A case of recurrent graft rejection

Dr. Anthrayose Kakkanat¹, Dr. Anil Radhakrishnan², Dr. Charles K. Skariah³, Dr. Freddy T. Simon⁴, Dr. Noel Moniz⁵, Dr. C.V. Radha Devi⁶, Dr. Tony Fernandez⁷, Dr. Thomas Kuriakose⁸

Case History

38 yr old male patient with a history of defective vision & watering both eyes since 8 yrs of age, underwent Penetrating Keratoplasty four times in his right eye and three times in the left. The surgical procedures and the sequence in which he underwent them is enumerated below:

1 RE – Penetrating Keratoplasty 22 yrs back at Little Flower Hospital, Angamaly
2 LE – Penetrating Keratoplasty 21 yrs back at Little Flower Hospital, Angamaly
3 RE - Combined cataract extraction with Intraocular lens implantation & Penetrating Keratoplasty 15 yrs back at Little Flower Hospital, Angamaly
4 LE – Combined cataract extraction with intraocular lens implantation and penetrating keratoplasty 14 yrs back at Little Flower Hospital, Angamaly
5 RE – Penetrating keratoplasty 5 yrs back at Little Flower Hospital, Angamaly
6 LE – Penetrating keratoplasty on 3/8/03 at Jubilee Mission Medical College, Thrissur
7 RE – Penetrating keratoplasty on 7/1/06 with graft of a 7 yr old donor with good endothelial count at Jubilee Mission Medical College, Thrissur

Visual acuity in January 2006 was 6/36 in the right eye (immediate postoperative period), and counting fingers at one metre in his left eye.

There was no history of similar illness in his family. The patient did not give any history suggestive of herpes or diabetes.

Postoperatively the patient was treated with topical and systemic steroids and followed up regularly. At present, the patient maintains a visual acuity of counting fingers at 1 metre in both his eyes. Slitlamp biomicroscopy showed diffuse corneal haze involving the stroma and endothelium suggestive of rejection.

There was no evidence of vascularisation, intraocular inflammation or glaucoma in the right eye. The left eye on examination revealed an opaque cornea, minimal peripheral vascularisation and no evidence of glaucoma or inflammation.

Urinary screening test for Mucopolysaccharidosis – Negative

We request your expert opinion regarding:

1. Reasons for recurrent graft failure in this patient
2. Further management in this case
3. In case of attempting repeat penetrating keratoplasty, precautions to prevent graft failure
4. Comments on recurrent graft failure
5. Any other systemic or metabolic causes for repeated

Fig. 1. Fullface photograph showing irritable and congested eyes, narrowing of palpebral aperture and bilateral opaque corneas
Expert Comments

Dr. J K Reddy

In this very unfortunate patient two important things to look at are,
1) The etiology or the diagnosis of the primary corneal condition that necessitated the corneal transplant and
2) The intraocular pressure during the post operative period.

Each condition has its own success rate for corneal transplant. Looking at the details given, I am of the opinion the patient may be a case of Congenital hereditary endothelial dystrophy. In that case the short term corneal survival rates are good, but the long term prognosis is not good.

These patients need multiple grafts in their life time. As the number of re-grafts goes on the inflammation is more and chances of failures are more. Very often these young patients are steroid responders and the intraocular pressures goes up to very high levels and results in graft failures.

The IOP assessment rather than measurement in the both eyes is to be done as the cornea is very hazy and edematous. In case the intraocular pressures are normal, we can plan for PKP with trabeculectomy in the left eye. During the pre operative waiting period he can be put on topical Prednisolone eye drops 6 times a day in the left eye and IOP should be measured after one month. Regarding the Donor selection, I like to go for A grade cornea with Blood group match. Regarding the Surgical technique, interrupted suture with 10-0 prolene or merselene is preferable for re-grafts. In the immediate post operative period the patient should be put on systemic steroids or / and immuno-suppressants like Cyclophosphamide or Azathioprine for 4 to 6 months, and then tapered with low dose maintenance. Topical Cyclosporine or Tacrolimus are to be instilled along with a potent topical steroid like prednisolone. Accurate measurement of post operative IOP is very important.

