienced a recurrence of infectious keratitis, one of whom developed gradual endothelial functional decompensation for secondary glaucoma, and one who developed persistent epithelial defects and conjunctival invasion. Collectively, the final percentage of clear grafts in the final was 36.7% (11/30) in Group 2 (Figure 1 and Table 3).

The high-risk characteristic showed no difference between groups during follow-up. For the high-risk characteristics of Group 1 graft rejection patients, two had large-diameter grafts, and three of them had repeat transplantation, eccentric grafts, or CNV (no more than quadrants). In Group 2 graft rejection patients, seven had with large-diameter grafts, four had repeat transplantation, one had eccentric grafts, and three had CNV (Table 4). Typical photos are shown in Figures 2 and 3.

Kaplan-Meier curves were plotted according to rejection episodes and graft failure within the follow-up period. The combination of systemic MMF with topical FK506 and corticosteroid eyedrops showed a statistically significant superiority to treatment with topical immunosuppresants alone in preventing graft rejection (rejection-free survival: Group 1 *vs* Group 2, 82.5% *vs* 37.1%, log-rank test, $P<0.01$; Figure 4) and in

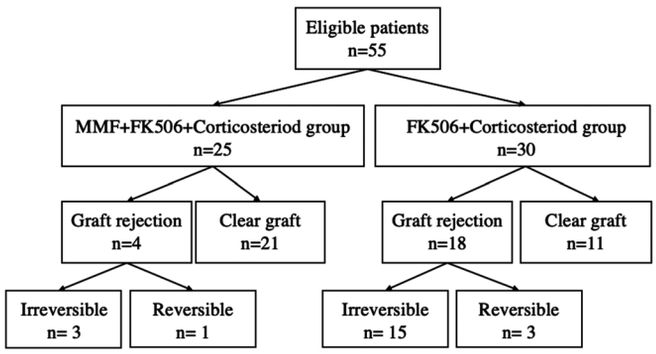


**Figure 1 Flow chart of the HSK patients' grouping and outcomes.** MMF: Mycophenolate mofetil; HSK: Herpes simplex keratitis.

**Table 2 Indications for high-risk penetrating keratoplasty**

| Diagnosis | Group1 (MMF+FK506) | Group 2 | Total |
|---|---|---|---|
| Corneal leukoplakia | 8 | 3 | 11 |
| Corneal perforation due to ulcers | 7 | 12 | 19 |
| Fungal keratitis | 0 | 3 | 3 |
| Bacterial keratitis | 1 | 2 | 3 |
| Acanthamoeba keratitis | 0 | 1 | 1 |
| Graft failure due to irreversible rejection | 6 | 6 | 12 |
| Graft failure due to trauma | 0 | 1 | 1 |
| Graft failure due to recurrence of Infectious keratitis | 2 | 1 | 3 |
| Alkali burn | 1 | 1 | 2 |

**Table 3 Efficacy of combination treatment with or without systemic MMF**

| End points | Group1 (*n*=25) | Group 2 (*n*=30) | *P* |
|---|---|---|---|
| Graft rejection | 4 (16%) | 18 (60%) | 0.002[a] |
| Reversible | 1 | 3 | |
| Irreversible | 3 | 15 | |
| Graft failure cause | 4 (16%) | 19 (63%) | 0.001[a] |
| Rejection | 3 | 15 | |
| Epithelial problem | 1 | 1 | |
| Secondary glaucoma | 0 | 1 | |
| Recurrent keratitis | 0 | 2 | |

[a]Log-rank test. MMF: Mycophenolate mofetil.

**Table 4 Number of high-risk characteristics and graft rejection between groups**

| High-risk characteristics | Group 1 | Group 2 | Total |
|---|---|---|---|
| Two or more quadrants of CNV | 6 (1) | 8 (3) | 14 (4) |
| Re-graft | 9 (1) | 9 (4) | 18 (5) |
| Large diameter grafts | 4 (2) | 9 (7) | 13 (9) |
| Eccentric grafts | 6 (1) | 4 (1) | 10 (2) |

Fisher's exact test for high-risk characteristics: $P=0.542$, no statistical difference. CNV: Corneal neovascularization.

