Simultaneous penetrating keratoplasty and amniotic membrane transplantation in eyes with a history of limbal stem cell deficiency

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Simultaneous Penetrating Keratoplasty and Amniotic Membrane Transplantation in Eyes with a History of Limbal Stem Cell Deficiency

Simultaneous Corneal and Amniotic Membrane Transplantation in Limbal Deficiency

Kératoplastie Transfixiante combinée à une Greffe de Membrane Amniotique au cours du Syndrome d'Insuffisance en Cellules Souches Limbiques

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ABSTRACT

Purpose: To describe the outcome of simultaneous penetrating keratoplasty (PK) and amniotic membrane transplantation (AMT) performed both as a ring-shaped graft and as a temporary patch in eyes with a history of limbal stem cell deficiency (LSCD).

Methods: Prospective observational case series including 48 simultaneous PK/AMT procedures (48 patients) in eyes with a history of partial or total LSCD. Patients with total LSCD were first treated with limbal stem cell transplantation. The preoperative diagnosis was regraft in 58.3% of cases. Most recipients (89.6%) were at high risk for rejection.

Results: The mean graft reepithelialization time was 29.2±30.8 days. Graft reepithelialization was achieved in 30 days in 70.8% of cases. No AMT-related adverse events were observed. The mean keratoplasty-to-last visit time was 84.5±54.5 months. The 3-year graft survival estimate was 62.5%. Recurrence of corneal epithelial defects after graft reepithelialization (47.9%) was associated with lower graft survival ($p = 0.004$). In eyes with successful grafts at last visit, the mean LogMAR visual acuity was 1.90 (20/1575) ± 5 lines before keratoplasty and 0.89 (20/155) ± 10 lines at 5 years. A ring of amniotic membrane was observed between the graft stroma and the corneal epithelium on slit lamp examination and optical coherence tomography in all successful cases.

Conclusions: In this series of eyes with a history of LSCD and at high risk of rejection, simultaneous PK and AMT were associated with satisfying graft survival and no additional adverse events.

Key-words: amniotic membrane transplantation; epithelial defect; graft survival; keratoplasty; limbal deficiency

RESUME

Introduction: Notre but est de rapporter les résultats de la kératoplastie transfixiante (KT) combinée à une greffe de membrane amniotique (GMA) en anneau et en patch temporaire au cours du déficit en cellules souches limbiques (DCSL).

Matériel et méthodes: Il s'agit d'une série prospective observationnelle incluant 48 chirurgies combinées KT/GMA (48 patients) réalisées dans des yeux ayant des antécédents de DCSL partiel ou total. Les patients ayant un DCSL total ont été traités préalablement par une greffe de cellules souches limbiques. L'indication de la greffe était un échec de greffe dans 58,3% des cas. La plupart des receveurs (89,6%) étaient à haut risque de rejet.

Résultats: Le temps moyen de réépithélialisation du greffon était de 29,2±30,8 jours. Le greffon était réépithélialisé au cours du premier mois dans 70,8% des cas. Aucun effet indésirable lié à la GMA n'a été observé. Le délai moyen entre la greffe et la dernière visite était de 84,5±54,5 mois. Le taux de survie du greffon à 3 ans était de 62,5%. La récurrence de défects épithéliaux après réépithélialisation du greffon (47,9%) était associée à une survie du greffon diminuée (p = 0.004). En cas présence d'un greffon clair lors de la dernière visite, l'acuité visuelle LogMAR moyenne était de 1,90 (0,013) ± 5 lignes avant greffe et 0,89 (0,13) ± 10 lignes à 5 ans. Un anneau de membrane amniotique était visible à la lampe à fente et en tomographie à cohérence optique entre le stroma du greffon et l'épithélium cornéen dans tous les cas de succès de la greffe.

Conclusions: Dans cette série d'yeux à haut risque de rejet ayant un DCSL, la chirurgie combine KT/GMA a permis une survie du greffon satisfaisante sans créer d'effet indésirables supplémentaires.

