LIMBAL TRANSPLANTATION: MULTICENTER RETROSPECTIVE CASE SERIES ANALYSIS

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ABSTRACT

Purpose: To report the results of limbal transplantation (LT) in patients with limbal stem cell deficiency (LSCD) in the context of ocular surface diseases.

Materials and methods: A multicenter (5 centers) retrospective case series analysis of patients who underwent LT between 1996 and 2004 was performed. Data were collected by the same researcher using a customized database. Success was defined by the absence of a persistent corneal epithelial defect, ongoing inflammation or recurrence of a pterygium.

Results: Data from 72 LT performed in 61 patients (65 eyes) with a mean follow-up of 20.8 months (SD 23.5; range, 3-115) were analyzed. There were 33 males and 28 females with a mean age of 55.8 years (SD: 15.6; range, 20-89). Fifty-eight (80.6%) LT were autografts (40 pterygia, 12 alkali burns, 3 iatrogenic cases, 2 viral infections, 1 neoplasia case) and 14 (19.4%) were allografts from cadaver.

RESUMEN

Objetivo: Revisar el resultado de los trasplantes de limbo (TL) realizado en pacientes con Síndrome de insuficiencia límbica (SIL) en el contexto de varias enfermedades de la superficie ocular.

Materiales y métodos: Se realizó un estudio retrospectivo y multicéntrico (cinco centros) de los TL realizados entre 1996 y 2004. Los datos fueron recogidos por el mismo investigador, en una base de datos especialmente diseñada para el estudio. Se consideró como «éxito» del TL a la ausencia de: defectos epiteliales, inflamación y recurrencia del pterygium cuando éste fue la causa del TL.

Resultados: Se analizaron un total de 72 TL realizados en 61 pacientes (65 ojos) con tiempo de seguimiento de 20,8 meses (DS 23,5; rango, 3-115). Hubo 33 hombres y 28 mujeres, con una media de 55,8 años (DS: 15,6; rango, 20-89). Se realizaron 58 (80,6%) TL autólogos (40 pterygia, 12 quemaduras por alcali, 3 casos iatrogénicos, 2 infecciones virales, 1 caso neoplásico) y 14 (19,4%) fueron alólogos de cadáver.

Received: 20/4/07. Accepted: 26/5/08.
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This paper was presented at the LXXXI Congress of the Spanish Ophthalmology Society (Saragossa, 2005), and as a poster at the Association for Research in Vision and Ophthalmology (ARVO) (Fort Lauderdale, USA, 2005).

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INTRODUCTION

The location of corneal epithelial stem cells in the limbo has been a far-reaching finding (1). A large number of diseases and external aggressions may lead to a destruction of these cells, causing a condition known as limbal stem cell deficiency (LSCD). In this situation limbal stem cells are lost partially or totally, so the regenerating capacity of the corneal epithelium is altered (2). LSCD is associated with chronic inflammation, recurrent ulceration (which may become persistent), corneal vascularization, and growth of the conjunctival epithelium towards the cornea, known as conjunctivalization. All these changes have an impact on the patient’s quality of life, and/or an important reduction in visual function (2-4).

The management of these patients has been a major challenge for specialists in ocular surface pathologies. When tackling this condition, it is essential to determine the etiology of the process, for then initiating a conservative medical treatment which may reduce inflammation to a minimum (3,4). Once the inflammation process has been ‘cooled down’, and diagnosis is confirmed for an irreversible LSCD, with its corresponding structural damage to the ocular surface, a number of techniques are considered, such as: sequential epitheliectomy (5), amniotic membrane transplant, and the different variations of limbal transplantation (LT) proposed for reconstruction. For full, strictly unilateral LSCD, limbar tissue may be transplanted from the contralateral healthy eye. For partial unilateral occurrence, e.g. pterygium, limbal tissue may be used from the same eye. Both procedures are known as autograft LT (6-10). However, when the limbo has been partially or fully destroyed bilaterally, allografts are needed for LT, either from family donors (10-12) or from cadaver donors (10-13).

Current success rates for these transplants vary largely, as many factors seem to be involved, such as: 1) the donor source for LT, 2) the etiology of LSCD, 3) the inflammatory status of the receptor bed, 4) the association to other transplants, such as corneal transplantation, 5) the number of consecutive transplants performed, or 6) the follow-up time for each series (14).

The objective of this study is to evaluate the global results of LT carried out on LSCD patients at a number of different centers in the Iberian Penin-
sula, in order to find out failure rates and their main causes in our context. The current work aims at becoming the basis for multi-centre prospective studies, which are already in progress.

SUBJECTS, MATERIAL, AND METHODS

A retrospective multi-centre case series has been carried out at five centers, all of them included in the Carlos III Health Institute’s Thematic Network for Collaborative Research in Ophthalmology (CO3/13). Four centers are located in Spain, and one in Portugal: IOBA (Institute for Applied BioOphthalmology) and Valladolid University Hospital, University of Valladolid; IBILI (Biomedical Institute for Research in Light and Imaging), University of Coimbra, Portugal; CHUS (Santiago de Compostela Hospital Complex), University of Santiago de Compostela; Castroviejo Institute and San Carlos Hospital, Complutense University of Madrid, and VISSUM, Alicante.

