Thieme

Clinical Course of Different Types of Immune Reactions following Keratoplasty

Klinischer Verlauf von verschiedenen Typen der immunologischen Abstoßungsreaktion nach Keratoplastik

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ABSTRACT

Background Immune-mediated corneal graft rejection (IR) is a leading cause of corneal graft failure. The endothelium, stroma, epithelium, or a combination can be affected. Little is known about the long-term outcomes of different types of IR.
Methods We reviewed the medical records of all keratoplasties that had been performed at our eye centre between 2003 and 2016 (n = 3934) for any kind of IR that occurred between the surgery and 2019. All patients with a definite diagnosis of IR and sufficient clinical data were included in the analysis. IRs were grouped according to the affected part of the graft (endothelial, stromal, epithelial, and mixed). We analysed the dynamics of recovery and the clinical outcomes.
Results We identified a total of 319 patients with IR. Twenty-seven of those were lost to follow-up and were excluded from further analysis. Of the IRs, 89% affected the endothelium. Endothelial IR resulted more frequently in a considerable loss of endothelial cell density than other forms of IR. Stromal IR showed a lower relapse rate and a better visual recovery than other types of IR and resulted less often in a failure of the graft.
Conclusions We herein report comprehensive data about the prognosis regarding functional recovery after different types of IR following keratoplasty. Our data underline that timely recognition and correct classification of IR are important because they determine the clinical course and prognosis.

ZUSAMMENFASSUNG

Hintergrund Die Transplantatabstoßung ist ein Hauptgrund für ein Transplantatversagen nach Hornhautübertragung. Dabei können das Endothel, das Stroma, das Epithel oder eine Kombination davon betroffen sein. Über die klinischen Verläufe nach den verschiedenen Abstoßungsformen ist wenig bekannt.
Methoden Es wurden die Krankenakten von sämtlichen Patienten nach Hornhauttransplantation analysiert, die zwischen 2003 und 2016 in unserer Klinik durchgeführt wurden (n = 3934). Alle Patienten mit einer immunologischen Transplantatabstoßung wurden in die Analyse aufgenommen. Die Patienten wurden nach der führenden Abstoßungskomponente gruppiert (endothelial, stromal, epithelial und gemischt). Wir analysierten die klinischen Ergebnisse im Verlauf.
Ergebnisse Insgesamt wurden 319 Patienten mit einer immunologischen Abstoßungsreaktion identifiziert. 27 Patienten wurden mangels Nachuntersuchungsdaten nicht weiter ausgewertet. 89% der Immunreaktionen betrafen das Endo-

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thel. Endotheliale Abstoßungsreaktionen führten häufiger zu einem signifikanten Verlust von Endothelzellen als andere Formen der Abstoßung. Stromale Immunreaktionen zeigten eine geringere Rezidivrate und eine schnellere Erholung der Sehschärfe als andere Formen von Immunreaktionen und führten auch seltener zu einem Transplantatversagen.

Schlussfolgerungen Wir berichten hier über die Prognose nach verschiedenen Arten der Immunreaktion nach Keratoplastik. Die rechtzeitige Erkennung und korrekte Klassifizierung von Immunreaktionen sind wichtig für die Prognose des klinischen Verlaufs.

Introduction

Immune-mediated corneal graft rejection in keratoplasty

Corneal graft rejection is defined as an immune-mediated alteration of the graft by the host's immune system. Immune-mediated corneal graft rejection (IR) has been identified as the leading cause for corneal graft failure, accounting for up to 50% of all failures [1, 2].

Most IRs occur within the first 18 months following transplantation. Guilbert and co-workers reported an average keratoplasty-to-IR time of 19.8 ± 20.4 months (range from 0.5 to 158 months) in a cohort of predominantly penetrating keratoplasties [1209 cases of penetrating keratoplasty (PK), 165 cases of anterior lamellar keratoplasty (ALK), 64 cases of Descemet's stripping endothelial keratoplasty (DSEK)]. Rejection episodes in this cohort led to graft failure in 53.8% of the cases [3].

In the literature, the reported incidence of IR ranges from 2.3 to 68% [4]. In avascular corneal beds without inflammation at the time of surgery, IR-free graft survival 2 years after keratoplasty exceeds 80% [5, 6]. Williams and co-workers reported a 34% IR rate (analysing 14622 medical records) in the Australian corneal graft registry, including a heterogeneous cohort of normal- and high-risk keratoplasties [2]. Registry data from selected high-risk keratoplasty cohorts (e.g., repeat keratoplasties, PK after infectious diseases such as herpetic keratitis/acanthamoeba infections, limbo-keratoplasty, or PK in children) show notably higher IR rates, up to 90% [4, 7, 8].

