

Comparative Study of Graft Rejection in Keratoconus Patients With and Without Self-Reported Atopy

Elvin H. Yildiz, MD,*† Mesut Erdurmus, MD,*‡ Kristin M. Hammersmith, MD,*
Christopher J. Rapuano, MD, Peter R. Laibson, MD,* and Elisabeth J. Cohen, MD*

Purpose: To compare the incidence and characteristics of graft rejection in keratoconus patients with and without self-reported atopy.

Methods: From November 2007 to February 2008, 75 consecutive keratoconus patients who had undergone penetrating keratoplasty completed a questionnaire regarding personal history of atopy. The questionnaires were reviewed without knowledge of history of graft rejection. The patients were divided into three groups: nonatopic (group 1), mild atopic (group 2), and severe atopic (group 3). Charts were retrospectively reviewed with regard to the incidence and characteristics of graft rejection episodes for each patient.

Results: There were 13 patients in group 1, 36 patients in group 2, and 26 patients in group 3. The incidence of graft rejection (rejection episodes per 100 person-years) in groups 1, 2, and 3 was 7.3, 15.8, and 13.4, respectively. Although the mild and severe atopic groups had roughly twice the rate of rejection of the nonatopic group, there was no statistically significant difference among the groups ($P = 0.25$). The mean age of 37.1 ± 8.9 years at the time of surgery was significantly lower ($P = 0.004$) and the rate of bilateral penetrating keratoplasty was significantly higher (40/55 eyes 73%) ($P = 0.01$) in group 2 than in the two other groups. The majority of first graft rejection episodes were mild or moderate in atopic patients (69% in group 2 and 94% in group 3) and severe (50%) in nonatopic patients; however, no significant difference was found among the groups ($P = 0.13$).

Conclusions: Atopic conditions may increase the incidence of graft rejection in keratoconus patients, and the characteristics of graft rejection in atopic patients may differ from those in nonatopics because of the immune alteration, but differences in this study were not statistically significant.

Key Words: keratoconus, graft rejection, atopy

(*Cornea* 2009;00:000–000)

Received for publication August 20, 2008; revision received November 18, 2008; accepted December 4, 2008.

From the *Wills Eye Institute, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania; †Ankara Training and Research Hospital, 1st Eye Clinic, Ankara, Turkey; and ‡Department of Ophthalmology, Fatih University Medical School, Ankara, Turkey.

Reprints: Elisabeth J. Cohen, MD, Cornea Service, Wills Eye Hospital, 840 Walnut Street, Suite 920, Philadelphia, PA 19107 (e-mail: ecohen@willseye.org).

Copyright © 2009 by Lippincott Williams & Wilkins

INTRODUCTION

The high incidence of atopy in keratoconus patients has been documented in the literature.^{1,2} It has been postulated that one cause of keratoconus is eye rubbing, stimulated by ocular itching or discomfort, resulting from atopic disease.³ Additionally, patients with high levels of IgE, as seen in atopic conditions, have been thought to be more prone to corneal graft rejection.² High IgE levels lead to an increase in expression of complex immunological mediators in atopic disease, including prostaglandins and interleukins.

Classically, corneal allograft rejection is mediated by a type 1 T helper (Th 1) cell response. However, promoting type 2 T helper (Th 2) immune response has been shown to cause eosinophil-mediated rejection of cardiac and skin allografts in mice.^{4,5} Similarly, Th 2 cell mediated corneal allograft rejection in a mouse model with a Th 2 cell immune bias has been reported.⁶ Since atopy causes an increase in Th 2 cell immunity, it may play an important role in corneal allograft rejection in atopic keratoconus patients.

Although the expected graft clarity rate is >95% in keratoconus patients undergoing penetrating keratoplasty,^{7–10} clinical experience suggests that a history of allergic disease may affect graft survival. Recently, Reinhard et al. showed that atopic dermatitis negatively affects graft prognosis in keratoconus eyes.¹¹ In this study, our aim was to compare the incidence and characteristics of graft rejection in keratoconus patients with and without self-reported atopic disease, defined as allergic rhinitis, perennial allergy, asthma, and atopic dermatitis.