Mots-clés: greffe de membrane amniotique; kératoplastie; déficit en cellules souches limbiques

Introduction

Reepithelialization of corneal graft is an important step after penetrating keratoplasty (PK). Post-keratoplasty persistent corneal epithelial defects and chronic ulcer are common in eyes with impaired limbal stem cell function [1]. Diagnosis of limbal stem cell deficiency (LSCD) can be made on clinical features such as late and irregular fluorescein staining of the corneal epithelium, persistent epithelial defects (PED), and superficial corneal vascularization [2]. Confocal microscopy, impression specimen cytology, and histology can provide evidence of LSCD by showing presence of goblet cells in the corneal epithelium [3,4,5,6]. Long standing epithelial defects are associated with increased risk of infectious keratitis and stromal melting [7]. They may lead to corneal scarring, thinning, neovascularization, and progressive stromal ulceration. Perforation may complicate this severe corneal condition and lead to loss of the eyeball.

Eyes with a history of LSCD requiring keratoplasty for optical indication are at high risk of post-operative epithelial complications [8]. In addition they are often at high risk of rejection due to previous development of stromal vascularization and/or failure of conventional keratoplasty. In these high-risk eyes featuring a history of irreversible graft rejection or at least 2 quadrants of stromal vascularization, high post-operative steroid regimen is necessary to prevent graft rejection [9]. However this steroid regimen is associated with longer graft reepithelialization [10].

The past two decades have witnessed the revival of amniotic membrane (AM) transplantation [11]. AM has numerous properties that render it extremely useful in ocular surgery. It has been shown to promote epithelialization, to inhibit fibrosis, inflammation and angiogenesis, and to feature antibacterial properties [12,13,14,15,16,17,18]. Due to all these properties, AM was used for treating persistent epithelial defects (PED) with a reported success rate ranging from 50%

to 90% [12,19,20,21,22].

In the context of penetrating keratoplasty, AM transplantation can improve PED healing before or after keratoplasty [23,24]. AM transplantation may also prevent PED occurrence when combined with penetrating keratoplasty, thus preventing several post-keratoplasty complications (i.e., corneal ulcer, stromal scars, and infectious keratitis).

In high-risk recipients, the results reported by Seitz and al. suggest a better short and intermediate term prognosis after simultaneous AM transplantation (temporary patch) and PK than after non-combined PK procedure [25,26]. We hypothesized that simultaneous PK and AM transplantation performed both as a ring-shaped graft and as a temporary patch could improve the prognosis of surgery in eyes with a limbal deficiency history. The objective of the present study was to describe the short and long term outcome of simultaneous penetrating keratoplasty and amniotic membrane transplantation in eyes with a history of limbal stem cell deficiency.

Methods

Study Design

The study design was a retrospective observational case series. The study was designed from a consecutive series of simultaneous PK and AM transplantation performed in a tertiary eye hospital (Centre Hospitalier National d'Ophtalmologie des 15-20, Paris, France). Informed consent was obtained from patients before surgery. Patients were followed prospectively after keratoplasty as required by French regulation. No modifications to French standards of treatment or follow-up were made. IRB approval was obtained from the Ethics Committee of the French Society of Ophthalmology (IRB 00008855).

Inclusion criteria were the following: simultaneous PK and AM transplantation procedures performed by one surgeon between 2000 and 2012 for optical indication in eyes with a history of partial or total LSCD. Diagnosis of limbal stem cell deficiency was based on clinical findings [27]. All patients had late fluorescein staining of corneal epithelium associated with superficial corneal vascularization in at least one peripheral quadrant. Patients with late fluorescein staining of corneal epithelium associated with superficial corneal vascularization on the whole corneal surface (i.e., total LSCD) were first treated with limbal stem cell transplantation, either limbal tissue or cultured limbal stem cell transplantation (Table 1). For cultured limbal stem cell transplantation we used a human amniotic membrane scaffold to grow and deliver limbal stem cells. All corneas featured at least mild corneal endothelial impairment. Corneas with healthy corneal endothelium were treated with deep anterior lamellar and not penetrating keratoplasty; they were not included in the present study.

