An Attempt to Optimize the Outcome of Penetrating Keratoplasty in Congenital Aniridia-Associated Keratopathy (AAK).

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Research Article

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Abstract

Purpose

To propose an optimized microsurgical and medical approach to reduce the risk of complications after penetrating keratoplasty (PKP) in patients with aniridia-associated keratopathy (AAK).

Methods

Retrospective observational case series of 25 PKP performed in 16 patients with AAK. Preoperative indications were endothelial decompensation and vascularised scars (68%) or graft failure (32%) due to limbal stem cell deficiency. The optimized approach included a combination of a small corneal graft size (around 7.0mm), interrupted 10-0-Nylon sutures, simultaneous AMT as a patch, large bandage contact lens, temporary lateral tarsorrhaphy, postoperative autologous serum eye drops, and systemic immunosuppression. Main outcome measures included: Visual acuity, transplant survival and complications encountered during follow-up of 107 weeks on average.

Results

A complete modified keratoplasty scheme was used in 10 of 25 PKP (group 1), while at least one of the modifications was missing in the other 15 PKP (group 2). After 8 weeks follow-up, the epithelium was closed in 23 eyes. Visual acuity improved in 19 eyes at 6 months follow-up, and remained stable in 6 eyes. None of the eyes showed a decrease in visual acuity. At the last post-operative follow-up, this visual improvement persisted in 14 eyes and graft survival rate after 156 weeks (3 years) was 69% in group 1 vs. 44% in group 2 ($p = 0.39$, logrank test). Secondary corneal neovascularisation (8%), scarring (4%), ulcer (4%) or graft rejection (8%) happened mostly in the second group which was missing at least one of the suggested modifications.

Conclusions

PKP in congenital aniridia must be considered as a high-risk keratoplasty. An optimized therapeutic approach seems to be promising in order to reduce the postoperative complication rate in these most difficult eyes.

Key Messages

- Patients with congenital aniridia-associated keratopathy are likely to develop low vision that significantly affects their quality of lives.

- An optimized high-risk penetrating keratoplasty approach seems to be promising to reduce the postoperative complication rate in those most difficult eyes.

Introduction

First described as “congenital irideremia” in the 19th century, congenital aniridia is a rare (1:60.000 to 1:90.000) pan-ocular disease that can be differentiated in 2 major categories depending on the presence of a PAX-6-Gene mutation [1, 2, 3, 4]. Among those with a mutation of the PAX-6-Gene, frequent mutations involve point mutations and deletions. A deletion of the short arm of chromosome 11(p13) may be autosomal dominant or sporadic, and to a lesser extent autosomal recessive, e.g. in the Gillespie-Syndrome [5]. A PAX-6-Gene mutation is more frequently associated with ocular complications compared to aniridia triggered by other mutations than the PAX-6-Gene [2, 3]. Congenital aniridia is linked to different malformations such as iris, macular or optic nerve hypoplasia, but also to deteriorating progressive major ocular dysfunctions such as limbal stem cell deficiency, premature onset of cataract, and secondary glaucoma that can lead to blindness throughout life [2, 3, 6, 7, 8]. Aniridia-associated keratopathy (AAK) increases with age affecting about 20–30% of those patients, and leads to corneal opacities, scarring and vascularisation due to a unique form of limbal stem cell deficiency [2, 9, 10, 11].

With time, it may result in corneal ulcers, dense vascularised scars or endothelial decompensation (especially after complicated cataract and/or glaucoma surgery), where a penetrating keratoplasty makes sense [8]. Persisting epithelial defects, suture loosening, and an increased risk of graft rejection are typical postoperative complications in those high risk keratoplasties [8]. We hypothesized that the combination of a small corneal graft size, interrupted sutures, simultaneous amniotic membrane transplantation (AMT) as a patch [12, 13], large bandage contact lens, temporary lateral tarsorrhaphy, postoperative autologous serum eye drops [14], and systemic immunosuppression may improve the outcome after PKP in congenital aniridia.

Patients And Methods

This study is a retrospective observational case series of 25 penetrating keratoplasties (PKP) performed in 20 eyes of 16 patients with AAK at the Department of Ophthalmology of the Saarland University Medical Center in Germany between 2012 and 2019. All procedures were performed in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments. Transplantation had been proposed as a last resort therapy in endothelial decompensated corneas with or without vascularised scars (68%) and graft failure (32%), due to limbal stem cell deficiency with no success of conservative treatments.

