Published in final edited form as:

Cornea. 2016 July ; 35(7): 1008–1014. doi:10.1097/ICO.0000000000000893.

Tear Matrix Metalloproteinases and Myeloperoxidase Levels in Patients with Boston Keratoprosthesis Type I

Marie-Claude Robert, MD^{1,2,3}, Samer N Arafat, MD^{1,2}, Sandra Spurr-Michaud, MS¹, James Chodosh, MD MPH², Claes H Dohlman, MD PhD², and Ilene K Gipson, PhD¹

¹Schepens Eye Research Institute, Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, MA

²Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA

³Centre Hospitalier de l'Université de Montréal, Department of Ophthalmology, Université de Montréal, Montréal, Canada

Abstract

Purpose—To investigate the tear levels of matrix metalloproteinases (MMPs), myeloperoxidase (MPO) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in eyes after Boston keratoprosthesis type I (B-KPro) implantation and to correlate these markers with established B-KPro prognostic categories.

Methods—Tear washes were collected from forty patients (seven with autoimmune disease, two with chemical burn and 31 patients with other non-cicatrizing diagnoses). Tear MMPs, MPO and TIMP-1 levels were quantified using multianalyte bead-based enzyme-linked immunosorbent assays. Total MMP activity was determined using a fluorimetric assay. The analytes were compared to underlying diagnosis and other clinical factors.

Results—MMP-8, MMP-9 and MPO levels were markedly elevated in eyes with B-KPro (80 ± 31 pg/ μ g, 291 ± 77 pg/ μ g and 244 ± 33 pg/ μ g, respectively). Chemical burn was associated with significantly higher tear MMP-8 (474 ± 376 pg/ μ g) and MMP-9 levels (1300 ± 635 pg/ μ g) compared to non-cicatrizing diseases (MMP-8: 41 ± 15 pg/ μ g, $p = 0.02$ and MMP-9: 196 ± 57 pg/ μ g, $p = 0.02$) and higher MMP-9 levels compared to autoimmune diseases (MMP-8: 96 ± 65 pg/ μ g, $p = 0.21$ and MMP-9: 306 ± 196 pg/ μ g, $p = 0.04$). Similar analyte levels were observed in the B-KPro eye and contralateral non B-KPro eye of patients with bilateral diseases. MMP-8, MMP-9 and total MMP activity correlated strongly with each other.

Conclusions—In eyes with B-KPro, tear MMP-8 and MMP-9 levels appear related to the underlying ocular surface pathology and not significantly influenced by the presence of the prosthesis.

Keywords

Boston keratoprosthesis type 1; matrix metalloproteinase; chemical burn; Stevens-Johnson syndrome; keratolysis; tears

Introduction

The Boston Keratoprosthesis type I (B-KPro) can provide visual rehabilitation in patients with corneal blindness. Eyes with more severe ocular surface diseases, particularly cicatrizing diseases, have poorer outcomes after B-KPro. The increased need for postoperative repair procedures and the faster visual attrition rates observed in these disease categories form the basis for the well-recognized prognostic categories for B-KPro surgery.¹ Eyes with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have the poorest outcomes while eyes with mucous membrane pemphigoid and chemical burn have intermediate outcomes when compared to eyes with non-cicatrizing conditions. Recent case series continue to support this prognostic hierarchy.²⁻⁷ Corneal melting (sterile keratolysis) after B-KPro also occurs in up to 19% of eyes with non-cicatrizing diseases.² Corneal melting of the B-KPro carrier graft is a critically important cause of B-KPro failure as it may lead to periprosthetic leaks, B-KPro extrusion and several other sight- and globe-threatening complications.³

Matrix metalloproteinases (MMP) are enzymes responsible for the corneal collagen and extracellular matrix degradation seen in sterile corneal ulceration.⁴ A subset of these enzymes, neutrophil collagenase (MMP-8) and gelatinase-B (MMP-9), as well as myeloperoxidase (MPO), a microbicidal enzyme released during the respiratory burst of neutrophils, have been found in high levels in the tears of patients with SJS/TEN and mucous membrane pemphigoid.⁵ High tear MMP levels have also been found in several other conditions that may eventually necessitate B-KPro surgery.⁶⁻⁹ As such, the purpose of this study was to assess tear levels of MMP, MPO and tissue inhibitor of metalloproteinase-1 (TIMP-1) in eyes with prior B-KPro and to correlate these enzymes with the underlying diagnoses and B-KPro prognostic categories.