High-risk recipients were defined as having a vascularized cornea (> 2 quadrants of corneal stromal vascularization) and/or a history of irreversible graft rejection in the operated eye. ABO-compatible donor corneas were used for high-risk recipients. Forty-eight surgical procedures were included in the study. Characteristics of recipients are shown in Table 1.

Surgical Procedures

All transplantations were performed at a single institution. The donor cornea was punched from the posterior corneal surface with the Hanna device. Donor and recipient trephination diameters are shown in Table 1. Two AM grafts were prepared. The first AM graft was trephined with the trephine used for the donor cornea. The second AM graft was trephined with a 15-mm trephine. The first AM graft was placed epithelial side up on the top of the corneal graft. The AM and corneal grafts were secured with 10/0 nylon as follows: 8 interrupted sutures and a 16-bit running suture in 41 cases (86%), 24 interrupted sutures in 4 cases (8%), and 24-bit running suture in 3 cases (6%). The 4-mm central part of the AM graft was then removed with scissors. This resulted in a ring-shaped graft firmly adherent to the corneal graft (Fig. 1). The second AM graft was placed epithelial side down on the ocular surface and secured to the conjunctiva with 8 Vicryl 8/0 interrupted sutures (Fig. 2). A therapeutic contact lens was placed on the ocular surface at the end of surgery. It was removed when the second AM graft fell off and the first AM graft was completely recovered by the recipient corneal epithelium.

Combined procedures included cataract surgery in 5 eyes, tarsorrhaphy in 3 eyes, IOL exchange in 2 eyes, and trabeculectomy in 1 eye.

All patients were treated with topical dexamethasone (1 mg/ml) and neomycin (3400 IU/ml). This treatment was tapered for several months without standardization of postoperative corticosteroid management. The initial corticosteroid regimen was 1 drop hourly in high-risk recipients and 1 drop every 6 hours in low-risk recipients. Corticosteroid use was never stopped in the former patients, and it was discontinued when all the sutures were removed in the latter.

Transplantation Outcome

Patients were hospitalized up to the time of graft reepithelialization. They were then examined prospectively at 1 and 2 weeks, 1, 3, 6, 9, 12, 18, 24, 30, and 36 months, and 3, 4, 5, 6, 7, 8, and 10 years after surgery. Manifest refraction (with spectacle-correction), corneal central

thickness (ultrasound pachymetry), fluorescein staining, and graft transparency were recorded at each examination. The criteria for graft failure were irreversible graft stromal edema and corneal opacification. At various post-operative time points, the graft was evaluated using wide-field specular microscopy and optical coherence tomography.

Statistical Analysis

Graft survival was analyzed with the Kaplan-Meier method. The log-rank test was used to compare the groups of patients. For statistical analysis of visual acuity, astigmatism, corneal thickness, intraocular pressure, and endothelial cell density, only eyes with clear grafts were included. Visual acuity was converted to Log MAR units before statistical analysis. Qualitative variables were analyzed using the χ^2 test. Statistical analysis was performed using a software program (Statistica version 6.1; StatSoft France, Maisons-Alfort, France).

Results

Corneal Epithelium Outcome

The mean graft reepithelialization time was 29.2±30.8 days. The median graft reepithelialization time was 14 days. Graft reepithelialization was completed in 7 days in 14 eyes (29.1%), in 8-30 days in 20 eyes (41.7%), in 31-60 days in 7 eyes (14.6%), and in 61-90 days in 7 eyes (14.6%). Corneal epithelial defects occurred after graft reepithelialization was completed in 23 eyes (47.9%). The occurrence of post-operative corneal epithelial defects was not significantly associated with presence of PED at the time of keratoplasty ($p = 0.41$) nor with the grade of superficial corneal vascularization before keratoplasty ($p = 0.55$). No AM transplantation-related adverse events were observed.