The mean age during keratoplasty was 52 ± 8 (from 26 to 64) years and most (92%) eyes had a history of previous surgeries (**Table 1**). We defined a small graft as ranging from 6.0 to 7.5 mm diameter. Two types of nylon 10 – 0 sutures were used as follows: double running cross-stitch sutures according to Hoffmann vs. 24 to 32 interrupted sutures (Fig. 1). Amniotic membranes were collected from healthy women with

their consent and properly processed in the eye bank before transplantation [15]. They were transplanted at the end of the keratoplasty as a single 16-mm layer membrane with the stromal side facing the corneal graft and fixed with a running 10 – 0 nylon episcleral suture as a patch [12]. The membrane was covered with a large 17-mm bandage contact lens and removed after a period of 4 to 6 weeks. Temporary lateral tarsorrhaphy was performed using 5 – 0 silk and left typically for 4–6 weeks after PKP.

100%-concentrated autologous serum eye drops were prepared after exclusion of systemic infectious diseases [16, 17]. During the first postoperative days, they were applied hourly alternating with preservative-free hyaluronic acid containing artificial tear eye drops. A topical antibiotic coverage for at least 4 weeks with Ofloxacin or Moxifloxacin eyedrops was necessary to prevent infections until complete epithelial healing was achieved, and the bandage contact lens was removed. An additional long-term therapy with topical and systemic acyclovir was given to patients with history of herpetic keratitis.

Postoperative topical and systemic corticosteroid (Prednisolon, Prednisolonacetat and Methylprednisolon) were slowly tapered over 4 weeks. A systematic preoperative evaluation of long-term use of immunosuppression with Mycophenolate mofetil or Cyclosporin-A, and systemic follow-up was established in collaboration with the family physician, and doses being adapted to the general condition of the patient.

Combined transplantations with simultaneous cataract or vitreoretinal surgeries were adapted to the cases and their individual requirements. Characteristics of recipients are shown in **Table 1**. The optimized approach included a combination of a small corneal graft size (around 7.0mm), interrupted 10-0-Nylon sutures, simultaneous AMT as a patch, large bandage contact lens, temporary lateral tarsorrhaphy, postoperative autologous serum eye drops, and systemic immunosuppression. Main outcome measures included: Visual acuity, transplant survival and complications encountered during the follow-up of 107 weeks on average.

For statistical analysis, eyes were separated into two groups based on the therapeutic scheme. A complete, modified keratoplasty scheme was used in 10 of 25 PKP (group 1). The second group included all other PKP, where at least one modification of the modified keratoplasty scheme was missing (group 2). Techniques used are illustrated in **Table 2**.

Statistical analysis was performed using SPSS v. 20.0.0 (IBM Corp., Armonk, NY, USA). Visual acuity was recorded in decimal and converted into logMAR before analysis. Graft survival was analysed using the Kaplan-Meier method and logrank test.

Results

Visual acuity

At 6 months follow-up, the mean visual acuity (VA) improved from logMAR 2.18 to logMAR 1.65. None of the eyes showed a decreased VA. On the other hand, the VA remained unchanged in 6 eyes and improved

in 19 eyes. The last examination was made on average 107 weeks postoperatively with a mean VA of logMAR 1.69 in group 1 and logMAR 1.77 in group 2.

All patients reported a subjective improvement in their visual acuity, either by increased clarity of the image, or by decreased visual discomfort caused by corneal optical phenomena such as glare. This visual improvement persisted in 14 eyes until the end of follow-up (107 weeks on average).

Graft survival

At 8 weeks follow-up, the epithelium was closed in 23 eyes and only 2 eyes needed a second AMT. Graft survival was referred to as a clear and transparent graft, without endothelial decompensation scars or corneal opacities. Graft survival is demonstrated in a Kaplan-Meier chart **(Figure 2).** The mean postoperative follow-up was 119 weeks in group 1 and 216 weeks in group 2. Graft survival rate after 156 weeks (3 years) was 69% in group 1 and 44% in group 2 ($p = 0.39$, logrank test). The median graft survival time was 97 weeks in group 1 and 81 weeks in group 2.

Adverse events

While only 2 eyes needed a repeat keratoplasty in group 1 and 3 eyes in group 2, severe corneal complications such as graft rejection (8%), anterior segment fibrosis syndrome (4%) or graft ulcer (16%), mostly occurred in group 2. Other, less severe corneal complications such as persistent epithelial defects (20%) and premature suture removal/replacement (28%) were present in both groups **(Table 3)**.