In the unlikely event of further failure of the graft with in a short period, say 4 to 6 months, the only option is Keratoprosthesis.

Dr. Noel Moniz

This young man has undergone multiple graft failures. It may not be possible to revive the present graft since I am sure intensive measures would already have been tried. It is best to wait for at least 6 months before going in for a regraft. IOP should be checked at all times ideally with an instrument like the tonopen. It would be best to get a specular count of the donor eye with an eye bank specular microscope. I usually prefer to do smaller grafts than the previous size. The multiple grafts have already made us go in for large grafts and enlarging it further would be going too close to the limbus and also a cause for glaucoma. Care should be taken to form the anterior chamber well to prevent postoperative glaucoma. I would start the patient on systemic steroids on the day of surgery and intense topical steroids from the 1st postoperative day. As the steroids are being tapered around day 10 – 15 I would start the patient on Azathioprine (Imuran 50mg) OD and wean the patient off systemic steroids. Imuran can be continued for a long time and so also topical steroids. Any signs of vascularisation should be watched for. We should always keep a look out for the complications of topical and systemic steroids and also imuran.

The above regimen has worked well in my hands.
The only other newer drug I would like to try is cyclosporine drops. I would also like to give tear substitutes.

**Dr. Thomas Kuriakose**

This sort of scenario is not uncommon when one has been doing cornea for some time and one wonders if PKP is the final answer for corneal blindness and is it time we changed the practice whenever possible.

The reasons for recurrent graft failure are not hard to find because once a graft rejection has occurred then all the factors which normally gave the corneal graft its privileged position goes. Blood vessels, increased amounts of antigen presenting cells (APC) in the periphery, sensitization of the system etc increases the chances for recurrence. I doubt if any of the measures like trying to close the vessels of by laser etc really helps.

If facilities are available then the best chance for survival of a regraft in this patient would be to do a repeat keratoplasty using a HLA matched, high quality donor material. Systemically he should be managed like any other solid organ transplantation and should be put on systemic Cyclosporine or Tacrolimus in addition to steroids. If one is not used to this one can enlist the help of a nephrologist well versed in its use for renal transplantation.

I am not being funny when I say that the best way to prevent a graft rejection is by not doing one. Thus I discourage patients with corneal opacity in only one eye to undergo PKP I think when possible one should do only a Deep Anterior Lamellar Keratoplasty using the big bubble technique so as to reduce the chances for rejection. Besides systemic immuno-suppression one should also use systemic long term antiviral to prevent graft failure if there is a viral etiology.

**Dr. S. Tony Fernandez**

The reasons for graft failure.

From the history it is obvious that this is an Immune mediated graft rejection. The reasons are the following. Such graft reactions occur mainly in the young people. The first graft was done 22 years ago and the other eye was grafted the next year. The rejection started late. When the first graft rejects the possibility of recurrent graft rejections are possible. Other risk factors, like vascularisation, loose sutures, infection glaucoma, or eccentric and large grafts are not there. Such graft rejections does occur in about 16 to 30 % of people. Though normally the cornea is an immune privileged tissue and therefore transplantations are regularly successful, some young patients have a tendency for Immune mediated graft rejections. They should be treated and managed with extreme caution and care we give to kidney or other organ transplantations. As stated here the following precautions can be undertaken.

Tissue typing and ABO typing can be done. As we take blood from the cadaver as a routine procedure such tests are worth starting in institutions where transplants are routinely done. Though this is not necessary in normal cases, in such case it might be of value.

Selection of better donor material preferably preserved in M.K.Medium should be used. An endothelial study should be done before this is selected.

Removal of epithelium from the donor material is suggested by some, but it is controversial. As the recipient cornea is not healthy enough I feel it should not be done because epithelial defect can occur.

Immunosuppressants should be used as in other organ transplantations and they should be monitored carefully. Regular follow ups are necessary.

**Dr. Freddy T Simon**

The apparent reasons for rejection in this case are

1. In the Collaborative Corneal transplant Studies (CCTS) the number of previous grafts proved to be a strong risk factor for graft failure. With each additional graft the risk increases by 1.2%. Since the host is presensitized the subsequent graft rejections can occur sooner as in this case.