Materials and Methods

This cross-sectional study was approved by the Human Studies Committee at the Massachusetts Eye and Ear (MEE) and Schepens Eye Research Institute (SERI) in Boston, Massachusetts. The study procedures complied with the Health Insurance Portability and Accountability Act (HIPAA) and adhered to the tenets of the Declaration of Helsinki. Patients were informed of the nature and design of the study prior to recruitment and signed informed consent was obtained.

Study population

Study participants included patients with prior B-KPro implantation that were followed at the MEE cornea clinic. Subjects were deemed eligible for inclusion if they were above 18 years of age and had at least one month of stable postoperative follow-up after B-KPro

surgery. Subjects were excluded if they had undergone any intraocular surgery in the preceding month. Pregnancy and breastfeeding were additional exclusion criteria. Study participants were recruited and their tears were collected during their regular scheduled visits at the MEEI cornea clinic between January 2013 and May 2014. Additional tears were collected at subsequent clinic visits for a subset of participants, depending on the availability of the researchers. A complete ocular history and slit lamp examination were performed on the day of tear collection. Slit lamp photographic images were taken of eyes with active corneal melting. Medical records were reviewed to identify the participants' demographics, prior ocular diagnoses and surgeries as well as ocular and systemic medications. Patients were grouped by diagnostic category (autoimmune diseases, chemical burns, non-cicatrizing diseases) based on the classic prognostic hierarchy following B-KPro implantation.¹

Tear collection and processing

A tear wash method was chosen for collecting tear proteins because it allows harvest of tear fluid from patients with little tear production and increases protein recovery from the ocular surface to allow multiple analyte assays.¹⁰ Tear collection was performed in the afternoon (between 1 and 4 pm) to control for diurnal variation in MMP levels¹¹¹² and prior to any ocular surface manipulation. The tear wash method involved instillation of 60 μ L of sterile 0.9% saline solution onto the ocular surface using a sterile plastic micropipette. Participants were instructed to rotate their eyes without blinking, allowing the saline to contact different areas of the ocular surface and thus, collect more protein. The tear wash fluid was collected from the inferior fornix using a micropipette, transferred to a sterile polypropylene tube and kept on ice. All samples were centrifuged at 15 000 RPM (21 130 $\times$ g) at 4°C for 30 minutes to remove cellular and mucoid debris. Supernatants were recovered and stored at -80°C for further processing. The tear wash process was performed on both eyes and each eye was analyzed separately.

Given the variable volume yield using the tear wash method, quantification of analytes was normalized to protein content, determined using a Micro BCA Protein Assay Kit (Pierce, Rockford, IL), according to the manufacturer's instructions.

Analysis of matrix metalloproteinase, myeloperoxidase and tissue inhibitor of matrix metalloproteinase-1

Total (proform and active) tear levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 and MMP-12 content were analyzed simultaneously using the Fluorokine Human MMP MultiAnalyte Profiling Kit (R&D Systems, Minneapolis, MN). Tear MPO and TIMP-1 levels were quantified together using the Fluorokine Human Cardiac MultiAnalyte Profiling Kit (R&D Systems, Minneapolis, MN). Samples were processed following the manufacturer instructions and analyzed using a Bio-Rad Bio-Plex analyzer (Bio-Rad, Hercules, CA) powered by Luminex 100 xMAP technology (Luminex Corp., Austin, TX). Median fluorescence intensities were used to calculate the analyte concentrations from a standard curve (in picograms per milliliter) and standardized to micrograms of total protein loaded in the assay. Analyte levels are reported in picograms of analyte per microgram of total protein (pg/ μ g).

Analysis of total matrix metalloproteinase activity

A modified protocol of the MMP-9 Fluorometric Drug Discovery Kit (Enzo Life Sciences, Plymouth Meeting, PA) was used as previously described to quantify total MMP activity.⁵ Briefly, a quenched OmniMMP RED fluorogenic substrate (Enzo Life Sciences) was added to each diluted tear sample. Fluorescence was measured at 1-minute intervals for 45 minutes at 540 nm (excitation) and 590 nm (emission) using the Synergy Mx monochromator-based multimode microplate reader (Biotek U.S., Winooski, VT). The slope values were derived from the linear portions of the background and sample curves and total MMP activity was calculated as the sample slope minus the background slope (in relative fluorescence units per minute (RFU/min)) and standardized to microgram of protein loaded in the assay.