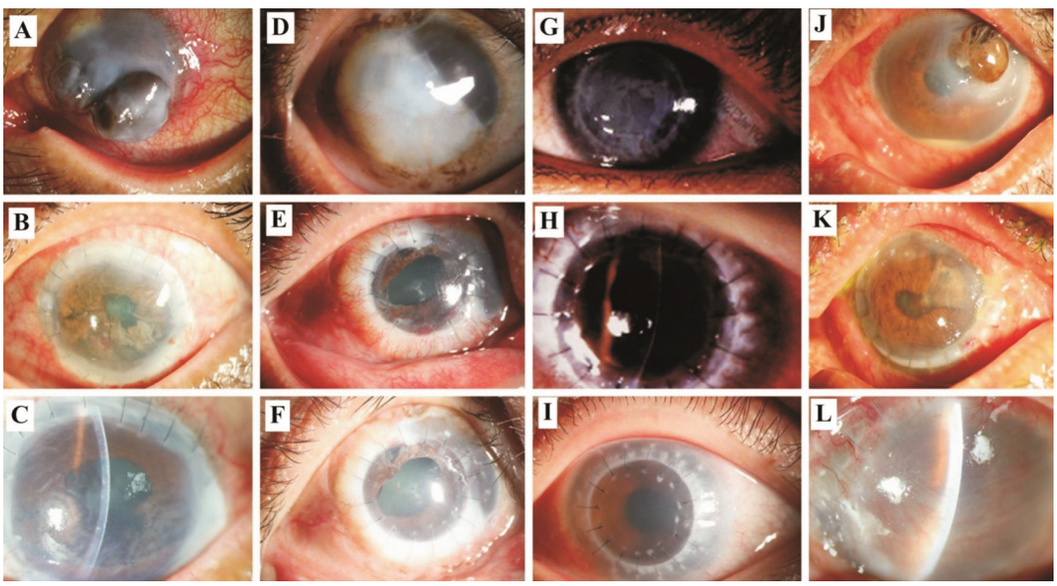


**Figure 2 Typical cases that experienced graft rejection** A-C: Photograph of Group 1 patient (a 46-year-old female) with a large diameter graft at preoperative (A), one week after surgery (B), and 6mo after surgery (C). D-F: Photograph of Group 1 patient (a 43-year-old male) with previously failed graft due to rejection at preoperative (D), one week after surgery (E), and 6mo after surgery (F). G-I: Photograph of Group 2 patient (a 22-year-old male) with previously failed graft due to rejection at preoperative (G), one day after surgery (H), and 7mo after surgery (I). J-L: Photograph of Group 2 patient (a 58-year-old female) with a large diameter graft at preoperative (J), one week after surgery (K), and 2mo after surgery (L).