2. Higher incidence of graft rejection is seen in younger recipients.

3. The picture given shows a large graft, again a predisposing factor.

4. Previous anterior segment surgeries were also found in CCTS to predispose to graft failure
The other causes to be kept in mind are anterior synechiae, vascularization more than 2 quadrants, and raised intraocular pressure. The IOP should ideally be measured with a tonopen and the discs checked for any damage.

The treatment options for this patient are

1. Treat the present rejection episode aggressively and save the graft if possible.
2. If a further graft is required
   a. Use a smaller graft
   b. Use ABO and HLA matched donor if possible
   c. Use an organ culture stored cornea, (if available) since cornea stored for sometime loses some of its antigenicity.
   d. It is most important in this case to use immunosuppression either cyclosporine A or Mycophenolate mofetil. CSA needs close monitoring of the blood levels to keep it within 120-150 ng/ml to avoid side effects, MMF was found to be as effective as CSA while being more cost effective and safer. (Alexander Reis et al).

It is important that the patient is followed up closely and aim for vision only in one eye.

**Recurrent graft failure - Comments**

**Dr. Charles K. Skariah**

Going through the history of this patient his symptoms started early in childhood and the first keratoplasty was done by the age of 16 years in the right eye and 17 years in the left eye. The preoperative diagnosis or a photograph showing the corneal condition at that period is not available. Being a bilateral disease in young age with symptoms of watering, foreign body sensation and defective vision, the possibility of stromal dystrophies, metabolic storage disease, keratoconus or even rarely HSV infection are the possibilities to be considered. Keratoconus and HSV rarely warrant bilateral keratoplasty at the age of 16 years. So in all probability the original diagnosis could have been either corneal dystrophy or a metabolic storage disorder like mucopolysaccharidoses both of which are likely to recur following keratoplasty.

From the clinical history, the regrafting was done after 7 years which was coupled with cataract surgery. There is no mention in the history as to how long the graft remained clear. Even after repeated graft failure there are no signs of inflammation and deep vascularisation in the graft. So it is unlikely to be a case of graft rejection and hence most probably this is a case of recurrence of disease leading to graft failure.

**What may be the disease recurring in this graft?**

? Stromal dystrophy
? Schies syndrome
?? HSV infection

Most of the stromal corneal dystrophies tend to recur after 3-12 years following PKP. Among the stromal dystrophies, macular dystrophy tends to produce early onset of visual impairment than others and warrants early PKP. Although most of the corneal dystrophies show a tendency to recur after PKP, the most common type to recur is Lattice and then macular dystrophy. Among the storage disorders the most common to produce early corneal clouding and which recurs after surgery is Scheie’s syndrome (MPS type-1 S). This is produced by deficiency of the enzyme alpha – L iduronidase leading to deposition of Heparan sulfate and Dermatan sulfate in the cornea. Unlike Hurlers disease, Schies syndrome may not have all the classical features of MPS type-1 and may be missed by a casual examination. The only features may be claw hand deformity and bony changes in the feet. Although there is no history of HSV infection, this possibility has also to be thought of in all cases of recurrent graft failure of unexplained etiology.

**Risk factors for rejection:-** Several host factors have been identified as conferring “high risk” status to the host leading to graft rejection or failure. These include:-

1. Young recipient age
2. Large grafts
3. Previous failed / rejected grafts (especially when two or more grafts have previously failed)
4. More than two quadrant vascularisation with associated lymphatics
5. Herpes simplex keratitis
6. Uveitis
7. Preoperative glaucoma
8. Multiple surgical procedures at the time of grafting
9. H/O previous anterior segment surgery
10. Anterior iris synechiae
11. Silicon oil keratopathy
12. Vitreous adhesions
13. Blood group ABO incompatibility

Graft rejection is largely mediated by the major histocompatibility antigens, minor antigens, and perhaps blood group ABO antigens and some cornea-specific antigens. Just as rejection is mediated by active immune mediated events, the lack of rejection (tolerance) is also sustained by active immune regulatory mechanisms. The anterior chamber associated immune deviation (ACAID) and probably, conjunctiva associated lymphoid tissue (CALT) induced mucosal tolerance, beside others, play an important role.