Statistical analysis

Statistical analysis was performed using Stata/SE 13.1 (StataCorp, College Station, TX). Results were reported as means $\pm$ standard errors of the mean. The non-parametric Mann-Whitney U and Kruskal-Wallis tests were used to compare analyte levels across diagnostic categories. The Wilcoxon matched-pairs signed-rank test was used to compare paired data between the two eyes of patients with bilateral disease and unilateral B-KPro as well as those of patients with bilateral B-KPro. Analyte levels were converted to logarithmic values for correlation analyses using the Spearman rank correlation test. Analysis of variance (ANOVA) was used to identify clinical parameters associated with MMP-8, MMP-9 and MPO levels and MMP activity. The regression model included patient age, gender, prognostic category, time elapsed since B-KPro surgery, the presence of a glaucoma drainage device, sterile keratolysis, retroprosthetic membrane or contact lens (CL) deposits, CL type and the use of topical medications (antibiotics, steroids, prostaglandin analogues and/or anti-glaucoma) and/or systemic medications (doxycycline, statins, angiotensin converting enzyme inhibitors (ACEi) and/or systemic immunomodulation therapy). Univariate and multivariate analyses were performed. A similar multivariate model was built to explore relationships between the different analyte levels and MMP activity. Statistical significance was determined when the two-tailed P values were below the 0.05 threshold.

Results

Study population

Forty patients participated in this study. The mean age was 65 ± 3 years (range: 26 to 97 years). Sixty-one percent of patients were male. Thirty-three patients had unilateral B-KPro while 7 patients had bilateral B-KPro. The underlying diagnoses leading to B-KPro surgery are outlined in Table 1. Twenty-eight patients (70%) had bilateral underlying corneal disease (twenty-one patients with unilateral B-KPro and seven patients with bilateral B-KPro).

The interval between B-KPro surgery and tear collection was 6.9 ± 0.5 years (range: 1 month to 15.6 years). At the time of tear collection, all B-KPro eyes were on topical antibiotic prophylaxis (B sulfate-trimethoprim, moxifloxacin and/or vancomycin 14 mg/mL with benzalkonium chloride). Fifty-three percent of eyes were on prednisolone acetate 1% used once to four times per day. Sixty-two percent of eyes were on topical glaucoma medication at an average of 1.5 medications per eye. Prostaglandin analogues were used in

28% of eyes. Other medications with potential impact on MMP levels included doxycycline (15%), statins (45%) and ACEi (36%).¹³¹⁴ Fifteen percent of eyes were on systemic immunomodulatory therapy.

Eighteen eyes (38%) had undergone prior glaucoma drainage device implantation. Nineteen eyes (83%) had a CL in place. Twenty-three percent of CL had visible deposits on the day of tear collection. Previous B-KPro related complications included retroprosthetic membrane in 27 eyes (57%) and sterile keratolysis (melt) in 13 eyes (28%). Four eyes (9%) had active melting at the time of tear collection.

Tear sampling

The tear wash method yielded an average of 39 ± 2 μ L of fluid from B-KPro eyes and 37 ± 2 μ L of fluid from non B-KPro eyes ($p = 0.89$). The average protein yield was 44.6 ± 6.2 μ g in B-KPro eyes and 40.3 ± 5.2 μ g in non B-KPro eyes, respectively ($p = 0.61$).

Matrix metalloproteinases, myeloperoxidase and tissue inhibitor of metalloproteinase-1 levels

Mean tear levels of MMPs, MPO and TIMP-1 from eyes with B-KPro are presented in Figure 1A. Low levels of MMP-1 (0.3 ± 0.1 pg/ μ g), MMP-2 (3 ± 1 pg/ μ g), MMP-3 (12 ± 3 pg/ μ g), MMP-7 (34 ± 5 pg/ μ g), MMP-12 (0 ± 0 pg/ μ g) and TIMP-1 (32 ± 2 pg/ μ g) were detected. Conversely, MMP-8 (80 ± 31 pg/ μ g), MMP-9 (291 ± 77 pg/ μ g) and MPO (244 ± 33 pg/ μ g) levels were markedly elevated.

