

Limbal Stem Cell Transplantation

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Case presentation

HPI: A 29 year-old woman h/o Stevens-Johnson syndrome (SJS) with severe ocular involvement occurring six months earlier (10/97)

Treated with topical lubrication drops and topical antibiotics.

S/P amniotic membrane grafting (AMG) for a persistent epithelial defect OS

H/o recurrence of persistent epithelial defect OS

Experienced increased conjunctival inflammation, tear film deficiency, and posterior lid margin keratinization. Patient started on topical steroids without response.

Referred for consultation regarding management.

Examination: (see figures 1,2)

4/98

Visual acuity 20/100 OD and 20/60 OS.

Lids: +marked posterior lid margin keratinization of upper and lower lids OU

+marked blepharitis

Tear: +decreased aqueous tear production

Conjunctiva: 1+ conjunctival injection OU

Cornea: OD: conjunctivalization (superficial neovascularization and decreased clarity);

OS: marked corneal epitheliopathy.

Anterior chamber: formed OU

Lens: poor view secondary to corneal surface

Fundus: poor view secondary to corneal surface

B-Scan: normal OU

Assessment:

Chronic conjunctivitis following SJS OU:

Differential: 1) chronic infection

2) Inflammation secondary to posterior lid margin keratinization

3) immunologically-mediated chronic infection

Limbal stem cell deficiency OU

Tear film deficiency OU

Posterior lid margin keratinization OU

Recommendations:

Conjunctival swabbing for cultures

Trial scleral lens fitting

Conjunctival biopsy

Serum for ESR, CRP, sIL2R

Results:

Conjunctival cultures: Gram positive cocci

Scleral lens fitting: increased comfort; however, patient unable to stay in Boston for personal fitting

Conjunctival biopsy: 3+ staining of vessel walls with complement, IgG

Elevated ESR, CRP, and sIL2R

The results suggested that the persistent inflammation was at least in part due to an ongoing immune-mediated process. Recommendations were to begin systemic immunosuppression to control active inflammation, and continue vigorous lubrication.

The patient returned for reconsultation four months later

8/98

Medications: Imuran 50 mg PO QD

Cyclosporine 100 mg PO QD

Examination: (see figures 3,4)

Lids: Posterior lid margin keratinization OU

Blepharitis OU

Trichiasis OS

Tear: Decreased aqueous tear production
Conjunctiva: 2+ injection OU
Cornea: OD - conjunctivalization
OS - frank central epithelial defect
Plan: 1) Increase immunosuppression therapy - the doses were inadequate
2) Continued lubrication
3) Continued antibiotic drops OS

The patient decided to continue follow-up with our service in Boston.
9/98

Conjunctival injection was markedly decreased. Patient still had marked ocular discomfort and photophobia.

10/98

Mucous membrane grafting (MMG) and amniotic membrane grafting (AMG) with tarsorrhaphy OS.
Limbal stem cell transplantation with amniotic membrane grafting OS (donor: sister after HLA matching done of several family members)

Epithelialization with cornea-type cells occurred 9 days following LSCT.

1/99 Va OS : 20/100, stable corneal surface

2/99 Va OS : 20/50, stable corneal surface

MMG/AMG OD

6/99 LSCT / AMG OD

Va OD: 20/200 (limited by stromal scarring), stable ocular surface

11/99 PK OD

12/99 Va OD 20/60 OS 20/30

Limbal stem cell transplantation

History

Thoft and Friend first brought up the concept of the "ocular surface" in 1977. They are credited for first describing the use of autologous free conjunctival grafting for resurfacing chemically burned corneas. However, the first descriptions of the use of autologous limbal transplantation were described many years earlier by Strampelli in 1960 and subsequently by Barraquer in 1964.

Background

The concept of limbal stem cell transplantation was based initially on the conjecture that it was the limbal epithelium that contained the stem-cell population for corneal epithelial cellular proliferation and differentiation, which differed greatly from the conjunctival epithelium phenotype. Evidence supporting this theory included models of burn injuries to rabbit eyes demonstrating that removal of 2/3 of the limbal epithelial cells in rabbit models resulted in cornea conjunctivalization, vascularization, and chronic keratitis, so-called limbal stem cell deficiency (LSCD). It was noted that an intact limbus was required to restore the corneal epithelium.