Risk factors for IR include properties of the graft (e.g., antigen mismatch, storage time in organ culture [9]) and features of the recipient [10, 11]. Most importantly, vascularization of the graft bed leads to enhanced trafficking of antigen-presenting cells as well as adaptive immune cells and thus increases the risk of graft rejection. Inflammation of the graft bed due to previous or ongoing infections or ocular surface diseases is associated with increased immune-competent cells that can trigger an immunological reaction against the transplanted tissue. Unrelated ophthalmologic or medical conditions (e.g., glaucoma, uveitis, atopic dermatitis) also lead to an increased risk of graft rejection [4, 7]. Last but not least, young age also makes recipients more prone to IR [8].

The rate of reversibility of IR is indicated to be approximately 60 to 70% after 3 months, given early diagnosis and appropriate therapeutic measures [12–14]. Molter and co-workers did not see a significant difference in visual acuity before the occurrence of the IR compared to 3 to 12 months after IR [14].

Aims of this study

We herein present long-term follow-up data of a single-centre cohort of 292 patients who suffered at least one episode of IR following penetrating or posterior lamellar keratoplasty. We analysed dynamics and outcomes of different types of immune rejection (endothelial, stromal, epithelial, and mixed) and putative trigger factors for IR.

Material and Methods

2.1 Corneal IR registry

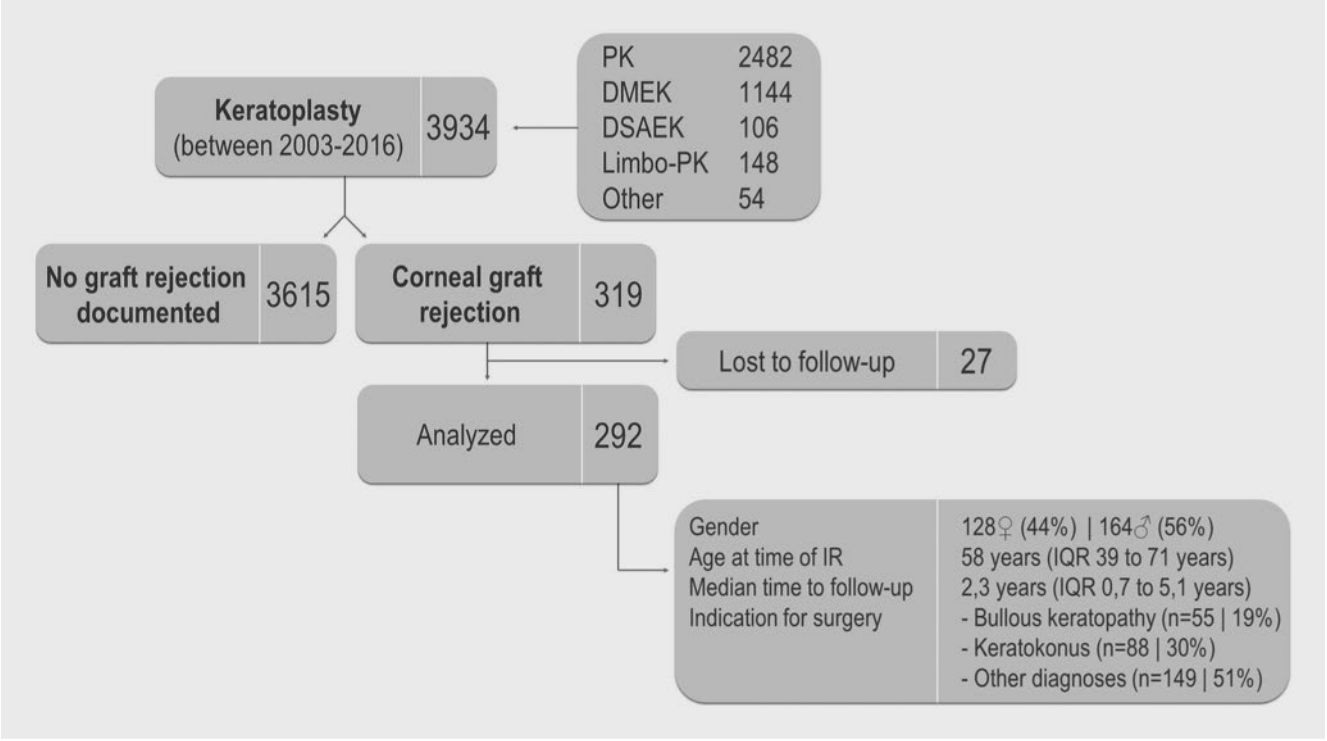
In this retrospective, single-centre, longitudinal observational study we analysed clinical charts from 3934 patients (see ▶ **Fig. 1**). All keratoplasties were performed between 2003 and 2016 at the Eye Center, Faculty of Medicine, University of Freiburg, Germany and included PKs (n = 2482), Descemet's membrane endothelial keratoplasties (DMEKs, n = 1144), limbo-PKs (n = 148), Descemet's stripping automated endothelial keratoplasties (DSAEKs, n = 106), and others (n = 54). Only patients with signs of IR after penetrating and/or posterior lamellar keratoplasty were enrolled. All data from patients with IR were compiled in a relational database (for further details see ▶ **Fig. 1**).