Corneal Graft Survival

The mean keratoplasty-to-last visit time was 84.5±54.5 months. The average follow-up time (from surgery to failure for unsuccessful grafts or from surgery to the date of last visit for successful grafts) was 34.4±32.3 months. The 1-year, 3-year, and 5-year graft survival estimates were respectively 72.1%, 62.5%, and 43.6% (Fig. 3). At the last visit 24 grafts (50.0%) had failed and 24 (50.0%) were clear. Post-operative complications are shown in Table 2. Graft survival was not significantly associated with preoperative diagnosis ($p = 0.64$), recipient rejection status ($p = 0.57$), etiology of limbal stem cell deficiency ($p = 0.28$), previous limbal stem cell transplantation ($p = 0.08$), the level of superficial corneal vascularization ($p = 0.80$), and presence of persistent epithelial defect at the time of keratoplasty ($p = 0.40$). Conversely, recurrence of corneal epithelial defects after graft reepithelialization was associated with lower graft survival ($p = 0.004$) (Fig. 4).

Outcome of Successful Grafts

In eyes with successful grafts at last visit, the mean LogMAR visual acuity was 1.90

(20/1575) ± 5 lines before keratoplasty, 0.89 (20/155) ± 7 lines one year after keratoplasty, 0.85 (20/142) ± 9 lines at 3 years, and 0.89 (20/155) ± 10 lines at 5 years. The average endothelial cell loss was 45.1±20.2% at 1 year and 53.1±18.0% at 5 years. The average manifest cylinder was 4.8±2.0D at 1 year and 3.2±1.4D at 5 years. The average central corneal thickness was 506±42µm at 1 year and 541±41D at 5 years. A ring of amniotic membrane was observed between the graft stroma and the corneal epithelium on slit lamp examination and optical coherence tomography in all successful cases whatever the post-operative time point was. The AM ring was either complete (Fig. 1) or partial (Fig. 5) when AM fragmentation had occurred during the first post-operative months.

DISCUSSION

We report the results of a large series of simultaneous penetrating keratoplasty and amniotic membrane transplantation performed both as a ring-shaped graft and as a temporary patch with long follow-up of patients. Graft reepithelialization was achieved in 30 days in 70.8% of cases and the graft survival estimate was 62.5% at 3 years. Graft survival was not dependent on the preoperative evaluation but it was higher for patients who had no corneal epithelial defects after graft reepithelialization. Two AM grafts were used for each keratoplasty. The first AM specimen was used as a ring-shaped graft trephined at the same diameter as the donor cornea and sutured firmly on the top of the corneal graft with the epithelial side up so that the recipient corneal epithelial cells could re-epithelialize the AM graft. The center of the AM graft was removed so that vision could not be impaired by the AM graft. This AM graft was integrated to the ocular surface in the long term in case of successful surgery. Our hypothesis was that long-term maintenance of AM could improve the microenvironment of the corneal epithelium as it has been demonstrated *in vitro* [28,29,30,31]. The second AM graft was used as a temporary patch sutured to the conjunctiva with the epithelial side down. The objectives were to protect the corneal epithelium during healing and to provide the corneal epithelium with growth factors. This patch fell off during the first 2 weeks after surgery so that its effect was limited to the early post-operative period.

Seitz and al. reported the short and mid-term results of simultaneous PK and AM transplantation in eyes with corneal melting disorders [25,26]. The AM transplantation was performed as a patch that fell off during the first 2 weeks after surgery. They found a higher rate of graft reepithelialization at 30 days with simultaneous PK and AM transplantation compared with PK without AM transplantation, respectively 90% for the former group versus 60% for the latter [25]. Differences between both groups were not significant for graft survival and the rate of PED recurrence.

Several studies have reported the results of AM transplantation in partial LSCD without simultaneous PK [32,33,34]. The mean reepithelialization time after AM transplantation (3 to 4 weeks) was similar to that observed in the present study.[32] For total LSCD additional stem cell transplantation is needed [[33],[34]]. Eberwein et al. have reported the results of allogenic central penetrating limbo-keratoplasty in conjunction with conjunctivoplasty, mitomycin C, and AM transplantation in 20 patients with bilateral limbal stem cell deficiency [35]. All patients were under systemic immunosuppression. The mean follow-up time was 20 months. Healthy corneal epithelium was observed in 70% of eyes and a clear graft was maintained in 70% of cases 2 years after keratoplasty. In our series the 2-year graft survival estimate was 63% and no patients were under systemic immunosuppression after keratoplasty.