Other evidence supported the limbal location of corneal stem cells. Clinically, one often observed the movement of pigment lines spreading from the limbus centrally in certain types of corneal epithelial injury. Studies on mitotic index demonstrated higher mitotic activity in the peripheral cornea when compared to the central cornea. Finally, it was noted that conjunctival intraepithelial neoplasia, a precancerous condition which presumably develops from stem cells, typically occurs at the limbus.

Eventually, studies based on tritiated thymidine incorporation localized the stem cells to the basal epithelium of the limbus. There are several proposed mechanisms of how these stem cells develop into daughter cells that either replace mother stem cells or continue to differentiate into corneal epithelial cells. It is felt that differentiating cells first develop into a "transient amplifying cell", which are then capable of further differentiation.

Etiologies

There are many potential causes of limbal stem cell deficiency. The entity most commonly encountered by ophthalmologists is probably chemical and thermal injury. However, multiple surgeries, severe infections, and immunologically mediated diseases like SJS and atopic

keratoconjunctivitis can also cause LSCD. A list of these etiologies is provided:

- Traumatic destruction of LSC
- Chemical injury
- Thermal injury
- Multiple surgeries or cryotherapies affecting the limbal region
- Chronic CL-associated epitheliopathy
- Inflammatory destruction of LSC
- SJS / TEN
- Severe microbial keratitis
- Loss of LSC due to insufficient stromal support
- Aniridia
- Keratitis associated with multiple endocrine deficiencies
- Neurotrophic keratopathy
- Peripheral inflammatory keratopathy or limbitis
- Idiopathic

Diagnosis

The gold standard in the diagnosis of LSCD is demonstration of the conjunctival epithelium phenotype. Typically, this is done by impression cytology. Findings consistent with LSCD are the presence of goblet cells, which are normally present in conjunctiva, but not in corneal epithelium. However, it is not practical (nor necessary) to perform impression cytology on every patient with potential LSCD. Tseng described three clinical characteristics for the diagnosis of LSCD, as seen in rabbit models: conjunctivalization, vascularization, and chronic inflammation. He also noted that since vascularization and chronic inflammation can be seen in other disorders, conjunctivalization is considered more specific, and can be considered a sign of LSCD in the appropriate clinical scenario. Persistent epithelial defects can also be the presenting clinical sign of LSCD, but one must remember that other conditions (e.g. profound sicca) can also cause PEDs despite intact limbal stem cell function.

Types of LSCT

Types of LSCT differ based on the donor source of the stem cells. Autologous (same or opposite eye) has the benefit of eliminating the possibility of graft rejection; however, this type of LSCT is restricted to unilateral or localized conditions. Allografts (live related donor or cadaver source) have also been performed; this type of LSCT is necessary in conditions in which both eyes are affected.

There is no consensus on which of the types of allograft, living related donor or cadaver donor, is superior. Both offer advantages and disadvantages: living related donors decrease the chance of rejection. This is important since both experimental and clinical evidence suggest that rates of rejection of LSC grafts are higher than that seen in corneal grafts. However, it has been noted that rejection can occur even in HLA-matched donor. Rao and colleagues suggested that systemic immunosuppression may be necessary even in living related donor cases.

An obvious disadvantage of living related donor is that a healthy individual has to undergo eye surgery. It should be noted that no case series have reported complications to the donor when 180 degrees of tissue or less have been used. Some studies suggest even more limbal tissue may be harvested without inducing an iatrogenic LSCD.

Using cadaver donor LSC has its own potential merits. The greatest benefit is due to the ability to harvest 360 degrees of limbal stem cells. In addition to the increased number of LSC transplanted, there may be an additional benefit in the form of a "barrier effect". Some authors believe that the limbal stem cell environment produces factors that actively prevent the migration of conjunctivally derived epithelium over the limbus onto the cornea, hence a "barrier effect". Clinical evidence supporting this are small case series demonstrating LSCT in pterygium surgery prevents recurrence. A disadvantage with using cadaver donor material is the high chance of graft rejection. Systemic immunosuppression use has been described by experts in most series using cadaver donor material.