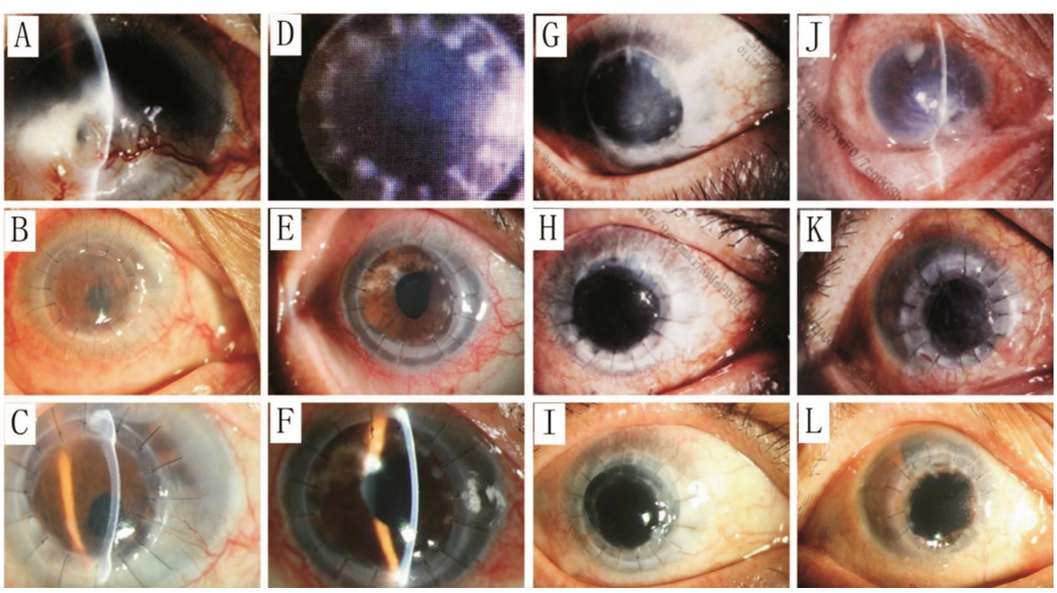


**Figure 3 Typical cases without experiencing graft rejection** A-C: Photograph of Group 1 patient (a 46-year-old female) with 4 quadrants of CNV at preoperative (A), one week after surgery (B), and 9mo after surgery (C). D-F: Photograph of Group 1 patient (a 71-year-old female) with previously failed graft due to rejection at preoperative (D), one week after surgery (E), and 2mo after surgery (F). G-I: Photograph of Group 2 patient (a 46-year-old male) with previously failed graft due to rejection at preoperative (G), one day after surgery (H), and 12mo after surgery (I). J-L: Photograph of Group 2 patient (an 80-year-old male) with eccentric graft at preoperative (J), one day after surgery (K), and 12mo after surgery (L). CNV: Corneal neovascularization.

the survival rate of clear graft (Group 1 *vs* Group 2, 83.6% *vs* 36.7%, log-rank test, $P<0.01$; Figure 5).

## DISCUSSION

In this study, we found that the incidence of graft rejection was significantly lower in HRK patients who applied triple combination use of systemic MMF, topical FK506, and corticosteroid eyedrops (16.0%), than in those treated with only topical FK506 and corticosteroid eyedrops (60.0%). Our study suggests that a triple combination regimen should be recommended for patients with HRK.

The prevalence of corneal graft rejection in HRK ranges from 32.4% to 77.6%, including patients who receive tapering

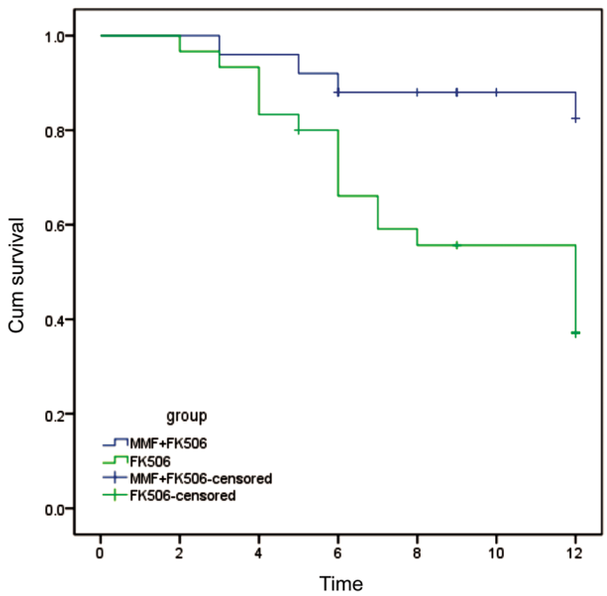


**Figure 4 Kaplan-Meier survival plot of rejection-free graft survival** Log-rank test: *P*=0.002. MMF: Mycophenolate mofetil.