Patient characteristics

A total of 319 patients with IR were identified; 27 were lost to follow-up (see ▶ **Fig. 1**). Of the remaining 292 patients, 128 were female (44%) and 164 were male (56%). The medium age at the time of IR was 58 years (lower quartile 39 years, upper quartile 71 years). The medium time of follow-up was 2.3 years (lower quartile 0.7 years, upper quartile 5.1 years). Indications for keratoplasty were Fuchs endothelial dystrophy and bullous keratopathy (n = 55; 18.8%), keratoconus (n = 88; 30.1%), and others (n = 149; 51.1%). Surgical techniques were PK (n = 235; 80.5%), limbo-PK (n = 29; 9.9%), DSAEK (n = 7; 2.4%), DMEK (n = 10; 3.4%), and others (n = 11; 3.7%). The "other" group includes optical or nonoptical emergency keratoplasties. We also perform DALK in our hospital, but, so far, there are no patients in our registry who show clear signs of IR following DALK.

Clinical signs of graft rejection

Corneal graft rejection was diagnosed clinically by slit lamp examination. Rejections were grouped into endothelial, stromal, epithelial, or mixed rejection forms defined as follows: Epithelial IR was defined as a line of leukocytes located near engorged limbal vessels, with migration across the graft-recipient interface (with or without the presence of punctate keratitis) when other causes (e.g., dry eye) seemed unlikely. Stromal IR was diagnosed when



▶ **Fig. 1** Study details. In the study presented here, records of 3934 patients were retrospectively analysed; immune-mediated graft rejection was documented in 319 patients. The relevant further details can be obtained from the figure.

deeper cellular infiltrates/Krachmer spots and/or involvement of stromal keratocytes could be detected. Endothelial IR was defined by identification of keratitic precipitates, an endothelial line of leukocytes (Khodadoust line) with or without stromal oedema, or detection of leukocytes in the anterior chamber. Graft rejection following DMEK was confirmed in cases with precipitates on the graft. Mixed IR was defined when at least two signs of different IR subtypes were present, e.g., endothelial and stromal or epithelial and stromal IR.

Inclusion/exclusion criteria

All patients presenting at our eye centre are seen by a resident as well as by a cornea consultant who establishes the definite diagnosis. Keratoplasty patients were identified from the computer database of our cornea bank. The charts were reviewed manually. All patients with a definite diagnosis of IR and sufficient clinical data were included in this analysis. For the DMEK records, this was supported by natural language processing using custom software in combination with the open-source program "Apache OpenNLP" and a formal grammar. However, all matches were reviewed by one of the authors.

Ethics approval

The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the local institutional ethic review board (approval number 88/12). It adheres to German federal and state laws. Patient data were anonymised prior to analysis.

Statistical analysis

Statistical calculations were performed using the R platform. We used the kernel density estimate, which is a smoothed version of the histogram, to graphically compare the temporal dynamics of incidence rates between groups (which is referred to as the probability density function). This method derives temporal probabilities from discrete events. Event rates were estimated using the Kaplan-Meier method.

Results

Baseline data and rejection subtypes

We reviewed a total of 3934 patient files in this study. This study comprises low- as well as high-risk keratoplasties. We discovered a total of 319 IRs. Twenty-seven patients had to be excluded due to loss of follow-up, 292 IRs remained for detailed analysis. Of the IRs, 89% were classified as endothelial (n = 260), 3% (n = 10) as stromal, and 3% (n = 9) as epithelial IR, while 4% (n = 13) were mixed. The indications for the initial keratoplasty were Fuchs' endothelial dystrophy or bullous keratopathy in 55 eyes, keratoconus in 88 eyes, and other in 149 eyes. The medium age at the time of IR was 58 years (lower quartile 39 years, upper quartile 71 years).