Clinical histories were revised for all patients of LSCD who underwent LT in the period between April 1995 and September 2004, with a minimum follow-up time of three months. Data was collected by the same researcher (JT), not involved in patient surgery or follow-up. Data was then loaded onto a database specifically designed for this by one of the authors (MFM) (Microsoft Access 2000). The following variables were analyzed: age, sex, etiology of LSCD, origin of donor material, concomitant earlier and later surgeries, follow-up time, earlier and final visual acuity (VA), and success or failure of LT. ‘Success’ for LT was defined as the absence of the following: inflammation symptoms of the ocular surface, such as photophobia, foreign body eye sensation, or pain, and persistent epithelial defects, or recurrence of pterygium when this was the cause for LT. ‘Success’ for LT was defined as the absence of the following: inflammation symptoms of the ocular surface, such as photophobia, foreign body eye sensation, or pain, and persistent epithelial defects, or recurrence of pterygium when this was the cause for LT. All pterygium cases were from the same centre (Coimbra), and were operated on by two surgeons (MJQ and JM) using the same surgical technique (LT autograft, from the ipsilateral eye, and no amniotic membrane). In other centers both autografts and allografts were used for LT, and applied to other pathologies outside pterygium. The time for the rings of the material source from cadaver donor was 24 hours for allograft-based LT, between extraction and implantation. Allografts were preserved until the time of implantation, using a wet chamber, with a tobramycin antibiotic solution at 4°C.

Cyclosporine A (5 mg/kg weight/day), was administered orally for patients as an immune modulator treatment.

The studies were approved by the Ethics Committee of all centers involved.

In order to statistically assess the relationship between qualitative variables, Pearson’s chi-square contrast was used. The McNemar-Bowker test was applied when samples were paired. For quantitative variables means were compared between groups using the parametric analysis of variance when feasible, and the non-parametric Kruskal Wallis equivalent when the hypothesis for normality was not verified. For comparison between two groups, the non-parametric Mann Whitney U contrast was used. A logistic regression model was adjusted in an attempt to explain the success or failure of LT in relation with all variables collected. A ‘forward’ algorithm was used to select the best model. Statistical analysis was performed using SPSS software, version 13.0 (SPSS for Windows, SPSS Inc., Chicago, USA), by another one of the authors (IF). Values were considered significant when p<0.05.

RESULTS

Data was collected for 72 LT on 61 patients (65 eyes), with an average follow-up time of 19.8 months (SD: 23.5; range, 3-115). The group included 33 men and 28 women, with an average age of 55.8 years (SD: 15.6; range, 20-89). The male group was significantly younger than the female group (Mann-Whitney U 255.5; p<0.0001). 34 out of 72 LTs (47.2%) were performed on women, with 38 LTs (52.8%) on men.

The diseases causing LSCD included: 40 pterygions, 18 alkali burns, seven immune inflammations (four Stevens-Johnson syndromes, one ocular rosacea, one ocular cicatricial pemphigoid, and one atopic keratoconjunctivitis), four iatrogenic ones (two medicamentous, two surgical), two viral infections, and one eye with neoplasia (fig. 1). The average age for LSCD patients due to alkali burns and iatrogenic was below 45, which is a significantly lower value than that for the rest of etiologies, where average age was above 55 (Kruskal Wallis 17.53; p=0.004).

ARCH SOC ESP OFTALMOL 2008; 83: 417-422
58 (80.6%) of the 72 LTs were autografts, and 14 (19.4%) were allografts from cadaver donors (fig. 1). Females received 50.9% of autograft LTs, and 33.3% of allograft LTs (Chi square 1.21; p= 0.270).

The average number of LTs on the same eye was 1.1: 1 LT was performed in 59 cases (90.8%), two LTs were performed in 5 cases (7.7%) (in three of these an autograft LT replaced a previous autograft LT, with successful results for two of the three cases; there was one case where an autograft LT was carried out first, followed by an allograft LT, both of which failed; finally, there was a case where an allograft LT was performed twice, with no success), and lastly three autograft LTs were carried out in one case only (1.5%), where the first two failed and the last one succeeded.

An associated surgical technique was carried out together with LT for 20 cases: two penetrating keratoplasties (2.8%), and 18 amniotic membrane transplantations (25.0%).

As regards final results, 48 out of 72 LTs (66.7%) could be considered as a success. The success proportion was associated with earlier diseases, with high values in pterygium cases (32/40; 80.0%), and low values for immune pathologies (1/7; 14.3%) (Chi square 19.02; p= 0.002) (Fig. 1).

Significant differences in success rates were noticed according to receptor gender: 27/48 successful LTs were performed on females (87.1%), and 19/48 (55.9%) were carried out on males (Chi square 7.63; p= 0.006).