Significant differences were noted in the levels of MMP-2, MMP-8 and MMP-9 when comparing the different prognostic categories. MMP-2 levels in eyes with autoimmune diseases were 5-fold higher than eyes with non-cicatrizing diseases ($p = 0.02$). MMP-8 and MMP-9 levels were 12- and 7-fold higher, respectively, in eyes with chemical burn compared to eyes with non-cicatrizing diseases ($p = 0.02$). MMP-9 was 4-fold higher in eyes with chemical burn than in eyes with autoimmune disease ($p = 0.04$).

With respect to specific diagnoses across the B-KPro cohort, the highest levels of MMP-8, MMP-9 and MPO were seen in B-KPro eyes with chemical burns (474 ± 376 pg/ μ g, 1300 ± 635 pg/ μ g and 393 ± 19 pg/ μ g, respectively; 3 eyes), topical anesthetic abuse (360 pg/ μ g, 1406 pg/ μ g and 554 pg/ μ g; 1 eye), neurotrophic/exposure keratopathy (163 ± 81 pg/ μ g, 549 ± 217 pg/ μ g and 304 ± 80 pg/ μ g; 3 eyes) and SJS/TEN (141 ± 116 pg/ μ g, 426 ± 356 pg/ μ g and 366 ± 177 pg/ μ g; 4 eyes). The lowest levels of MMP-8, MMP-9 and MPO were seen in eyes with keratoconus (3 ± 3 pg/ μ g, 27 ± 26 pg/ μ g and 21 ± 20 pg/ μ g; 4 eyes), uveitis-induced corneal edema (0 ± 0 pg/ μ g, 7 ± 2 pg/ μ g and 47 ± 21 pg/ μ g; 2 eyes), mechanical trauma (5 pg/ μ g, 31 pg/ μ g and 74 pg/ μ g; 1 eye) and aniridia (6 ± 0 pg/ μ g, 19 ± 5 pg/ μ g and 165 ± 72 pg/ μ g; 2 eyes).

Total matrix metalloproteinase activity

Total MMP activity is shown in Figure 1B. There were no statistically significant differences between the classic prognostic categories ($p = 0.20$). The specific diagnoses with the highest total MMP activity were the same as those that exhibited markedly elevated levels of

MMP-8, MMP-9 and MPO: chemical burns (381 ± 267 RFU/min/ μ g; 3 eyes), topical anesthetic abuse (495 RFU/min/ μ g; 1 eye), neurotrophic/exposure keratopathy (244 ± 16 RFU/min/ μ g; 3 eyes) and SJS/TEN (103 ± 71 RFU/min/ μ g; 3 eyes). Those with the lowest total MMP activity were atopy (0.8 ± 0.8 RFU/min/ μ g; 3 eyes), aniridia (0.6 ± 0.6 RFU/min/ μ g; 2 eyes) and keratoconus (3.2 ± 1.8 RFU/min/ μ g; 4 eyes).

Correlation between tear levels of MMP-8, MMP-9 and MPO

Figure 2 depicts the correlation between MMP-8, MMP-9 and MPO levels in eyes with B-KPro for all three diagnostic categories. MMP-8 and MMP-9 were highly correlated across all eyes with B-KPro regardless of the diagnostic category ($r = 0.95$, $p < 0.001$). Strong and statistically significant correlations were also found for each of the diagnostic groups. Strong and statistically significant positive correlation was found between MMP-9 and MPO and between MMP-8 and MPO across all B-KPro eyes and in eyes with non-cicatrizating diseases. With smaller samples sizes failing to reach statistical significance, eyes with autoimmune diseases or chemical burn showed only minimal to moderate correlation between MMP-9 and MPO as well as between MMP-8 and MPO.

Regression analysis

Univariate analysis did not identify any clinical factor with a statistically significant association with MMP-8, MMP-9 and MPO levels or MMP activity. Using multivariate analysis, the clinical factors explained only a small fraction of the analyte levels: MMP-8 ($R^2 = 0.47$, $p = 0.67$), MMP-9 ($R^2 = 0.55$, $p = 0.46$), MPO ($R^2 = 0.68$, $p = 0.08$) and MMP activity ($R^2 = 0.46$, $p = 0.70$). The multivariate model identified a significant association between prognostic category and MMP-8 level ($p = 0.05$). Interestingly, a past or current history of sterile keratolysis was not associated with higher MMP levels.