Dynamics of rejection

The estimated probability of IR peaked within the first 10 to 15 months after surgery for the whole group. The probability for

IR after DMEK showed a second smaller peak 25 months after surgery (data not shown). Epithelial IRs were not seen after 20 months following surgery. Endothelial and mixed IRs included delayed events occurring up to 60 months after surgery (see ▸ **Fig. 2**).

Identification of risk factors

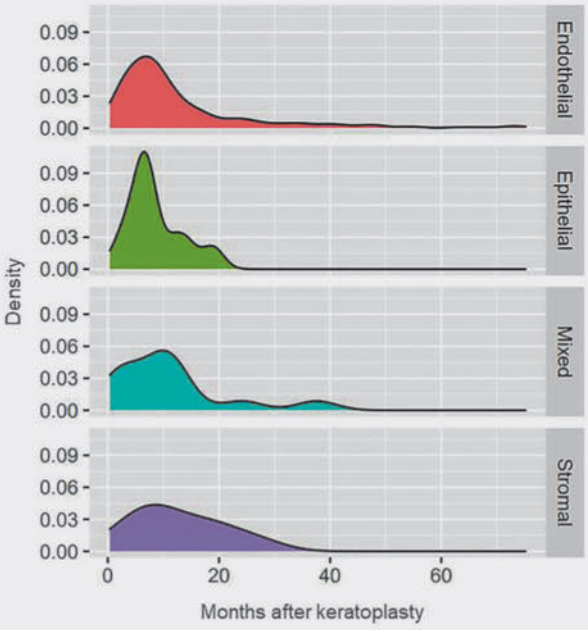
We analysed the temporal correlation between suture removal and time to event for all IR types. Across all types of IR, the percentage that experienced anterior segment surgery (including suture removal) ranged from 20% (epithelial IR) to 33% (stromal IR). The correlation between IR and time of suture removal was analysed for all PK, limbo-PK, and the group of "other" indications. The peak of the density estimates for IR at approximately 5 months before suture removal correlated with the general peak of probability. After suture removal, the probability for IR decreased over the following months and never exceeded the probability at the time of suture removal (data not shown).

Relapse-free graft survival and graft failure

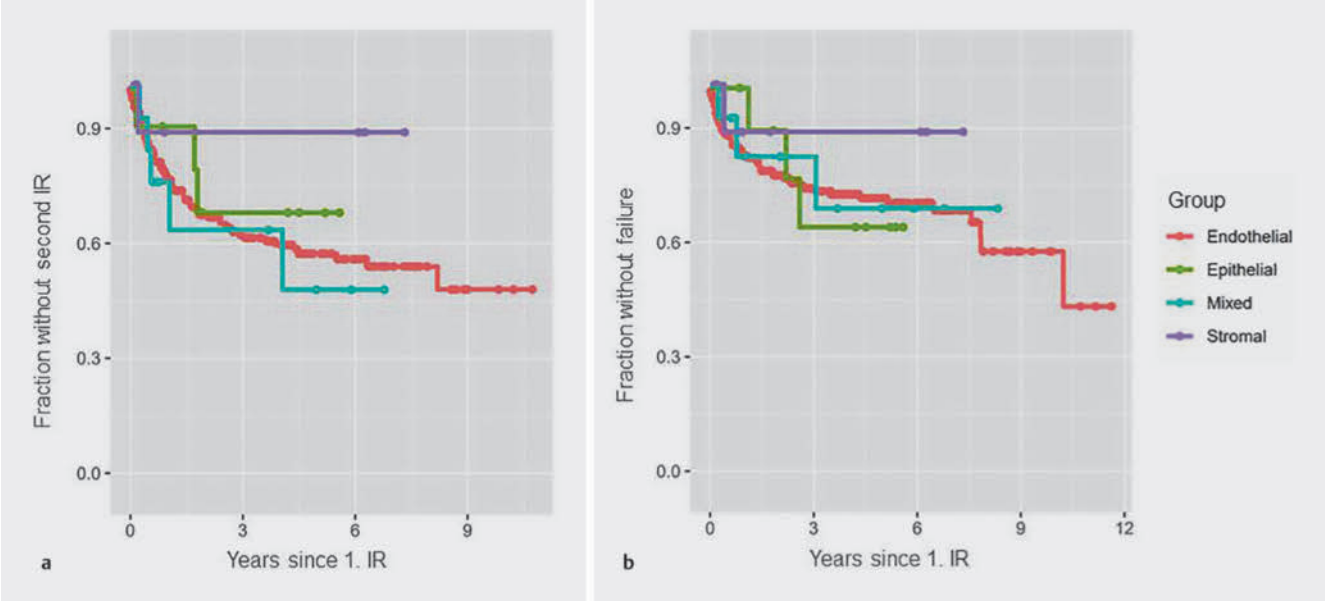
Our data suggest that 5 years after an initial IR, around 60% of eyes were relapse free. Approximately 50% of eyes with initial endothelial IR were still relapse free 10 years after the event. After stromal IR, a relapse seemed to be generally rare with recurrence-free eyes close to 90% after 6 years (see ▸ **Fig. 3a**). Irrespective of the type of IR, overall graft survival correlated closely with relapse-free graft survival (see ▸ **Fig. 3b**).