MATERIALS AND METHODS

The study was approved by the Wills Eye Institute Institutional Review Board and was in compliance with Health Insurance Portability and Accountability Act (HIPAA) regulations. The patients were enrolled over 4 months, between November 1, 2007, and February 28, 2008, when they came for a routine follow-up visit at the Wills Eye Institute, Cornea Service. All patients had undergone previous corneal transplant surgery for keratoconus, at the same institution, by one of the authors. We excluded patients with <2 years of follow-up after penetrating keratoplasty, a history of intraocular surgery before penetrating keratoplasty, a history of additional surgery at the same time or subsequent to penetrating keratoplasty, and a history of penetrating keratoplasty before presentation to the Wills Eye Institute, Cornea Service. All eligible patients were invited to participate in the study.

After informed consent was obtained, patients filled out a questionnaire regarding their personal history of atopic disease. The questionnaire was based on The European Community Respiratory Health Survey (ECRHS) and on The International Study of Asthma and Allergies in Childhood (ISAAC)^{12,13} and was used in previous studies reported from Wills Eye Institute, Cornea Service.^{14,15}

In February 2008, after patient recruitment was completed, the questionnaires were reviewed by one of the authors (KMH), who was blind to the patient's history of graft rejection. The patient's atopic status was determined on the basis of the presence of at least one of the following disorders: allergic rhinitis (hay fever or seasonal allergies), perennial allergies (year-long allergies to dust or mold), atopic dermatitis (eczema), and asthma. Patients were divided into three different groups according to the severity of the atopic conditions. Group 1 (nonatopic) included the patients without atopic conditions, group 2 (mild atopic) included the patients with allergic rhinitis and/or perennial allergies, and group 3 (severe atopic) included the patients with asthma or atopic dermatitis, in addition to allergic rhinitis and/or perennial allergies.

In February 2008, after the evaluation of questionnaires was completed, charts were retrospectively reviewed without knowledge of the atopic status of each patient, and the characteristics of graft rejection episodes compared between atopic and nonatopic patients. The diagnosis of graft rejection was made by one of the authors (KMH, CJR, PRL, or EJC), and the rejection episodes were further classified into three categories (mild, moderate, and severe). The mild rejection category included patients with epithelial rejection line and/or subepithelial infiltrates (SEIs), and the moderate rejection category included patients with keratic precipitates (KPs), with or without SEIs. The severe rejection group comprised patients with stromal edema, with or without KPs, and patients with stromal rejection.

All corneal transplant surgeries were performed by one of the authors (KMH, CJR, PRL, or EJC), using similar surgical techniques.¹⁶ All patients received postoperative topical antibiotics and topical prednisolone 1%, 4 times a day. Steroid frequency was usually decreased by 1 drop per month after the first or second postoperative month and tapered gradually over 1 year or longer. The dose and frequency of the steroid used at the time of the first rejection episode were also categorized in 4 potency groups (high, medium, low, and no steroid), which were described and used previously in another study by our group.¹⁶

The main outcome measure was the incidence of graft rejection episodes (episodes per year of follow up), classified into three categories (mild, moderate, and severe rejection), in three groups (nonatopic, mild atopic, severe atopic). Follow-up time was calculated from the surgery to the last follow-up visit. If the patient underwent any additional surgery after penetrating keratoplasty, then the end of follow-up was considered the last visit before this surgery. Patients who underwent any additional surgery less than 2 years after the penetrating keratoplasty were excluded. Secondary outcome measures included characteristics of first graft rejection, steroid potency at the time of first graft rejection episode,

seasonal distribution of the first graft rejection episodes, and the time interval between transplant surgery and first graft rejection episode. Other variables, including age, gender, and bilaterality of corneal transplantation, were reviewed.

Sample size and statistical analysis

The sample size was calculated by assuming (1) an incidence rate of rejection of 30 per 100 person-years¹⁷ among the atopic subjects, (2) a 50% rate of atopy in the sample, and (3) a 5-year average follow-up for each person. To have 80% power to detect an incidence rate ratio (atopic vs. nonatopic) with a *P* value of <0.05 in Poisson regression would require data from 88 eyes.

