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Limbal stem cells transplantation in the reconstruction of the ocular surface: 6 years experience

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PURPOSE. *To analyze the graft survival rate and stability of the corneal surface in patients who underwent limbal stem cell transplantation. Three surgical techniques were performed based on the origin of the ocular surface lesion: conjunctival limbal autograft (CLAU), living-related conjunctival limbal autograft (lr-CLAL), and keratolimbal allograft (KLAL) transplantations.*
METHODS. *Nonrandomized consecutive comparative case series study. Eighty-four patients (90 eyes; 31 women and 53 men; age range: 11–78 years) were included in the study. Mean follow-up was 31.2 months (range: 6–72 months). Patients were divided into three groups: CLAU, lr-CLAL, and KLAL, comprising 21, 26, and 43 eyes, respectively. Graft survival rate and clinical success of the stem cell transplantation was confirmed by impression cytology. The Kaplan Meier survival curve and generalized Peto tests were used for the analyses.*
RESULTS. *Graft survival rate and the regularity of the corneal surface differed significantly between the allo- and autografts. The 3-year and 6-year graft survival rates were 76.1% and 61.9%, respectively, for the autologous transplantation group, and 59.4% and 46.3%, respectively, for the allogeneic transplantation group. Corneal surface restoration correlated with positive staining for corneal epithelial cells in impression cytology.*
CONCLUSIONS. *Significantly better long-term outcomes were achieved with autotransplantation of the limbus compared with allogeneic limbal grafts from living-related and cadaveric donors. (Eur J Ophthalmol 2008; 18: 886-90)*

KEY WORDS. *Corneal epithelium, Limbal stem cells, Limbal transplantation*

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INTRODUCTION

The human cornea, despite its complicated collagen layer and nerve fiber structure, is an avascular tissue that remains transparent, which is a unique phenomenon in the body. The anatomic structure and physiologic function of the ocular surface is very important for maintaining corneal transparency and visual acuity. Primary and secondary ocular diseases may cause dysfunction of the limbal microenvironment and conjunctivalization of the cornea, a pathology referred to as limbal stem cell insufficiency. The disease is characterized by chronic inflammation of the cornea, and migration of the conjunctival epithelium over the cornea with vascularization (1). Limbal stem cell insufficiency is more often acquired than congenital (2). Limbal stem cell insufficiency caused by injuries, chemical and thermal burns, and iatrogenic disorders are commonly encountered by ophthalmologists.

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Limbal stem cell deficiency can arise from congenital insufficiency, however, such as from aniridia, sclerocornea, endocrinologic disturbances, and from some acquired disorders (ocular cicatricial pemphigus, Stevens-Johnson syndrome). These diseases are rare and difficult to treat and have a poor prognosis. Surgical methods to restore the anatomic structure of the ocular surface include limbal cell transplantation from living or cadaveric donors. The most straightforward treatment for limbal stem cell deficiency is with an autologous graft from the contralateral eye (3). If an autologous graft from the contralateral eye is not possible, a family-related donor graft is then required. The most complex keratolimbal grafts require cadaveric donor tissue. Modern methods of corneal surface restoration by transplantation with cultivated corneal epithelium appear to be the future of corneal surgery.

METHODS

This was a nonrandomized consecutive comparative case series study. Eighty-four patients (90 eyes; 31 women and 53 men; mean age: 62.52 years, range: 11–78 years) were eligible for the study. The mean follow-up was 31.2 months (range, 6–72 months). Three surgical techniques, based on the origin of the ocular lesion, were performed: conjunctival limbal autograft (CLAU), living related–conjunctival limbal allograft (lr-CLAL), and keratolimbal allograft (KLAL) transplantations. The three groups comprised 21 eyes (CLAU), 26 eyes (lr-CLAL), and 43 eyes (KLAL). Best-corrected visual acuity (BCVA) before surgery was 0.09±0.06 (range: 0.01–0.2) in the CLAU group, 0.21±0.11 (range: 0.03–0.3) in the lr-CLAL group, and 0.04±0.02 (range: 0.01–0.1) in the KLAL group. The causes of poor vision were defined and classified into three groups: A) vascular pannus on the corneal surface, B) corneal surface irregularity, and C) symblepharon-dependent corneal astigmatism. Based on this classification, the CLAU group comprised 52.3% A, 23.8% B, and 23.8% C; the lr-CLAL group comprised 76.9% A, 11.5% B, and 11.5% C; and the KLAL group comprised 88.3% A, 2.3% B, and 9.3% C. In 2 KLAL recipients (2 eyes), surgical removal of eyelid cicatrices with eyelid margin reconstruction for lagophthalmia resulting from thermal burns was performed prior to limbal transplantation. In two lr-CLAL recipients, a palpebral lesion was corrected during surgery with removal of the symblepharon involving the eyelid margin. To describe the severity of the ocular surface disorders, the number of quadrants affected by conjunctival fornix synechiae was evaluated: 1.34±0.9 (range: 0–4 quadrants) in the CLAU group, 0.51±0.21 (range: 0–3) in the lr-CLAL group, and 2.02 ± 1.34 (range: 0–4) in the KLAL group. In general, the conjunctival status (in order of severity) was worse in graft recipients with corneal burns, ocular cicatricial pemphigoid, Stevens-Johnson syndrome, and post-cryotherapy limbal insufficiency. No changes in the conjunctival fornices were observed in inflammatory ocular surface lesions or aniridia. Graft survival rate and the clinical success of stem cell transplantation was confirmed by cytologic examination. The surgery was considered to be successful when conjunctival goblet cells were absent in impression cytology of the central cornea and good corneal transparency was noted. Cytologic examination was performed at least twice a year for the first 2 years after surgery and at least once a year beyond 2 years after transplantation. The Kaplan Meier survival curve and generalized Peto tests were used. CLAU was performed in 21 eyes for monocular lesions resulting from chemical or thermal burns (76.1%) and postinflammatory limbal disorders (23.8%). Living-related CLAL was performed in 26 eyes for corneal burns (61.5%), ocular cicatricial pemphigoid (11.5%), Stevens-Johnson syndrome (7.6%), post-cryotherapy limbal insufficiency (7.6%), postinflammatory limbal insufficiency (7.6%), and aniridia (7.6%). HLA matching (class I and II) was performed for all cases of lr-CLAL to determine the best donor candidate. Living-related donors were qualified as donors if no more then two HLA antigen mismatches were detected. HLA matching was performed in a laboratory certified by the Polish Transplantation Agency (Poltransplant: HLA and Immunogenetics Laboratory, Regional Blood Center, Katowice, Poland). KLAL, performed in 46 eyes, was the most common procedure performed for the most severe cases or when other procedures could not be performed. The main indications for surgery were corneal burns (72.0%), ocular cicatricial pemphigoid (13.9%), Stevens-Johnson syndrome (6.9%), and postinflammatory limbal insufficiency (6.9%).