So how do we proceed from here?

A complete immunological work up and Tissue matching for HLA and ABO compatibility will be beneficial to reduce the incidence of rejection. A detailed multi disciplinary clinical examination and biochemical work up may be worthwhile to rule out any metabolic disease contributing to the recurrent graft failure. Conjunctival biopsy can confirm classic histopathology. Specific diagnosis requires biochemical assay for enzymes in tears, leucocytes, cultured fibroblasts, or amniotic cells and for elevated urinary excretion of Heparan sulfate and Dermatan sulfate. The corneal deposits stain with alcin blue and colloidal Iodine. An enzyme linked immunosorbant assay (ELISA) can measure sulfated keratan which may be useful in diagnosis of macular dystrophy.

Prognosis

The chances of graft failure following another surgery are very high as this falls in the very high risk category. Prognosis is very much guarded as abnormal storage material may accumulate again in the graft. Matching the patient and donor blood type (ABO compatibility) and HLA compatibility might be effective in improving the patient outcome. Explaining and educating the patient about the high chances of failure, a repeat grafting of smaller size may be done with a fresh cornea with normal endothelial count.

Although one is not sure of the cause of failure in this case, immunosuppression with high dose of topical and systemic corticosteroids and Cyclosporine are indicated. Good patient compliance and close follow up are the keys to successful corneal transplantation in high risk individuals. If signs of rejection are identified, pulsed intravenous methyl prednisolone and/or oral prednisolone may be initiated at the earliest evidence of rejection.

In cases of storage diseases, regression of corneal clouding following successful donor stem cell bone marrow transplantation has been reported.

Dr C V Radha Devi

Comments on graft failure

Despite the improving results in penetrating keratoplasty, graft failure still remains a significant problem. It is important to differentiate between graft failure and graft rejection. Graft failure can occur due to various causes any time after keratoplasty. Immediate graft failure is due to some defect in the donor material. Technical problems can also lead to graft failure but are not frequent. Even after good evaluation and selection of donor cornea, graft failure can occur. Some damage or fault in the donor corneal endothelium will lead to thickening of the graft and haziness. This may be associated with local inflammation and topical steroids may be helpful. Sub-conjunctival or systemic steroids may not be of much help. Prolonged shallow anterior chamber or adherence of iris or vitreous can also lead to graft failure. Endothelial dysfunction can occur due to surgical trauma. Excessive irrigation and improper handling of the graft can lead to endothelial damage.

Intraocular pressure should be monitored and if raised, should be controlled appropriately. Any chance of pupillary block can be prevented by iridotomy and/or adequate medication postoperatively. Antiglaucoma drugs if used prior to surgery should be continued postoperatively.

In the late postoperative period, even with uneventful keratoplasty, graft failure can occur due to attrition of endothelial cells. Postoperative inflammation can be an added cause. Common and leading cause of graft failure in the late post operative period is graft rejection.
This is specifically due to immunologically mediated process. Here, the graft remains clear in the initial stages for several weeks, and suddenly becomes oedematous, associated with inflammatory signs. If the symptoms are identified early and adequate treatment given with topical steroids supplemented with systemic steroids and if required, with immunosuppressants, reversal of the graft reaction may be possible.

Following points are important and should be remembered in keratoplasty. Vascularisation of the recipient cornea is one of the main factors for allograft reaction. This should be controlled prior to surgery. Peritomy may be helpful in some cases. This can be done before or during keratoplasty. Sutures invite vascularisation and this should be noted on postop review and if present, timely treatment should be given. Smaller the graft size usually gives better results. Large size grafts are associated with more chance of graft failure. Associated conditions like cataract, or glaucoma calls for additional procedures which may increase the chances of graft failures. Also longer duration of surgery and older recipient age can lead to graft failure.

It is important to remember that in repeated keratoplasty, successive grafts will not have as good a prognosis as the initial grafts. Associated conditions like cataract, or glaucoma calls for additional procedures which may increase the chances of graft failures. Selection of good donor material, sutures, technique and careful tissue handling are important to obtain clear grafts. Review of the case should be done daily in the initial postoperative stage and treatment instituted as and when required. Use of topical steroids or immunosuppressants and if required, systemic steroids and or immunosuppressants, in the postoperative period may be of great help to prevent graft failure.