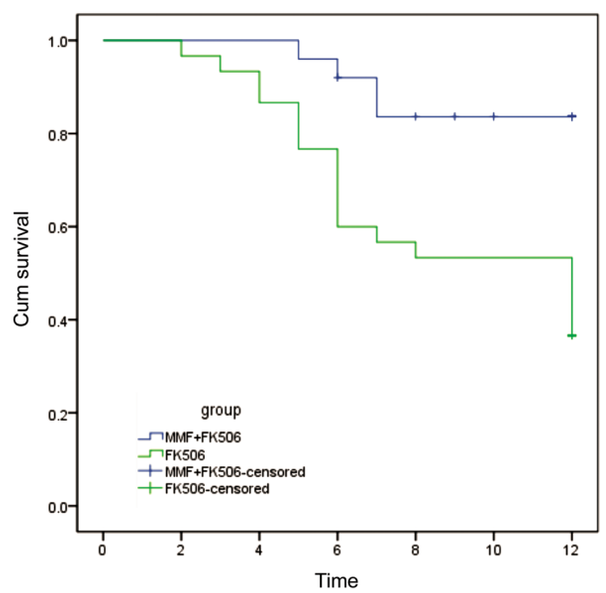


**Figure 5 Kaplan-Meier survival plot of clear graft survival** Log-rank test: *P*=0.001. MMF: Mycophenolate mofetil.

systemic and topical steroids alone or in combination with topical FK506[9,11-12,16]. In line with this, the incidence of corneal graft rejection in Group 2 was 60%. However, the incidence of corneal graft rejection in Group 1 was 16%. The difference between them is that Group 1 included contains treatment with systemic MMF. Systemic MMF is usually used as an anti-graft rejection drug after organ transplantation or for autoimmune diseases and is also used for HRK patients as a combination drug. In one multicenter randomized study with 2-year follow-up, the corneal graft rejection rate was 16% (8/50) in HRK patients treat with systemic MMF and fluocortolone combined with topical corticosteroid eyedrops[9]. In a prospective study with a two-year follow-up, the corneal graft rejection rate was 8% (8/98) in patient with systemic MMF combined with systemic prednisone and topical loteprednol eyedrops[11]. Our study is consistent with these results, suggesting that combined systemic MMF is an effective method for improvements in rejection-free survival and clear graft survival.

Topical corticosteroids are the basic treatment for preventing corneal graft rejection following keratoplasty[22]. To achieve optimal efficacy and avoid corticosteroid-related adverse events, tapering doses and combining them with immunosuppressive agents are suggested. Topical FK506 appears to be more beneficial as an adjunct to corticosteroids than topical CsA for incidences of corneal graft rejection in HRK[13,23]. However, graft rejection still occurs in 38.8% of recipients who receive topical FK506 and corticosteroids eyedrops[16]. The different rejection rates could be attributed to rejection risk discrepancies in different cohort studies.

For those at high risk of graft rejection, the results here indicate that the triple-combination regimen might have some advantages for large diameter grafts, regrafts, and vascularized transplant beds. Usually, patients who undergo large-diameter keratoplasty suffer from severe and large corneal ulcers before surgery[24], in which 43.8% to 65.0% of eyes develop graft failure[24]. Fifty percent of cases in Group 1 and 77.8% in Group 2 with large-diameter grafts experienced graft rejection. For regrafting, 11.1 % of grafts in Group 1 and 44.4% of grafts failed due to irreversible rejection. In a previous report, more than 20% of regrafts failed due to irreversible rejection[25]. For vascularized corneal transplant beds, 16.7% of grafts were rejected in Group 1, and 50% of grafts were rejected in Group 2. The literature shows that the survival of corneal grafts onto neovascularized transplant beds of two or more quadrants ranges from 50% to 66%[4]. Because of the small number of enrolled patients in subgroups, there was not enough for effective statistical analysis in this study.

The main side effects of systemic MMF are gastrointestinal and liver disturbances[14-15]. After using 1.0 g of MMF for the first month and 0.25 g for the next 6mo twice daily, gastrointestinal disturbances were reported in 26% of cases, and elevated liver enzymes were observed in 8% of cases. Thus, it is necessary to monitor liver function periodically during MMF treatment. No systemic side effects were recorded after the topical use of FK506 and corticosteroid eyedrops.

There are some limitations to this study, including its limited sample size, short follow-up period, and non-randomized grouping. Also, we did not collect blood after patients stopped taking MMF to observe levels of liver enzymes. A prospective large multicenter randomized clinical trial with long-term follow-up is needed to explore the effects of this combination strategy.

In conclusion, the combination of systemic MMF showed more benefits than topical immunosuppressants alone. The triple combination regimen with systemic MMF, topical

FK506, and tapering corticosteroid eyedrops could effectively prevent graft rejection and improve graft survival for HRK.

**ACKNOWLEDGEMENTS**

**Foundations:** Supported by Natural Science Foundation of Guangdong Province, China (No.2022A1515011140); China Postdoctoral Science Foundation (No.2023M744071); the Postdoctoral Fellowship Program of China Postdoctoral Science Foundation (No.GZB20230901); Regional Consolidated Fund—Youth Fund Project (No.2023A1515110531); Science and Technology Program of Guangzhou, China (No.202201020502).

**Conflicts of Interest: Zhang JY,** None; **Yu K,** None; **Jiang XY,** None; **Liang JX,** None; **Zhou SY,** None.

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