RESULTS

Graft survival rates and the regularity of the corneal surface differed significantly between allogeneic and autologous grafts. The 3-year and 6-year graft survival rates were 76.1% and 61.9%, respectively, in the autologous

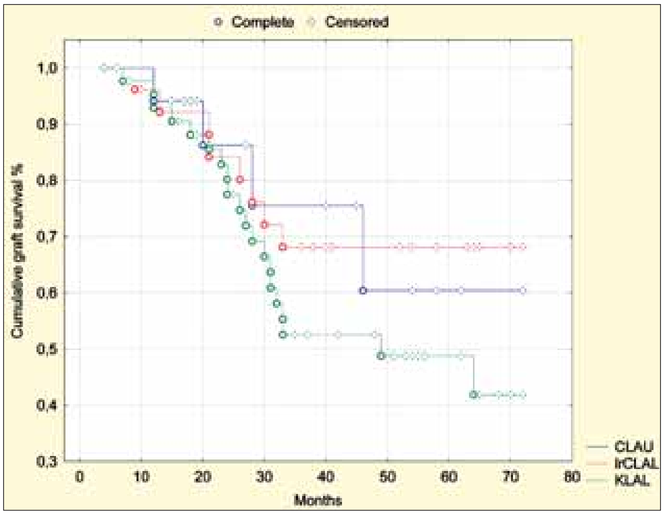

Fig. 1 - *Kaplan-Meier survival curve comparing autologous living-related and allogeneic graft survival rate after surgery.*

transplantation groups, and 59.4% and 46.3% in the allogeneic transplantation group. The Kaplan-Meyer survival curve for autologous living-related and allogeneic grafts is shown in Figure 1. Corneal surface restoration correlated with positive staining for corneal epithelial cells in impression cytology. CLAU resulted in the best long-term outcome. Vision improved (gain of 2 Snellen lines) in 15 eyes, and remained stable (no gain) in 6 eyes. In the lr-CLAL group, vision improved (gain of 2 Snellen lines) in 14 eyes, remained stable (no gain) in 10 eyes, and deteriorated in 2 eyes (Fig. 2). Only 3 patients had a BCVA better than 0.5. In 9 eyes, there was a persistent epithelial defect, and in 7 eyes the graft was rejected. The long-term results of the KLAL procedure were not consistent over time. Chronic rejection was observed in the majority of KLAL grafts. Improved vision was achieved in 23 eyes 6 months after surgery, but in only 15 eyes 12 months after surgery. Despite the administration of immunosuppressive drugs, the long-term results in all the patients were generally poor. Modern techniques of epithelial cell culture are promising as a new effective tool in treatment of ocular surface disorders. These procedures can be repeated several times and are supported by donor-recipient HLA matching.