A second AMT as patch was used to treat persistent epithelial defects in 5 eyes. Loose corneal sutures were removed as quickly as possible to prevent infiltrates and infections. Graft rejections were primarily treated with topical intracameral and systemic steroids, followed by a repeat keratoplasty, if needed. The two eyes that required a repeat keratoplasty after graft rejection had contraindications to systemic immunosuppressive therapy.

Extra-corneal complications, regardless of the chosen therapeutic scheme, were changes in ocular pressure (hypo- and hypertension) (20%), retinal detachment (4%), retinal vein occlusion (4%), and intraocular lens luxation (4%). Those complications were handled according to the respective German guidelines.

Discussion

The development of microsurgical techniques and the knowledge of limbal stem cell function has led to a considerable improvement in treatment and visual prognosis of eyes with congenital aniridia over the years [3–9, 18–20]. Each small improvement is helpful for those progressively visually impaired patients. The global therapeutic approach should always consider a high risk of concomitant glaucomatous damage with irreversible optic atrophy due to a mispositioning of the ciliary body processes toward the iris stump and a very short ciliary body [2, 7, 21].

In well selected cases, the treatment of AAK with PKP has shown to be beneficial, even though the post-operative complications turned out to be more frequent in those high-risk eyes [6, 8, 22]. Our modified treatment scheme appears to reduce the severe post-operative complications and improve visual prognosis as well as graft survival in the mid-term follow-up.

At first glance, the Kaplan-Meier curve may suggest a different trend between the groups, but the logrank test could not detect a statistically significant difference between the two groups in terms of graft survival ($p = 0.39$). We attribute this to the low number of cases, which is due to the rarity of the disease. Based on our clinical experience, we refrain from randomizing patients as we do not want to deprive them of what we believe to be the optimal treatment nowadays.

We explain this difference by the following preventive measures: the use of interrupted sutures allows a quick removal of loose sutures at the slit lamp without risking graft slippage, and thus reduces the risk of infections and secondary immune reactions. A temporary lateral tarsorrhaphy combined with simultaneous AMT as a patch and 17mm bandage contact lens considerably reduces postoperative epithelial defects due to a mechanical protective effect [12, 24, 25].

Furthermore, the amnion membrane supports epithelialization, it has anti-fibrotic effects (due to a reduced expression of TGF β1, β2, β3 isoforms and TGF-beta receptor II), and anti-inflammatory effects (through inhibition of proinflammatory cytokines). Moreover, anti-angiogenic effects (due to production of thrombospondin-1, endostatin and tissue inhibitors of metalloproteases), and immunomodulatory effects have been reported [12, 13, 15, 23]. Similar positive effects have been described for the autologous serum eye-drops [13, 15, 22]. Due to the higher risk of graft rejection, a systemic immunosuppressive therapy proved to be useful in most patients [26, 27].

The limitations of the study are the small sample size due to the rare condition and the lack of long-term observation periods to demonstrate a significant difference between groups. A prospective randomized study is not possible for ethical reasons. As our proposed therapeutic scheme does not directly address the cause of AAK, it could be combined with a prior transplantation of allogenic limbal stem cells. However, the survival curve looks promising, with a major separation after only 2 years of follow-up. We may expect an average graft survival of 50% at 5 years for the group which was treated with the complete scheme, and only 30% for the other group (Fig. 2).

In conclusion, an optimized high-risk PKP approach seems to be promising to reduce the postoperative complication rate in these most difficult eyes with congenital aniridia.

Declarations

Funding: No funding was received for this research.

Conflict of Interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in

speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Center for Limbal Stem Cell Research and Congenital Aniridia) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This manuscript did not involve any kind of animal research. All the authors consent to the publication of this manuscript in International Ophthalmology.

For this type of study formal consent is not required.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data availability: The data was attached to the manuscript as a supplementary file.

Author contribution:

CJF: collected data, conceived and designed the analysis, wrote the paper.

FNF: performed analysis, collected data.

LL: Contributed data or analysis tools.

BDD: Contributed data, designed the analysis.

BS: Designed the analysis, wrote the paper.

