

SHORT COMMUNICATION

Case report

Multilayer amniotic membrane transplantation in severe ocular graft versus host disease

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PURPOSE. *To clarify the usefulness of multilayer amniotic membrane transplantation in an unusual case of calcareous corneal degeneration in a patient with graft-versus-host disease.*

METHODS. *A 20-year-old Caucasian woman had bilateral calcareous corneal degeneration of one year of evolution, secondary to graft-versus-host disease. Treatment for both eyes with topical steroids and antibiotic ointment was not successful. Right eye had a spontaneous corneal perforation, and a three-layer circle amniotic membrane graft was applied to the whole cornea.*

RESULTS. *During a follow-up period of 20 months we observed stability of the corneal epithelium and stroma. The amniotic membrane-covered area showed rapid epithelization, reduced inflammation and suppressed fibrosis formation.*

CONCLUSIONS. *Multilayer amniotic membrane transplantation may be considered an alternative for reconstructing the ocular surface in a patient with severe dry eyes and calcareous corneal degeneration, even with little perforation. (Eur J Ophthalmol 2001; 11: 183-6)*

KEY WORDS. *Amniotic membrane transplantation, Graft-versus-host disease, Calcareous corneal degeneration*

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INTRODUCTION

Graft-versus-host disease (GVHD) is a major complication following allogeneic bone marrow transplantation. The disease results from the reaction of transplanted immunocompetent cells against the host. The GVHD may occur weeks to months after bone marrow transplant, and most often involves the skin, gastrointestinal tract and liver (1). Ophthalmic involvement is fairly common too, occurring in approximately 60% of cases (2). The most frequent ocular manifestations include keratoconjunctivitis sicca, cicatricial lagophthalmos and sterile conjunctivitis and uveitis.

The severe ocular complications of persistent corneal epithelial defects and stromal ulceration are related to the concomitant dry-eye condition (2) and can be man-

aged by conventional therapy including topical lubricants, lacrimal punctal occlusion and tarsorrhaphy. However, deep ulcers and corneal perforations after thinning require penetrating or lamellar keratoplasty, conjunctival homograft, suturing a scleral patch into the perforation, tarsorrhaphy, and sealing the perforation site with tissue adhesives (3). However, there is inherent danger of an immunologic reaction in the use of homologous tissue and application of tissue adhesives is not always easy (3).

A recent encouraging approach is amniotic membrane transplantation (AMT), which was initially reported for corneal surface reconstruction in a rabbit model with total limbal deficiency (4). Tsubota et al (5) used this technique, together with allograft limbal transplantation, to reconstruct the corneal surface in

patients with severe dry eye caused by ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Here we describe the use of AMT to treat spontaneous corneal perforation in a patient with severe dry eye and calcareous corneal degeneration secondary to GVHD.

Case report

A 20-year-old Caucasian woman developed paroxysmal nocturnal hemoglobinuria in 1996. She underwent allogeneic bone marrow transplantation (BMT) at "La Fe" University Hospital, on October 1, 1997. Before the transplantation, she was prepared using cyclophosphamide, 50 mg/kg, and total body irradiation for a dose of 800 rads. About three months after BMT, she developed GVHD with a skin rash, diarrhea and elevated liver enzymes. Despite treatment with prednisone (60 mg/day orally), chronic GVHD evolved by the sixth month, with major skin involvement, dry mouth and dry eyes.

She was transferred to our Ophthalmology Department for her ocular problems. In May 1998, visual acuity (VA) was 20/20 in both eyes. Initial ocular evaluation showed scattered punctate epithelial defects in both eyes. A Schirmer test, with topical anesthesia, showed 11 mm of wetting in the right eye (RE) and 7 mm in the left eye (LE) at five minutes. Treatment included artificial tears (HypoTears®, CIBA Vision).