Pre-op considerations

There are several considerations one must make before proceeding to surgery. One must address all issues that may affect the outcome of limbal stem cell transplantation. Ocular surface problems, such as severe dry eye, lid margin keratinization, and trichiasis, commonly seen in etiologies leading to LSCD, must all be addressed prior to proceeding to LSCT. Additionally, we feel strongly that in cases of chronic inflammatory diseases, that inflammation be controlled adequately before embarking on surgery. Finally, one must consider whether concomitant or subsequent surgery will be needed.

Technique

Preparation of host

A conjunctival peritomy is performed for 360 degrees, and the conjunctiva is allowed to recess. Then, abnormal tissue on the corneal surface is removed. Irregularities of the corneal surface are "smoothed over", but scraping of the anterior stroma is kept to a minimum. Similarly, the limbal beds for the LSC are similarly prepared.

Harvesting of donor cells

In cases of living related donor, the donor patient is ideally in an adjacent OR suite. The limbal stem cell lenticles are prepared by incising into clear cornea about 1 mm anterior to the limbus, for the appropriate width (e.g. width of 2 clock hours if harvesting 3 lenticles for total 6 clock hours). Then, conjunctiva is dissected 2 mm posterior to limbus with sharp scissors, up to the conjunctival insertion to the limbus. The lenticle is lifted by the conjunctiva edge, and then careful dissection is carried anteriorly with a 57 blade. The lenticle is finally removed with completion of the dissection with a 57 blade or sharp scissors. The donor material is immediately placed into BSS solution. When all lenticles are harvested, they are brought to the OR suite of the recipient. In cases of cadaver donor, typically the limbal stem cells are delivered as part of a corneal donor tissue. The cornea is removed using a large diameter cutter or by hand, leaving 0.5 to 1.0 mm of peripheral cornea. The donor tissue may be left as a large ring.

The LSCT are then sutured to the recipient limbal site. 10-0 nylons are used for the corneal surface, and 10-0 nylon or 10-0 vicryl for the conjunctival edge (suture to episclera or conjunctiva). Typically, a bandage contact lens is placed at the end of the case.

Post-op considerations

As stated before, maintenance of the ocular surface is critical to the success of the LSCT. Factors addressed before the surgery may become problematic again, and must be addressed quickly (e.g. recurrence of trichiasis).

Detection of rejection poses a difficult problem to clinicians. Tsubota and colleagues noted difficulty in assessing LSCT rejection in their large series, and opted only to count cases in which a concomitant corneal transplant had been done, relying on signs of PK rejection to determine the presence of LSCT rejection. Some signs described by authors as potential clinical signs of LSCT rejection include graft edema, graft neovascularization, vascularization over the graft onto the cornea, focal conjunctival injection, or focal corneal epithelial defect in the sector of rejection.

LSCT success rates

Kenyon & Tseng published the first series of LSCT in 1989. In this series, all were autografts, and consisted of 26 consecutive cases. Most were chemical and thermal injuries (22), but there were a few CL related cases (3) as well as one due to multiple surgeries. Follow-up was 2-45 months (mean 18). They noted epithelial resurfacing in 7-21 days in 22/26 patients, with visual improvement after LSCT alone to 20/100 or better in 9 patients and a 2 Snellen line improvement in another 8 patients. Fifteen patients had reduced or regressed neovascularization. Eight underwent subsequent LK or PK, of which all had visual improvement.

Rao and coauthors published the first large series of living related donor LSC grafts in 1999.