The results of PK in eyes with unilateral limbal stem cell deficiency treated with autologous cultivated limbal epithelial transplantation were reported in a series of 47 eyes [36]. The 1-year graft survival estimate was 66% in this series, close to our own result (72%), and it was higher with a 2-stage approach than with a 1-stage approach. In our series 25% of recipients had severe limbal deficiency that required limbal stem cell transplantation which was performed before keratoplasty (2-stage approach). As we grew limbal stem cells on human amniotic membrane, we could also combine the limbal stem cell transplantation and the penetrating keratoplasty as suggested by other authors who combined autologous limbal tissue transplantation with deep anterior lamellar keratoplasty [37,38]. However these studies did not include a control group with a 2-stage approach. Further studies are needed to determine whether limbal function should be restored before keratoplasty is performed.

Transplantation of cultured limbal epithelial stem cells human amniotic membrane has been reported to be efficient for improving the ocular surface condition and visual acuity in many studies. A meta-analysis reported the rate of success and 2-line improvement in best-corrected

visual acuity to be 67% (95% confidence interval, 59%-75%) and 62% (57%-66%) after this surgical procedure [39].

The causes for failure observed in the present study were first recurrence of corneal epithelial defects followed by infectious keratitis and rejection. Interestingly irreversible rejection represented only 1/3 of graft failures despite absence of systemic immunosuppression and a high proportion (90%) of high-risk recipients. High-risk recipients were treated with high topical steroid regimen and steroids were never discontinued in these patients. The impaired limbal function appeared to be the most important cause for failure. It is known to be associated with PED and increased incidence of infectious keratitis.[7] Recurrence of PED after graft reepithelialization was not associated with presence of PED at the time of keratoplasty or with the grade of superficial corneal vascularization before keratoplasty. This could be due to limbal stem cell transplantation that was first performed in case of severe limbal stem cell deficiency.

Limitations of this study include absence of control group and heterogeneity of the study population. However the study population was at high risk of graft failure because of limbal deficiency which was present in all cases and risk factors for rejection that were present in 90% of cases. In such a population graft survival after PK is usually poor.

In conclusion, we propose a new technique for simultaneous keratoplasty and amniotic membrane transplantation taking advantage of both the ring-shaped AM graft technique and the AM patch technique. In this series of eyes with a history of LSCD and at high risk of rejection, simultaneous PK and AMT were associated with satisfying graft survival and no additional adverse events. It may be considered as a therapeutic option for eyes at high risk of post-operative epithelial complications that maintain a level of limbal stem cell function sufficient to maintain renewal of the corneal epithelium. Further studies are needed to compare this technique with other therapeutic options such as the AM patch technique, autologous serum

eyedrops, umbilical cord serum eyedrops, or growth factor eyedrops combined with keratoplasty [26,40,41,42].

Figure legends

Figure 1. Ring-shaped amniotic membrane graft (arrows) one year after transplantation. The patient had been first treated with cultured autologous limbal stem cell transplantation for severe limbal deficiency after ocular burn. This amniotic membrane graft is re-epithelialized by the recipient corneal epithelium and integrated to the graft surface. In OCT scans it is visible in the peripheral zone of the corneal graft as a thin hyper-reflective layer covered by the corneal epithelium.

Figure 2. Amniotic membrane patch (overlay) covering the ocular surface and sutured to conjunctiva. This patch falls off during the first 2 weeks after surgery. In OCT scans it is visible as a hyper-reflective layer (large arrow) between the therapeutic contact lens and the corneal graft. The ring-shaped amniotic membrane graft (small arrow) is visible in the peripheral zone of the corneal graft between the amniotic membrane patch and the corneal graft.

Figure 3: Corneal graft survival after simultaneous penetrating keratoplasty and amniotic membrane transplantation.

Figure 4: Corneal graft survival after simultaneous penetrating keratoplasty and amniotic membrane transplantation according to the occurrence of corneal epithelial defects after graft reepithelialization (log-rank: $p = 0.004$).

Figure 5. Slit-lamp and OCT images of a fragment of the ring-shaped amniotic membrane graft still visible 8 years after transplantation in a successful case. Visual acuity is 20/25.

Conflicts of interest

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