Subsequent examination in July 1998, showed extensive corneal epithelial erosions and stromal thinning, with extreme keratitis sicca in both eyes. VA was then 20/60 in the RE and 40/60 in the LE. Frequent treatment with artificial tears continued. Despite therapy, three months later the patient complained of constant pain in the RE and VA had decreased to light perception with projection in the RE and 20/200 in the LE. Ophthalmic evaluation showed extensive corneal epithelial erosions and stromal thinning, with extreme keratitis sicca in both eyes. A continuous-wear soft contact lens was adapted in both eyes but was not tolerated in the RE. In December 1998, the patient came to our office because the pain in her RE had progressively increased. Slit-lamp evaluation showed extensive bilateral corneal involvement with stromal thinning and calcareous degeneration, specially in her RE. The sudden corneal ulceration in the RE had led to a 1.5 x 2 mm perforation with a knuckle of bulging iris, within 24 hours (Fig. 1). In the area before the perfo-

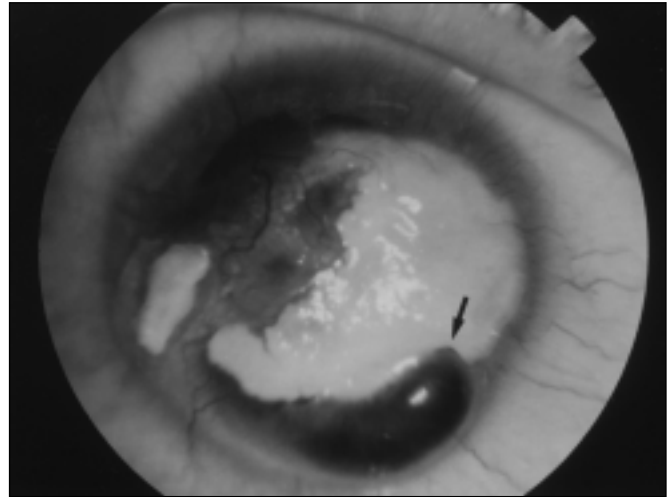


Fig. 1 - Slit-lamp preoperative photograph showing the calcareous corneal degeneration and 1.5 x 2 mm deep ulcer with a knuckle (arrow) of bulging iris in the right eye.

ration, the cornea was approximately 200 μ thick, as estimated by slit-lamp biomicroscopy. No microorganism was identified. The corneal perforation was treated initially by applying a therapeutic soft contact lens with topical fortified tobramycin (15 mg/ml) and eye patching. Because of intolerance to the contact lens and non-availability of a donor cornea at that time, it was decided to use an amniotic membrane (AM) graft to close the perforation. Informed consent was obtained from the patient before this treatment.

Human AM was prepared and preserved using a standard protocol (6, 7). The placenta was obtained from an elective cesarean section after the obstetricians obtained the donor's consent. Only tissues from mothers seronegative for human immunodeficiency virus, human hepatitis type B and C and syphilis were used. Under a lamellar flow hood, the placenta was cleansed of blood clots with sterile phosphate-buffered saline solution containing penicillin, 50 μ g/ml; neomycin, 100 μ g/ml; and amphotericin B, 2.5 μ g/ml. The amnion was separated from the chorion by blunt dissection and flattened onto nitrocellulose paper, with the epithelium-basement membrane surface facing away from the paper. The preserved AM was removed from the storage medium (Dulbecco's modified Eagle medium and glycerol, 1:1), cut to the same size as the whole corneal surface and peeled from the nitrocellulose paper.

The surgical goal included excision of calcareous plaques covering the cornea and careful removal of