Their series included 11 eyes of 9 patients, of which 7 eyes were affected by severe chemical burns and 2 eyes suffered from SJS. HLA-matching was performed in 7 patients, and were non-HLA matched close relatives in 2. Follow-up was from 3-33 months, with a mean of 17 months. They noted successful epithelialization, decreased vascularization, and increased ocular comfort

77.8%. Visual acuity however was 20/400 or better in only 2 eyes. Additionally, graft rejection was noted in three eyes; 2 of which were non-HLA matched. They also noted epithelial breakdown occurring in 2 eyes which underwent PK. The authors felt the data supported two recommendations: one, that systemic immunosuppression is probably still necessary in living related donor grafts; and two, that performing PK at the time of LSCT may enhance success by limiting ocular inflammation-mediated destruction of the LSC if performed at a second surgery. Tsubota and coauthors published the largest to date series of LSCT in 1999 in *the New England Journal of Medicine*. They used a cadaver source for the donor LSC, and all patients underwent concomitant amniotic membrane grafting. Their series was comprised of 43 eyes of 39 patients; 29 eyes were affected by OCP or SJS, while 14 were affected by chemical/thermal injury. Mean follow up was 3 years 68 days. The successful outcome measures were successful epithelialization with phenotypic corneal epithelium, presence of a clear cornea, and visual acuity. Success rates in the burn patients were 71%, 50%, and 0.04 (count fingers) respectively. Success rates in the OCP/SJS group were lower: 50%, 28%, and 0.02 (hand motion) respectively. Their conclusion was that LSCT/AMG as a surgical method of ocular surface reconstruction was successful in certain patients. We agree with the authors in that considering the alternatives for these patients (keratoprosthesis or blindness), these achievements result in increased patient quality of life. However, it is clear that much more research is warranted to increase rates of success of LSCT.

Conclusion

LSCT is a clear example of how knowledge of basic science and physiology can lead to a life-improving clinical treatment modality. It has allowed us to treat ocular conditions that were impossible to treat just 20 years ago. Continued understanding of the basic molecular biology and physiology of limbal stem cell function, their interaction with the ocular surface environment, and more clinical studies will help further our understanding of this technique, and lead to better ways of caring for patients with LSCT.

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Limbal Stem Cell Transplantation

David Chu, M.D.

1) Which one of the following is not a sign of limbal stem cell deficiency?

- A. Chronic keratitis
- B. Cornea conjunctivization
- C. Limbal flush
- D. Persistent epithelial defect

2) Which of the following suggests limbal location of corneal stem cells?

- A. Conjunctival intraepithelial neoplasm occurs at limbus
- B. Higher mitotic activity in the peripheral corneal epithelium compared to the central cornea
- C. Both
- D. Neither

3) Which of the following is not commonly associated with limbal stem cell deficiency?

- A. Sarcoidosis
- B. Alkali burn
- C. Multiple ocular surgeries
- D. Aniridia

4) True or False?

Diagnosis of LSCD requires impression cytology to demonstrate goblet cells in the corneal epithelium.

5) Which one of the following is the advantage of cadaver donor?

- A. ¿Barrier effectí
- B. Number of transplanted stem cells
- C. Availability
- D. All of the above

6) Which of the following statement is true regarding limbal stem cell transplant?

- A. No more than 3 clock hour of limbal stem cell can be removed from a living donor
- B. Limbal bed of the recipient must be smoothed over
- C. Corneal surface condition of the recipient is irrelevant
- D. When harvesting from the donor, incision into clear cornea must be avoided

7) Which of the following signs indicates rejection of transplanted limbal stem cells when combined with corneal transplantation?

- A. Graft edema
- B. Focal conjunctival injection

- C. Epithelial defect
- D. All of the above

8) Which statement concerning limbal stem cell transplant is true?

- A. HLA typing does not increase rate of successful transplant
- B. LSCT may be more likely to be successful if corneal transplantation is performed later
- C. Systemic immunosuppression is probably necessary in living related donor grafts
- D. Improvement in vision after LSCT is rare

9) True or False

In chronic inflammation-induced ocular surface disease, LSCT will reduce the inflammation

10) True or False

LSCT can improve outcome of pterygium excision

Answers 1-C, 2-C, 3-A, 4-False, 5-D, 6-B, 7-D, 8-C, 9-False, 10-True