When relating the success or failure of LT with all variables collected, it was found that the only independent factors associated to the success of LT were the source of the donor material (Odd Ratio 120.59; IC 95%; 8.03-1810.92) and gender (Odd Ratio 12.01; IC 95%; 1.40–103.37), so that success rates were higher for autograft LT and on females. These two variables were statistically independent of each other (Chi square 1.22; p= 0.270) and, in the case of sex, statistically independent on earlier disease (Chi square 4.20; p= 0.521).

Ranges of values of previous and final VA (in the last visit) are summarized on Table I below according to the base disease. Patients with alkali burns and viral infections managed to improve their VA. Besides, the number of patients with better VA after LT was significantly lower for immune-related pathologies (Chi square 19.23; p= 0.037).

**DISCUSSION**

Reconstructing the ocular surface and managing patients affected by serious LSCD is still a challenge for ophthalmologists. Ever since stem cells for the corneal epithelium were identified in the limbo-scleral niche, and since these cells were determined to be responsible for the regeneration of the corneal epithelium (1), was LT proposed as a surgical technique to reconstruct the damaged ocular surface. Autograft LTs are a safe and effective technique for managing patients of ocular surface diseases derived from traumas, such as thermal or chemical burns. This technique does not provide as many benefits to patients affected by immune diseases or bilateral LSCD, where the source of donor material must be an allograft (4).

Kenyon and Tseng were the first to publish results for autograft LT back in 1989. Results were obtained from a series of 26 patients with non-

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**Table I. Visual acuity intervals on a decimal scale, before and after limbal transplantation, according to the base disease**

<table>
<thead>
<tr>
<th>Earlier Disease</th>
<th>VA before LT</th>
<th>VA after LT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pterygium</td>
<td>0.4-0.6</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>Alkali Burns</td>
<td>FC</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>0.05-0.125</td>
<td>FC/0.05-0.125</td>
</tr>
<tr>
<td>Viral Infections</td>
<td>LP/HM</td>
<td>FC/0.05-0.125</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>0.4-0.6</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>Immune</td>
<td>HM</td>
<td>HM</td>
</tr>
</tbody>
</table>

inflammatory unilateral LSCD. After a 6-month follow-up, 20 out of 21 patients had shown a stable epithelium with no recurrence or epithelial defects (6). One year later Copeland and Char used autograft LT for reconstruction in two patients suffering from conjunctival squamous cell carcinoma. A more stable ocular surface and improved visual acuity were noted for both patients (15). In the current retrospective study, with 61 patients and 72 LTs, it was found that most autograft LTs were performed on pterygium (unilateral), and allograft LTs were carried out on patients with an immune-related inflammation base pathology (bilateral). Success rates for autograft LT (81.0%) were very similar to previously published results, although follow-up periods in these studies are shorter than the ones used for the current research (6-14 months) (6, 16-18). It is worth outlining that most of the successful cases in the current study correspond to patients with partial, unilateral LSCD, as is the case for pterygium, so that an autograft LT is the most suitable option. This outcome has led to the setting up of a prospective study, which is currently in progress, in order to confirm the results. The only choice for severe cases of bilateral LSCD (apart from an ex vivo expansion of tissue) is allograft LT, where the donor source may be a relative or a cadaver donor (10). Success rates for allograft LTs in different studies are very different, as they are dependent on largely varying factors, such as the definition of success and failure, or the follow-up time taken into consideration. It is thus difficult to compare results, although a success rate of 7.1% was shown in this study for allograft LTs, which is a low value if compared to other studies. A retrospective study by Shimazaki, for a series of 32 cases with total LSCD, caused by chemical and thermal burns, showed a total of 11 autograft LTs and 21 allogenic LTs performed, assessed over 67 weeks. Success was defined as corneal epithelialization. A success rate of 90.9% was achieved after the first autograft LTs, with 33.3% of successful allograft LTs (17). Samson et al analyzed a series of eleven eyes, with family donor allograft LTs performed on ten eyes, and autograft LT on one eye with inflammation pathologies-related LSCD. Five eyes failed (45%), with an average follow-up time of 35 months (19). Evidence was found for improvement of the clinical situation and the symptoms when comparing autograft- vs. allograft LTs (16,18).

The findings of the current study further reflect and support the remarkable difference in results between allograft- and autograft LT. The latter would be the most suitable option for unilateral and partial LSCD cases, where the stem cell population may recover, thus allowing for successful reconstruction of the ocular surface.

Allograft LT shows a disadvantage in that patients must be medicated with systemic immune suppressors over long periods of time in order to prevent rejection.

Once all variables were correlated, it was found that the factors most closely associated with a successful LT were the source of donor material and the patient’s sex, with a higher proportion of success amongst women with an autograft LT. The rather surprising finding that females showed a higher success rate may justify the relevance of designing a controlled prospective clinical study where the only variable to control would be receptor’s sex, in order to corroborate our results.

As a conclusion, the current retrospective study suggests that: 1) overall success rates for these LTs (66.7%) increased for autografts (81.0%) and decreased for allografts (7.1%); 2) success rates are lower for immune-related inflammation pathologies (14.3%), and higher for pterygium (80.0%); 3) the highest success rates were shown for autograft LTs and those performed on women.

REFERENCES