DISCUSSION

Significantly better long-term outcomes were achieved with autotransplantation compared to allogeneic limbal grafts from living-related and cadaveric donors. The 3- and 6-year graft survival rates were significantly higher in the autologous transplantation group (76.1% and 61.9%,

Fig. 2 - *A 17-year-old patient with endocrine deficiency and bilateral limbal stem cell deficiency. Preoperative view of corneal conjunctivalization* (top left). *3 x 7-mm superior limbal graft–margins marked by sutures* (top right). *Cornea covered with corneal epithelium, 1 year after transplantation best-corrected visual acuity 0.9* (bottom left). *Donor eye with no complications after donation* (bottom right).

respectively) than in the allogeneic transplantation group (59.4% and 46.3%, respectively). Maruyama-Hosoi et al reported a rejection rate of only 13.1% among 121 KLAL cases (4). In their study, 2 patients developed epithelialization disorders and 12 developed reconjunctivalization of the corneal surface. In our study, more than 50% of the grafts failed. The differences may be due to the indications for transplantation. In our group, over 80% of recipients had chemical or thermal burns of the ocular surface. All of those patients required reconstruction not only of the corneal surface, but also of the fornix of the conjunctiva. Coexisting damage of secretory tear ducts and dry eye syndrome, which was observed in the majority of eyes in our study, may have contributed to the poorer final outcomes. Another issue that may affect outcome is ineffective administration and noncompliance of drug and eyedrop treatment, which seems to be a major problem in postoperative ophthalmic care. Shimazaki et al achieved clear corneas in only 50% of eyes after thermal burns (5), the majority (66.6%) of which were covered with corneal phenotype epithelium. This result is comparable with our findings. Also, in another article Shimazaki et al analyzed corneal epithelial cell survival in patients who had undergone KLAL (6). The authors performed fluorescence in situ hybridization to detect X and Y chromosomes and polymerase chain reaction restriction fragment length polymorphism to detect HLA-DPBI antigens. Donor-derived epithelial cells were found in 60.0% of eyes in the fluorescence in situ hybridization analysis and in 77.8% of eyes in the restriction fragment length polymorphism analysis. In their study, the control group consisted of eyes that had penetrating keratoplasty. Nevertheless, limbal graft survival rates were similar to those in the present study. Solomon et al described the long-term outcomes of reconstruction of the ocular surface (7). They observed patients with total limbal stem cell insufficiency due to chemical burns, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, atopic keratoconjunctivitis, and aniridia. All of these patients underwent KLAL and, if necessary, penetrating keratoplasty using tissue from the same donor. KLAL procedures resulted in a higher survival of ambulatory vision at 2 years (86.1%) compared with the combined procedure (46.9%). The authors noted a progressive decline in the visual outcome and in the graft survival rate in association with postoperative decline in the number and mitotic potential of the donor limbal cells. Our results also indicate a decrease in the number of successful allografts over time. CLAU is associated with a more favorable outcome due to the use of homologous material. In cases of binocular burns, however, allogeneic grafts are necessary. The failure of KLAL can be reduced by using an HLA-matched donor from the patient's family. This procedure decreases the loss of donor cells in the recipient and thus improves the graft survival rate and the final visual outcome. It also allows for lower doses of immunosuppressive agents to be administered over a shorter period of time. Santos et al performed conjunctival limbal autografts and conjunctival limbal allografts from living HLA-matched donors (8). All patients underwent an additional amniotic membrane transplantation. Graft survival was 40% after 1 year and 33.3% after 2 years. A cumulative survival rate of 33% was noted after a mean follow-up period of 3 months. The authors noted that graft survival was significantly affected by Stevens-Johnson syndrome, dry eye, keratinization, eyelid abnormalities, and allogeneic conjunctival limbal transplantation. The application of an amniotic membrane may increase the number of successful autolimbal transplantations and decrease the rate of complications, as well as allow for the simultaneous correction of concomitant cicatricial abnormalities of the conjunctiva. Meallet et al reported that an amnion covering improved the regression of stromal vascularization and markedly improved corneal clarity (9). Eyes that received simultaneous symblepharon lysis and fornix reconstruction regained deep and stable fornices, which is necessary to maintain tear film on the reconstructed ocular surface. Despite the immune privilege of the corneal grafts, limbal stem cell transplantation should be considered a vascular graft. The limbal graft bed is a highly vascular site with multiple vessels of palisades of Vogt. Therefore, HLA matching seems to be one method for improving the outcomes of the lr-CLAL procedure. Wylegala et al reported an 83.6% graft survival rate in a 20.3-month follow-up (10). The prognosis for graft survival was definitely worse when eyelid abnormalities and conjunctival adhesions coexisted. In a subsequent article, Wylegala et al concluded that HLA non-matching is only one of many factors that may interfere with the postoperative course in recipients of an allogeneic limbus (11). Shimazaki et al compared autologous graft and allograft outcomes following chemical and thermal burns of the cornea (12). The autografts produced significantly better results than the allografts in corneal epithelialization and cornea transparency. The authors deduced that in cases with chemical or thermal corneal burns, autografting (if the contralateral eye is not affected) should be considered as a first-choice surgery. They also pointed out that it is safer to perform surgical ocular surface reconstruction first and to delay penetrating or lamellar keratoplasty as a secondary procedure, even if the corneal stroma is opaque immediately after injury.

In conclusion, autotransplantation of the limbus produces significantly better long-term outcomes compared to allogeneic limbal grafts from living-related and cadaveric donors. This procedure is recommended with HLA matching of donor-recipient pairs.

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