**Dr. Anil Radhakrishnan**

In any case of repeated graft failure it is imperative to know the reason, whether it is recurrence of primary disease, immunological rejection, endothelial decompensation or any other obvious pathology to address the cause.

The recurrence of primary disease as in corneal dystrophy presents with stromal haze [usually without oedema] with progressive increase in stromal deposits usually first seen along suture tracks. Immunological rejection is associated with stromal oedema, usually though not always associated with anterior chamber inflammation. Progressive depletion of endothelial cells or endothelial decompensation is also associated with stromal edema. An optical section by slit lamp biomicroscopy can help in differentiation. In stromal oedema, in addition to haze, the stromal thickness is increased along with appearance of Descemet’s folds. High pachymetry values also suggest stromal oedema. In this case, with the available information it appears more like a stromal haze with anterior stromal deposits rather than stromal oedema. The presence of stromal deposits initially along suture tracks, its centrifugal spread and early recurrence in a graft, in the absence of inflammation all suggest recurrence of primary disease, most likely a corneal dystrophy. Being fortunate to come across a few cases of homozygous granular dystrophy, I am tempted to presume it is so. However, the long interval between first and second graft in both the eyes and subsequent early graft failures suggest a diagnosis of immunological rejection.

Granular dystrophy is caused by mutation in the kerato-epithelin gene [type1 - Arg 555 Try, type2 - Arg 124 Hist, type3 - Arg 124 Leu / Arg 555Gln]. If it is a heterozygous mutation, as in most cases, typical phenotype of granular dystrophy is evident, characterized by bilateral symmetric bread crumb like stromal opacities separated from one another and limbus by clear stroma. If the mutation is homozygous, a severe phenotype with juvenile onset, placoid lesions and severe opacification of cornea and early recurrence following keratoplasty is seen. Genomic DNA analysis of blood leucocytes can pick up the presence of mutation. Phototherapeutic keratectomy or even scraping of epithelium and anterior stroma that may have to be repeated multiple times is effective for visual improvement, as the recurrence is initially confined to the epithelium with slow antero-posterior spread.

Gelatinous Droplet Like Dystrophy [GDLD] is another possibility. Primarily, it presents in the first or second decade of life with multiple, bilateral, mulberry-like elevations due to subepithelial accumulations of amyloid. It has been mapped to chromosome 1p due to mutation in M1S1 gene. Recurrence is the rule after corneal grafting. Mucopolysacchridoses [MPS] or mucolipoidoses [MLS] can also present with early recurrence, but unlikely in
this age group. MPS typically occurs in the first few years of life, has a family history and is associated with systemic involvement, presence of urinary glycosaminoglycans, and specific enzyme deficiency evident in blood or lacrimal fluid and positive conjunctival biopsy. Co-existent ocular pathology like retinitis pigmentosa or open angle glaucoma is present in most cases. Mucolipidosis also present with typical systemic features, quite often associated with mental retardation early in life.

Penetrating limbo-keratoplasty [limbal stem cell transplantation + keratoplasty] is a major step towards augmenting recurrence-free interval in corneal dystrophies, as the site of origin of mutated keratoepithelin gene is taken care of, but is not time-tested. The experience from available literature does not vehemently support the use of limbo-keratoplasty over routine keratoplasty probably due to progressive depletion in stem cell population over time. But, probably for GDLD and certainly for MPS it is justified. However, limbo-keratoplasty necessitates intensive prolonged follow-up and lifelong immuno-suppressive medications, for which cost is a major consideration in our set-up.