Decline of endothelial cell density

We compared endothelial cell density (ECD) loss between the different types of IR. Following an episode of graft rejection, only en-



▸ **Fig. 2** Dynamics of different types of immunological graft rejection. Data is presented as probability density functions for endothelial (n = 260), epithelial (n = 9), mixed (n = 13), and stromal (n = 10) immune reactions.



▸ **Fig. 3** Clinical course of different types of immunological graft rejections. Fraction of eyes without second immune reaction (IR) (**a**) and without graft failure (**b**) after endothelial, epithelial, stromal, and mixed IR.

dothelial and mixed IRs showed a subsequent drop of ECD below 500 cells/mm^2. However, the decline of ECD seemed to occur rather slowly: only every second eye with endothelial IR fell below 500 cells/mm^2 during the first 10 years after IR (Kaplan-Meier estimation; data not shown). Eyes with epithelial or stromal IR never showed ECD values under 500 cells/mm^2 during follow-up (data taken from Kaplan-Meier curve, not shown).

Visual recovery

Our data indicate that approximately 50% of eyes suffering from endothelial IR recover during the first year after IR (data taken from Kaplan-Meier curve, not shown). After 1 year, the prognosis for visual recovery worsens considerably. Stromal IR seems to confer a favourable prognosis regarding visual recovery in comparison to other forms of IR. However, due to the limited group size, our data do not allow a reliable prediction for visual recovery after epithelial, stromal, or mixed IRs.

Immunosuppressive therapy

We analysed the therapeutic regime at the time point of IR. In 70% of epithelial IRs, IR emerged under topical immunosuppressive therapy. In stromal, endothelial, and mixed IRs, the rates were 22, 32, and 38%, respectively (data not shown). Epithelial, endothelial, and mixed IRs were also observed in patients with systemic immunosuppressive therapy.

Discussion

We herein present a dataset of all IRs following keratoplasty from a specialised centre with long follow up. Our data are unique in that they offer a detailed analysis of the clinical course outcome of different types of IR after keratoplasty. Ultimately, our dataset can help to evaluate the prognosis at the time of IR and thereby facilitate decision-making regarding therapeutic measures.

Throughout the literature, the different types of corneal IR are not defined consistently. This is a common problem for large national keratoplasty registries. The single-centre approach of this study offers a consistent classification and diagnosis of IR.

IR is a relatively common complication of PK [3, 13]. The reported incidence of IR following DMEK seems to be considerably lower [15, 16]. Irrespective of surgical technique, however, episodes of graft rejection with absent or only subtle clinical symptoms can go unnoticed by the patient. These are possibly underdiagnosed and not part of our dataset as a matter of principle. This might have contributed to the large predominance of endothelial IRs in our data. Additionally, the definite diagnosis of epithelial IR is difficult to establish, as the clinical signs are highly variable and can resemble clinical signs of ordinary dry eye disease. Our data suggest that the probability for all types of graft rejection peaks within the first 12 months after keratoplasty. Epithelial graft rejection exclusively occurs within the first 2 years after transplantation, while stromal and endothelial rejections can occur much later. This is explained by the fact that the epithelium is replaced by the recipient's epithelium via limbal stem cells in the course of time [17]. Endothelial IR is not only the most common form of rejection in our cohort, it also bears a considerable risk of resulting in a drop of ECD below the critical threshold of 500 cells/mm^2. In our study, eyes that experienced stromal and epithelial IRs did not fall below this threshold, suggesting an accelerated loss of endothelial cells to be associated with endothelial IR. Interestingly, endothelial IRs do not result in substantially higher rates of graft failure and permanently impaired BCVA compared to stromal or epithelial graft rejections during the first 5 years after surgery. This finding suggests that endothelial cell loss due to IR alone does not result in graft failure. Importantly, IR is not the only reason for graft failure and chronic endothelial cell loss and resurfacing problems have been described as important contributors to eventual failure of corneal grafts [18, 19]. Eyes that receive corneal transplantation due to keratoconus show superior survival of endothelial cells than eyes that experienced corneal transplantation due to bullous keratopathy [20]. Since our cohort is heterogenous regarding indications for keratoplasty, our data are biased: the extent to which IR contributes to endothelial cell loss and graft failure diverges within our cohort.