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Tables

Table 1. Characteristics of 16 patients with congenital aniridia (25 keratoplasties)	
Patients	n = 16
- Males	(100%)
- Females	6 (37,5%)
Genetics	10 (62,5%)
- PAX-6	n = 16
- WAGR(O)	(100%)
- Genetically not analysed	7 (43,75%)
	1 (6,25%)
	8 (50%)
Type of Keratoplasty	n = 25
- First keratoplasty (with or without simultaneous pannus removal)	(100%)
- Repeat keratoplasty (with or without simultaneous pannus removal)	13 (52%)
- Classical triple procedure (combined with cataract-surgery and intraocular lens implantation)	5 (20%)
	4 (16%)
- Pole-to-pole surgery (keratoplasty combined with lens and vitreoretinal surgery)	2 (8%)
- HLA-typed keratoplasty	1 (4%)
History of previous surgeries before keratoplasty (Multiple choices possible)	n = 25
- Glaucoma surgery	(100%)
• Trabeculotomy	11 (44%)
• Cyclophotocoagulation	3 (12%)
• Ahmed Valve	9 (36%)
- Corneal surgery	5 (20%)
• Pannus removal (“pannectomy”)	12 (48%)
• Phototherapeutic keratectomy (PTK)	3 (12%)
• Penetrating keratoplasty (in domo)	1 (4%)
• Penetrating keratoplasty (ex domo)	5 (20%)
- Cataract surgery	3 (12%)
- Retinal surgery	8 (32%)
- None	6 (24%)
	2 (8%)

Table 2. Techniques used in 25 keratoplasties (Multiple choices possible)	
Diameter of recipient openings (diameter in mm)	n = 25 (100%)
- 6.5 mm	1 (4%)
- 7.0 mm	14 (56%)
- 7.5 mm	9 (36%)
- 12.0 mm (Limbo-Keratoplasty)	1 (4%)
Sutures	n = 25 (100%)
- Interrupted sutures	18 (72%)
- Double running cross-stitch sutures according to Hoffmann (1976)	7 (28%)
Use of simultaneous amnion membrane transplantation (Patch)	n = 25 (100%)
- Yes	24 (96%)
- No	1 (4%)
Use of simultaneous lateral tarsorrhaphy	n = 25 (100%)
- Yes	14 (56%)
- No	11 (44%)
Use of postoperative autologous serum eye drops primarily	n = 25 (100%)
- Yes	19 (76%)
- No	6 (24%)
Use of long-term immunosuppressive therapy	n = 25 (100%)
- Mycophenolate mofetil	20 (80%)
- Cyclosporin-A	3 (12%)
- None	2 (8%)

Table 3. Corneal complications during follow-up. n = 25 keratoplasties (100%) Group 1: Complete therapeutic modified scheme (n = 10) Group 2: Incomplete therapeutic scheme (n = 15)	
Persistent epithelial defect	n = 5 (20%)
- Group 1	3 (12%)
- Group 2	2 (8%)
Premature suture removal/replacement	n = 7 (28%)
- Group 1	3 (12%)
- Group 2	4 (16%)
Corneal endothelial decompensation	n = 4 (16%)
- Group 1	2 (8%)
- Group 2	2 (8%)
Immunological graft rejection	n = 2 (8%)
- Group 1	0 (0%)
- Group 2	2 (8%)
Graft ulcer / neovascularisation / scarring / anterior segment fibrosis syndrome	n = 5 (20%)
- Group 1	1 (4%)
- Group 2	4 (16%)

Figures

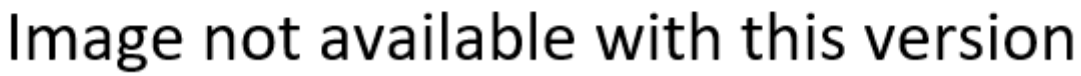

Figure 1

(Figure 1 a) Patient 1: Pre-operative picture with decompensated and severely vascularised graft, 6 years after penetrating keratoplasty. Visual acuity: Hand motion (logMar 2.7). (Figure 1b) Patient 1: 8 months post-operative picture with 26 interrupted sutures after repeat penetrating keratoplasty with Barron trephine (7.00/7.25 mm). Visual acuity has reached logMar 2.3. (Figure 1c) Patient 2: 64-year old woman with corneal decompensation, vascularised cornea, secondary amyloidosis and premature cataract in congenital aniridia. Visual acuity: Hand motion (logMAR 2.7). (Figure 1d) Patient 2: Clear corneal graft, 18 months after classical excimer laser assisted triple procedure (7.0/7.1 mm) and suture removal. Visual acuity improved to logMAR 1.0.

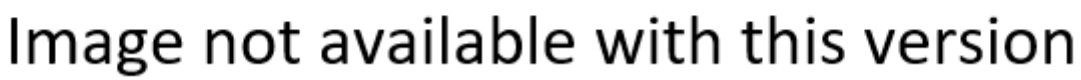

Figure 2

Survival of corneal grafts in group 1 (complete treatment) vs. group 2 (incomplete treatment). Graft survival rate after 156 weeks (3 years) was 69% in group 1 and 44% in group 2, (p = 0.39, logrank test).