On the other hand, if stromal oedema is the cause for opacification of the graft, it is either due to immunological rejection or endothelial decompensation. The latter is highly unlikely as the graft was recent and from a young donor. If immunological rejection is the cause for recurrent graft failure, long term immunosuppressive therapy in the form of systemic cyclosporine probably offers the best chance of graft survival after a re-graft. It is important to maintain adequate serum levels [trough levels of 120-150 ng/ml]. Co-administration of drugs like diltiazem can increase the serum levels by competitive protein binding and is a cost-effective way to do so without increasing the dose of expensive cyclosporine. If cost is still a major concern, azathioprine in combination with systemic steroid is a cheaper alternative. 2% topical cyclosporine is effective, without the side-effects of steroids, but difficulty in dispensing it in oil and incidence of superficial punctuate keratopathy makes it a less practical option. Considering his pseudophakic status I would like to maintain him on Prednisolone acetate eye drops once or twice a day after making sure that he is not a steroid -responder. Topical steroid therapy lowers the local population of immunologically active cells and reduces the expression of HLA class 2 antigens in the recipient cornea.

**Suggested management**

1] Blood – for genomic DNA analysis [a] for granular dystrophy [b] for GDLD
2] If no mutation is found in DNA analysis, medical consultation with an internist who is conversant with metabolic storage disorders to rule out mucopolysaccharidoses [MPS] or mucolipidoses [MLS]. This assumes greater significance if the visual acuity is not explainable by corneal opacity.
3] Specific enzyme assay for MPS or MLS in blood or lacrimal fluid / conjunctival biopsy – if the internist suspects a storage disorder
4] If nothing emerges out of the aforementioned, further management will depend on the histopathologic study of the corneal button, which can give precious information, if the patient desires another keratoplasty. This needs to be done by an experienced ocular pathologist in an ophthalmic institute where facilities for immunohistochemical study and special stains are available. Polymerase chain reaction for HSV DNA from the corneal button may also be done considering the remote possibility of HSV keratitis.

The management paradigm, as discussed before depends on whether it is recurrence of primary disease or immunological rejection. If it is a keratoepithelin gene associated dystrophy [granular/ lattice], I would like to perform a routine keratoplasty with the same sized or a smaller graft. I would prefer to follow him up closely [once a month] and perform debridement of epithelium at the earliest sign of recurrence. In the case of immunological rejection, local and systemic immnosupression, as detailed before hold the key.

In either scenario, being a high-risk graft, I would like to maintain him on topical steroids if the patient is not a steroid responder. Control of IOP and close follow up, monthly after the immediate postoperative period is desirable in this unfortunate patient.
Editors Comments

Graft failure following PKP is very common. Studies show that upto 30% of PKP patients have at least one episode of rejection with 5-7% of all grafts eventually failing because of rejection. Corneal graft failure can be primary due to donor endothelial damage or secondary, the most common cause of which being graft rejection.

The prognosis of keratoplasty depends on various factors like vascularisation, active corneal inflammation, glaucoma, immunological rejection, recurrence of the primary disease etc. Mucopolysaccharidosis (MPS) and certain corneal dystrophies are known to recur after keratoplasty.

Stromal vascularisation may be treated with photocoagulation with
Argon blue-green or yellow dye laser either preop or postop.

Mucopolysaccharidosis in graft rejection
Mucopolysaccharidosis can be detected by urinary screening tests
- Toluidine blue spot test and
- Turbidity tests using (i) Cetylpyridium chloride and
(ii) Acid-albumin.

The screening tests for mucopolysaccharidosis in urine are helpful indicators in predicting the chance for recurrence and rejection. The confirmatory test can be done with column chromatography.

High risk patients for recurrent graft rejection
According to Collaborative corneal transplantation study, high risk patients include those with
(a) 2 or more quadrants of stromal vascularisation and
(b) H/O previous graft failure

Approaches to prevent rejection in high risk patients are
(i) Making donor tissue less antigenic
  - Central corneal graft
  - Removal of donor epithelium
  - Pretreatment of donor tissue with UV light, hyperbaric O2, heterologous antibodies, storage in organ culture
  - Tissue matching-HLA & ABO
(ii) Suppressing host immune response
  - Glucocorticoids- topical & systemic
  - Azathioprine
  - Cyclosporine A- topical & systemic (in monocular high risk patients)

It is important to start immunosuppressant in the immediate post op period itself in high risk patients.

References

(v) Metabolic basis of inherited diseases-Scriver
(vi) Clinical diagnosis by laboratory methods-Davidson & Henry