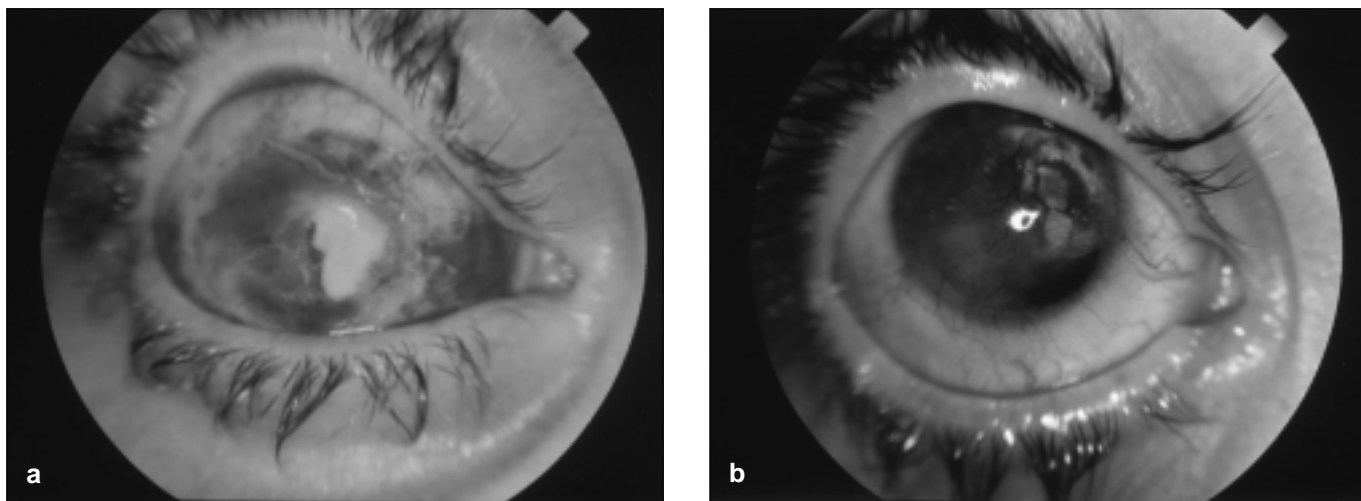


Fig. 2 - Slit-lamp postoperative photographs. **a.** On the first postoperative day a circle of three-layer amniotic membrane graft was sutured to the corneal limbus in the area of calcareous corneal degenerative thinning; **b.** Twenty months after placement of the amniotic membrane graft the perforation remains closed. Note the calcareous corneal degeneration is diminished.

all cellular debris from the base and walls of the ulcer, to expose healthy cornea. All loosened epithelium was removed from the rim of the ulcer with a hockey knife and fine forceps. This zone served as a firm base for attachment of the AM to neighboring healthy corneal epithelium. A circle of three-layer AM graft was stacked-one layer on top of the other-to fill the cavity of the perforation. A running 9-0 Vycril suture was placed over the limbal area with bites taken in the episclera to secure and stretch the three-layer AM graft (Fig. 2a). This membrane was then secured to maintain its physiological orientation (epithelium up and stroma facing the ulcer with the three layers). The surgical procedure was performed with the patient under general anesthesia.

Postoperatively the patient received 0.1% dexamethasone eyedrops, 3 to 4 hourly, tapered off over 6-8 weeks; 0.3% hydroxy-propyl-methylcellulose eyedrops 2 to 4 hourly; and 0.3% tobramycin five times a day. Thereafter, topical antibiotics and steroids were discontinued and treatment with artificial tears was instituted.

The AM was partly absorbed at six weeks after surgery and completely absorbed by five months and the cornea was completely healed. Anterior chamber depth was normal with neither aqueous leakage nor any signs of inflammation. During a 20-months follow-up, both epithelium and stromal thickness have remained stable, with no improvement of VA (Fig. 2b).

DISCUSSION

CVHD occurs in 50 to 70% of cases after allogeneic BMT (2), and frequently leads to major impairment of target organs, often with a fatal outcome. However, recent improvements in the systemic management of these patients has led to the recognition of ocular problems in a high percentage. Ocular problems arise in approximately 60% (2), the most frequent being keratoconjunctivitis sicca, cicatricial lagophthalmos and sterile conjunctivitis and uveitis. The severe ocular complications of persistent corneal epithelial defects and both noninfected and infected stromal ulcers are related to the concomitant dry-eye condition (2) and can be managed by conventional therapy including topical lubricants and antibiotics, lacrimal punctal occlusion and tarsorrhaphy. Deep ulcers and corneal perforations require penetrating or lamellar keratoplasty, grafting of conjunctiva or sclera, or tissue adhesive. The use of human tissue in deep ulcers and corneal perforations is hampered by rejection of the corneal graft and recurrent corneal thinning in the donor (3).