Atopy groups were compared with respect to demographic and clinical variables. The Kruskal-Wallis test was used to detect a difference among groups in continuous measurements, whereas Fisher's exact test was used to detect differences in categorical variables. The incidence rate for rejection events (total number of rejection events, divided by total follow-up time) was calculated for each atopy group. A generalized linear model, where the number of events was assumed to follow a negative binomial, was used to test for differences among groups in the incidence of rejection events. This comparison was adjusted for any differences in groups with respect to other important clinical and demographic variables. The Kaplan-Meier method was used to estimate the distribution of time to first rejection episode, and the log-rank test was used to test for differences among groups. Association of steroid potency and severity of rejection at the first rejection episode were evaluated with mixed-effects linear regression.

RESULTS

From November 1, 2007, to February 28, 2008, 75 consecutive keratoconus patients completed a questionnaire regarding their personal history of atopy. After the retrospective chart review was performed, it was determined that 31 patients underwent bilateral penetrating keratoplasty. Among these patients, 3 second eyes and 2 first eyes were excluded because of the following reasons: 2 of the second eyes received PK in 2007 (<2 years follow up); one of the second eyes and one of the first eyes received penetrating keratoplasty elsewhere; and one of the first eyes was lost to follow-up for 10 years. In the end, 106 eyes of 75 patients were included in this study. Group 1 (nonatopy) comprised 17 eyes of 13 patients, group 2 (mild atopy) comprised 55 eyes of 36 patients, and group 3 (severe atopy) comprised 34 eyes of 26 patients.

The mean age at the time of surgery was 45.7 ± 12.3 years in group 1 (range, 18 to 72 years), 37.1 ± 8.9 years in group 2 (range, 21 to 62 years), and 43.5 ± 8.9 years in group 3 (range, 26 to 63 years). The mild atopy group (group 2) was found to be statistically significantly younger than the other two groups at the time of surgery ($P < 0.004$). Male/female ratio was 6/7 in group 1, 25/11 in group 2, and 14/12 in group 3. No statistically significant difference was found between the groups ($P = 0.64$). Caucasian/noncaucasian ratio was 11/2 in group 1, 31/5 in group 2, and 21/5 in group 3. Mean follow-up time was 19.7 ± 9.8 years in group 1 (range, 2 to 33.3 years), 11.2 ± 7.7 years in group 2 (range, 2.4 to 32 years), and

TABLE 1. Demographic Characteristics of the Study Population

Characteristic	Nonatopic n = 13 (17 eyes)	Mild Atopic n = 36 (55 eyes)	Severe Atopic n = 26 (34 eyes)	Total n = 75 (106 eyes)	P Value
Age,* mean $\pm$ SD†	45.7 $\pm$ 12.3	37.1 $\pm$ 8.9	43.5 $\pm$ 8.9	40.6 $\pm$ 10.1	0.004§
Range	18–72	21–62	26–63	18–72	
Male/female	6/7	25/11	14/12	45/30	0.64
Follow-up, mean $\pm$ SD	19.7 $\pm$ 9.8	11.2 $\pm$ 7.7	8.8 $\pm$ 5.5	10.9 $\pm$ 7.5	0.54
Range	2–33.3	2.4–32	2–25	2–33.3	
Bilateral PK‡	9 eyes (53%)	40 eyes (73%)	18 eyes (53%)	67eyes (63%)	0.01

*Mean age in years at the time of surgery.

†Standard deviation.

‡Penetrating keratoplasty.

§Mild atopic group is statistically significantly younger than the other two groups.

8.8 $\pm$ 5.5 years in group 3 (range, 2 to 25 years). There was no statistically significant difference in terms of mean follow-up time between the groups ($P = 0.54$). Bilateral penetrating keratoplasties had been performed in 5 (38.5%) of 13 patients in group 1, 21 (58.4%) of 36 patients in group 2, and 10 (30%) of 26 patients in group 3. Bilateral penetrating keratoplasty was performed statistically significantly more often in the mild atopy group ($P = 0.01$) (Table 1).

The nonatopic group had the lowest incidence of rejection episodes (incidence rate [IR], 7.32 episodes per 100 person-years), whereas the mild and severe atopy groups had roughly twice the rate of rejection (IR = 15.8 and 13.4, respectively). However, there was no statistical difference among the groups ($P = 0.25$). After adjustment for differences in age at the time of surgery and bilaterality, the estimated rate of rejection in the nonatopic group increased slightly, whereas the rates in the other groups remained roughly the same. There was still no statistically significant difference among the groups ($P = 0.54$) (Table 2).