Understanding the underlying mechanism of graft failure is important when it comes to therapeutic measures. Granted that ECD loss is a central contributor to graft failure after endothelial IR, DMEK might be a viable option when PK fails due to an episode of endothelial graft rejection. To date, however, lamellar procedures do not show resounding results after failed PK [21, 22]. Regarding the visual outcome after endothelial IR, our data suggest that if the BCVA has not recovered approximately 1 year after the IR, the prognosis for visual recovery deteriorates. In those cases, a regraft might be evaluated to restore visual acuity.

Apart from host-specific risk factors like vascularisation of the graft bed, inflammation, or ophthalmologic comorbidities, several external factors that increase the risk for IR have been identified [23]. These include anterior segment surgery and discontinuation of immunosuppressive therapy [24]. We analysed whether the performance of anterior segment surgery (including suture removal) favoured a specific subtype of IR. Throughout all four types of IR, the rate of eyes that experienced anterior segment surgery ranged between 20 (epithelial IR) and 33% (stromal IR). Our data suggest that anterior segment surgery does not selectively increase the risk for any specific type of IR.

Published data concerning suture removal being a trigger factor for graft rejection is inconclusive. Epstein et al. reported that suture removal did not increase the risk for IR in a cohort of 23 eyes with graft rejection [25]. However, early removal of sutures was identified as a risk factor by Wiliams et al. within the Australian Corneal Graft Registry [2]. We investigated the temporal correlation between suture removal and the emergence of IR in the subgroup of PKs in our cohort (n = 141). Our data do not reveal an obvious correlation between the time point of suture removal and the time point of IR. We therefore see no evidence that suture removal triggers IR. However, our data do not control for a potential change in topical therapy following suture removal that might itself contribute to IR.

We analysed the therapeutic regimen (systemic and/or topical treatment) at the time point of IR to see whether the absence of immunosuppressive therapy rendered eyes more susceptible to a specific type of IR. The high percentage of epithelial IRs occurring under immunosuppressive therapy might be explained by the fact that epithelial IR is a rather early event after keratoplasty, when

topical immunosuppression is more likely to be administered. However, our data is biased since immunosuppressive therapy is not necessarily standardised and, for example, intensified in high-risk situations. Overall, our data do not provide evidence that the presence or absence of topical or systemic immunosuppressive therapy favours the emergence of a specific type of graft rejection. Generally, as reported previously, the absence of immunosuppressive therapy seems to render eyes more susceptible to any type of graft rejection [24, 26]. Therefore, our data support current clinical practice regarding the prophylactic long-term use of topical immunosuppressive therapy. Especially repeated episodes of IR can justify an intensified long-term administration of topical or systemic immunosuppressive drugs in order to maintain graft clarity. Notably, the individual risk for graft rejection varies highly and, for example, eyes that receive a corneal graft due to keratoconus usually do not require an equally intense immunosuppressive therapy as patients who receive an à chaud keratoplasty due to corneal ulceration [4, 6].

Summary

This study offers an in-depth analysis of the clinical course and outcomes of IR following different types of keratoplasty. Our data suggest differences in the clinical course of different subtypes of IR, with endothelial IRs bearing the highest risk for endothelial decompensation and stromal IRs conferring a favourable visual outcome. We did not find evidence that anterior segment surgery (including suture removal) triggers IR or favours the emergence of a specific type of IR. All types of IR can occur under topical and systemic immunosuppressive therapy. Our study broadens the clinical understanding of immune-mediated graft reactions following keratoplasty and can facilitate the assessment of therapeutic measures at the time of diagnosis.

Declarations

Ethics approval

The study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the local institutional ethic review board (Ethics committee of the Albert-Ludwigs-University of Freiburg, approval number 88/12). This study adheres to German federal and state laws. Patient data were anonymised prior to analysis.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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