Tissue adhesive is suitable for small perforations, less than 1.5 mm, but is not easy to apply and may be unsuccessful (3). For larger perforations, an alternative method must be considered. The recent understanding of the use of AMT in ocular surface reconstruction and the concept of limbal stem cell deficiency (8) has spurred several newer treatment pro-

ocols. Tseng et al (8) performed AMT with or without allograft limbal transplantation in a variety of clinical situations with various severities of cytologically proven limbal stem cell deficiency, and reported that AMT alone was sufficient to treat partial limbal deficiency, whereas additional allograft limbal transplantation was needed for total limbal deficiency.

We used an AM graft to close a 1.5 x 2 mm corneal perforation; the AM covering promoted normal conjunctival epithelization while preventing excessive fibrosis. The mechanism of AMT for the suppression of conjunctival fibrosis has not been completely explained (9).

Successful treatment of deep stromal defects or even perforations requires more than one layer of AM because a single layer disappears within a few weeks and a deep stromal defect cannot be filled by *novo* synthesis during this period (10). Consequently, stromal thinning remains when the single layer of AM disappears. It is difficult to establish how the size and depth of the ulcer are related to the success of AMT, and how many layers are required for each ulcer. In our case, a 1.5 x 2 mm perforated ulcer was successfully closed with a three-layer AM graft. On the other hand, the long-term effects of transplantation of multiple layers of AM remain unknown. This question needs to be answered by a longer follow-up and a larger number of patients.

Twenty months after the AMT our patient's eye is

quiet and closed, and the ocular surface is controlled well with artificial tears. One of the most interesting findings was that stromal thickness (approximately 450 μ , including the three layers, in our case) was maintained even when the three transplanted layers of AM had gradually dissolved. Based on continuous slit-lamp investigations. Kruse et al. reported that the AM graft in some cases was replaced by a relatively clear corneal stroma (10). They postulated that the *de novo* synthesis of corneal stroma takes place in the vicinity of the grafted membrane. Probably further investigations will reveal the AM capacity for modifying the proliferative and migratory behavior of stromal keratocytes.

We believe that AMT offers an additional method for closing small corneal perforations in special cases. It is easy to obtain, and its advantages include fast reconstruction of the ocular surface, very easy postoperative care and antimicrobial properties. In addition, because it does not contain live cells, immune rejection is not a concern.

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REFERENCES

1. Shulman HM, Sullivan KM, Weiden PL. Chronic graft versus host syndrome in man. A long term-clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; 69: 204-17.
2. Franklin RM, Kenyon KR, Tutschka PJ, Saral R, Green WR, Santos GW. Ocular manifestations of graft-vs-host-disease. *Ophthalmology* 1983; 90: 4-13.
3. Portnoy SL, Insler MS, Kaufman HE. Surgical management of corneal ulceration and perforation. *Surv Ophthalmol* 1989; 34: 47-58.
4. Kim JC, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea* 1995; 14: 473-84.
5. Tsubota K, Satake Y, Ohyama M. Surgical reconstruction of the ocular in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. *Am J Ophthalmol* 1996; 122: 38-52.
6. Lee S, Tseng SCG. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997; 123: 303-12.
7. Tseng SCG, Prabhaswat P, Lee S. Amniotic membrane transplantation for conjunctival surface reconstruction. *Am J Ophthalmol* 1997; 124: 765-74.
8. Tseng SCG, Prabhaswat P, Barton K. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol* 1998; 116: 431-41.
9. Shimazaki J, Yang HY, Tsubota K. Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. *Ophthalmology* 1997; 104: 2068-76.
10. Kruse FE, Rohrschneider K, Völcker HE. Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. *Ophthalmology* 1999; 106: 1504-11.