The majority of the first rejections were mild or moderate in atopic patients, whereas severe rejection was the most common type of rejection in the nonatopy group. There was no statistically significant difference in severity of rejection among the groups ($P = 0.13$); however, pairwise

comparisons indicated possible differences. Moderate rejection was seen more often in the severe atopy group than in the mild atopy group ($P = 0.058$) (Table 3). Distribution of the first graft rejection episodes by months showed that a majority of the first rejections in the mild and severe atopy group were during the allergy season from March to October (26/32 [81%] in the mild atopy group and 11/18 [61.1%] in the severe atopy group) (Figure 1).

Kaplan-Meier analysis for the time to first rejection episode by group indicates that the median time and 95% confidence interval (CI) to first rejection in the mild and severe atopy groups were 70 months (95% CI, 19–336) and 32 months (95% CI, 18– ∞), respectively. Although rejections occurred earlier in the severe atopic group than in the mild atopic group, there was no statistically significant difference ($P = 0.26$). The median time to the first rejection episode could not be estimated in the nonatopy group because only a small number of patients were nonatopic, and fewer than 50% of the eyes had a rejection episode.

There was some evidence of correlation among severity of rejection and potency of steroid ($P = 0.052$), with those on the highest dose of steroids having the greatest percentage of rejections categorized as mild (epithelial rejection and/or SEIs) (Table 4).

TABLE 2. Association of Atopy With Incidence of Rejection Episodes

Atopy Group	IR* CI†	Unadjusted IRR‡	Overall P value	Adjusted IR§ and CI	Adjusted IRR	Overall P Value
Group 0 (Nonatopy)	7.32 (3.1–17.2)	1	0.25	8.3 (3.7–19.0)	1	0.54
Group 0 (Nonatopy)	7.32 (3.1–17.2)	1	0.25	8.3 (3.7–19.0)	1	0.54
Group 1 (Mild atopy)	15.83 (11.1–22.5)	2.16 (0.9–5.4)		17.4 (12.0–25.1)	2.1 (0.8–5.2)	
Group 3 (Severe atopy)	13.42 (8.3–21.7)	1.83 (0.7–4.9)		14.0 (9.0–23.7)	1.8 (0.7–4.5)	

*Incidence rate (episodes/100 person-years).

†95% Confidence interval.

‡Incidence rate ratio.

§Adjusted incidence rate for age at the time of surgery.

||Adjusted incidence rate ratio for age at the time of surgery.

TABLE 3. Association of Atopy With Severity of First Rejection Episode

Atopy Group	Mild Rejection (Epithelial and/or SEI*)	Moderate Rejection (KP† with/without SEI)	Severe Rejection (Stromal Edema)
Non atopy	2 (33%)	1 (17%)	3 (50%)
Mild atopy	16 (50%)	6 (19%)	10 (31%)
Severe atopy	8 (44%)	9 (50%)	1 (6%)

P value for test of association: 0.13.

P values for pairwise group comparisons: nonatopy vs. mild atopy, 0.67; mild atopy vs. severe atopy, 0.058; nonatopy vs. severe atopy, 0.10.

*Subepithelial infiltrates.

†Keratic precipitates.

DISCUSSION

Our results showed that a majority of keratoconus patients enrolled in this study had atopic conditions (82.6%), higher than the expected 50% used in our sample size calculation, and that the most commonly seen allergic diseases were allergic rhinitis and perennial allergy, which were also found to be associated with a higher rate of bilateral PKP and a younger mean age at the time of surgery (in comparison with nonatopic patients). The association between keratoconus and atopy has been shown to be via eye-rubbing.^{2,3,17} Our results indicate that the progression of keratoconus may be faster in atopic patients, possibly because conjunctival itching leads to eye-rubbing.

Although the rate of clear graft after penetrating keratoplasty exceeds 95% in keratoconus patients, up to 31% of these patients can have at least 1 episode of rejection.^{18–20} Traditional belief indicates that Th 1 immune responses induce allograft rejection, whereas Th 2 immune responses induce allograft tolerance. However, preliminary evidence from Hargrave et al. showed a statistically significant increase in the number of eosinophils in the rejected grafts in keratoconus patients.⁷ Based on these findings, there may be a component of Th 2-mediated corneal allograft rejection in patients with Th 2 immune bias, such as atopics. We also found a tendency of first graft rejection episodes to occur between May and September (22/32; 68.7% of first rejections). This increase in first graft rejection episodes during the allergy season may result in promoting systemic Th 2 immune response to corneal graft donor alloantigens triggered by the common allergens in humans, such as pollen. Supporting these findings, a two times higher incidence of graft rejection was seen in atopic versus nonatopic patients, although the result was not statistically significant. We believe that this lack of statistical significance is due to the limited number of patients identified as nonatopic.

One of the secondary outcomes of this study was to analyze the difference between the groups with regard to time until first graft rejection episode. Our hypothesis was that the time to first graft rejection episode would be less in atopic patients because of the enhanced immunity caused by atopic conditions. However, we were unable to compare the groups because of the few graft rejection events in the nonatopic patients. We also thought characteristics of graft rejections would be different between the groups because of the enhanced immunity toward Th 2 cells in atopic patients. Conversely, our results indicated that nonatopic patients tend

to have more severe first rejection episodes than those in atopics.

We are aware of some weaknesses of this study. Although our original sample size calculation required data from 88 eyes and we included 106 eyes, the sample size was recalculated on the basis of an incidence rate of rejection of 7.3/100 person-years in the nonatopic group and a nonatopic rate of 15% (as found in this current study), and the result was 171 eyes. We believe higher numbers of patients could change the statistical analysis results. The diagnosis of atopy was based on the questionnaire, which may be subject to patient bias, and the study did not include any objective test such as skin sensitivity or serum IgE testing. However, Kim et al.²¹ reported that an elevation of serum IgE is usual but not always seen in atopic patients, and there is still no single objective laboratory test for the diagnosis of atopy. Moreover, the ISAAC questionnaire used in this study was previously found to be sensitive and specific for atopy.²² Another weakness of this study was that ocular information was obtained by retrospective chart review. However, the long mean follow-up time (19.7 years in nonatopics, 11.2 years in mild atopics, and 8.8 years in severe atopics) would be extremely difficult to achieve in a prospective study.

CONCLUSIONS

In conclusion, our results indicate that atopic conditions may increase the incidence of graft rejection in keratoconus patients who undergo penetrating keratoplasty. On the basis of

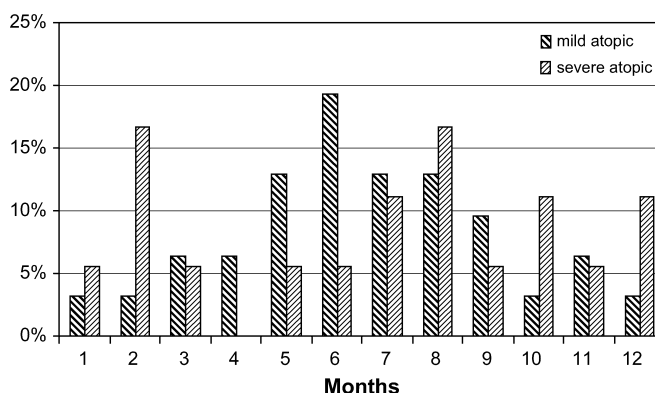


FIGURE 1. Distribution of first graft rejection episodes, by month (months 1–12 correspond to January–December).

TABLE 4. Association of Steroid Potency and Severity of First Rejection

Steroid Potency	Mild Rejection (Epithelial and/or SEI)	Moderate Rejection (KP $\pm$ SEI)	Severe Rejection (Stromal Edema)
No steroid	6	6	6
Low	7	6	5
Medium	5	3	1
High	8	1	2

P value for association (mixed-effects model): 0.054.

P value for difference of association by atopy group: 0.27.

our findings, we recommend treatment of allergy in keratoconus patients to try to decelerate the progression of keratoconus by preventing eye-rubbing and to perhaps reduce the incidence of graft rejection after penetrating keratoplasty. All graft patients need to be taught to seek immediate care for new symptoms that may indicate rejection. This is especially important for atopic patients who may attribute new symptoms to their allergies.

REFERENCES

- Wachtmeister L, Ingemansson SO, Moller E. Atopy and HLA antigens in patients with keratoconus. *Acta Ophthalmol.* 1982;60:113–122.
- Harrison RJ, Klouda PT, Easty DL, et al. Association between keratoconus and atopy. *Br J Ophthalmol.* 1989;73:816–822.
- Bawazeer AM, Hodge WG, Lorimer B. Atopy and keratoconus: a multivariate analysis. *Br J Ophthalmol.* 2000;84:834–836.
- Le Moine A, Flamand V, Demoor FX, et al. Critical roles for IL-4, IL-5, and eosinophils in chronic skin allograft rejection. *J Clin Invest.* 1999;103:1659–1667.
- VanBuskirk AM, Wakely ME, Orosz CG. Transfusion of polarized TH2-like cell populations into SCID mouse cardiac allograft recipients results in acute allograft rejection. *Transplantation.* 1996;62:229–238.
- Beauregard C, Stevens C, Mayhew E, et al. Cutting edge: atopy promotes Th2 responses to alloantigens and increases the incidence and tempo of corneal allograft rejection. *J Immunol.* 2005;174:6577–6581.
- Paglen PG, Fine M, Abbott RL, et al. The prognosis for keratoplasty in keratoconus. *Ophthalmology.* 1982;89:651–654.
- Ing JJ, Ing HH, Nelson LR, et al. Ten-year postoperative results of penetrating keratoplasty. *Ophthalmology.* 1998;105:1855–1865.
- Olson RJ, Pingree M, Ridges R, et al. Penetrating keratoplasty for keratoconus: a long term review of results and complications. *J Cataract Refractive Surg.* 2000;26:987–991.
- Sit M, Weisbrod DJ, Naor J, et al. Corneal graft outcome study. *Cornea.* 2001;20:129–133.
- Reinhard T, Moller M, Sundmacher R. Penetrating keratoplasty in patients with atopic dermatitis with and without systemic cyclosporin A. *Cornea.* 1999;18:645–651.
- Biino G, Rezzani C, Grassi M, et al. ECRHS screening questionnaire scoring: a methodological suggestion for asthma assessment. European Community Health Survey. *J Outcome Meas.* 2000–2001;4:740–762.
- Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J.* 1995;8:483–491.
- Rezende RA, Hammersmith K, Bisol T, et al. Comparative study of ocular herpes simplex virus in patients with and without self-reported atopy. *Am J Ophthalmol.* 2006;141:1120–1125.
- Rezende RA, Bisol T, Hammersmith K, et al. Efficacy of oral antiviral prophylaxis in preventing ocular herpes simplex virus recurrences in patients with and without self-reported atopy. *Am J Ophthalmol.* 2006;142:563–567.
- Epstein AJ, de Castro TN, Laibson PR, et al. Risk factors for the first episode of corneal graft rejection in keratoconus. *Cornea.* 2006;25:1005–1011.
- Rahi A, Davies P, Ruben M, et al. Keratoconus and coexisting atopic disease. *Br J Ophthalmol.* 1977;61:761–764.
- Lim I, Pesudovs K, Coster DJ. Penetrating keratoplasty for keratoconus: Visual outcomes and success. *Ophthalmology.* 2000;107:1125–1131.
- Brierly SC, Izquierdo L, Mannis MJ. Penetrating keratoplasty for keratoconus. *Cornea.* 2000;19:329–332.
- Sharif KW, Casey TA. Penetrating keratoplasty for keratoconus: complications and long term success. *Br J Ophthalmol.* 1991;75:142–146.
- Kim HJ, Honig PJ. Atopic dermatitis. *Curr Opin Pediatr.* 1998;10:387–392.
- Braun-Fahrlander C, Wuthrich B, Gassner M, et al. Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the school health services. SCARPOL Team: Swiss Study on Childhood Allergy and Respiratory Symptom with respect to Air Pollution and Climate; International Study of Asthma and Allergies in Childhood. *Pediatr Allergy Immunol.* 1997;8:75–82.