Multivariate analysis including the levels of each analyte and MMP activity had a better predictive value: MMP-8 ($R^2 = 0.98$, $p < 0.001$), MMP-9 ($R^2 = 0.97$, $p < 0.001$), MPO ($R^2 = 0.45$, $p = 0.34$) and MMP activity ($R^2 = 0.91$, $p < 0.001$). Significant association was seen between MMP-8, MMP-9 levels and total MMP activity. Multivariate models including only these three variables had R^2 values between 0.86 and 0.89 ($p < 0.001$). Finally, MMP-8, MMP-9 and total MMP activity did not show statistically significant association with MPO ($p = 0.918$, $p = 0.571$ and $p = 0.851$, respectively).

Bilateral disease

The analyte levels and total MMP activity in the tears of patients with bilateral disease and unilateral B-KPro are shown in Figure 3. Paired data was available for eyes with SJS/TEN (4 eyes), chemical burn (1 eye), vernal keratoconjunctivitis (1 eye), corneal edema (5 eyes), post-infectious scarring (2 eyes), keratoconus (2 eyes) and thermal burn (1 eye). Of these, the contralateral non B-KPro eyes had either no prior corneal surgery (8 eyes), phthisis (4 eyes) or corneal transplantation (4 eyes). MMP-1 levels were 2-fold higher in eyes with B-KPro (0.25 ± 0.06 pg/ μ g total protein versus 0.11 ± 0.02 pg/ μ g total protein, $p=0.04$). Interestingly, the other MMP levels were not significantly different between the B-KPro and contralateral non B-KPro eye. Both MMP-8 and MMP-9 tended to be higher in the non B-

KPro eye. Furthermore, there was no significant difference in analyte levels and total MMP activity between eyes of patients with bilateral disease and bilateral B-KPro (N=7).

Repeat tear sampling analysis

Tear collection was performed twice in 17 B-KPro eyes. The interval between repeat tear collections was 261 ± 35 days. Repeat tear collection and sample analyses were performed on 10 non B-KPro eyes. The interval between tear collections was 297 ± 41 days. There was no statistically significant difference between repeat measurements of MMP-8, MMP-9, MMP-12, MPO and TIMP-1 in either group.

Clinical correlation with sterile keratolysis

While MMP-8 levels correlated with the prognostic category for B-KPro, no other clinical parameter could be clearly associated with tear MMP levels, including the presence of active corneal melting. Figure 4 illustrates the slit lamp appearance and the variability of MMP-8 and MMP-9 levels of three eyes with active corneal melting. In one patient with aniridia, analyte levels in the setting of extensive, globe-threatening corneal melt (Figure 4B) were comparable to those obtained one year after repeat B-KPro surgery, in the absence of any clinical or optical coherence tomography evidence of melt (MMP-8: 64 pg/ μ g, MMP-9: 434 pg/ μ g and MPO: 2623 pg/ μ g).¹⁵

Discussion

This study is the first to report the MMP, MPO, TIMP-1 and total MMP activity levels in the tears of patients with B-KPro. We identified a correlation between MMP-8 level and the prognostic categories for B-KPro surgery as well the presence of particularly high levels of MMP-8 and MMP-9 in B-KPro eyes with chemical burn. MMP levels in tears may be more closely associated with the underlying diagnosis of ocular surface disease than with the presence of a B-KPro. Furthermore, the presence of clinically significant corneal melt did not clearly correlate with higher MMP levels.

Yaghouti and coworkers established a well-defined prognostic hierarchy for B-KPro.¹ Indeed, it is commonly held that severe and ongoing ocular surface inflammation and the associated overexpression of mediators including MMPs lead to an increased vulnerability to corneal melt.¹⁶ Our study identified that, contrary to the above prognostic hierarchy where autoimmune eyes fare the worse, B-KPro eyes with chemical burns had the highest tear levels of MMP-8 and MMP-9. This difference may be explained by the small sample sizes for the individual diagnostic categories and by the use of systemic immunosuppression in five of the seven patients with autoimmune disease.

Specific diagnoses with high tear levels of MMP-8 and MMP-9 included disease with a tendency for ocular surface breakdown. The absence of an intact epithelial barrier predispose the exposed underlying stroma to ulceration by MMP dysregulation and their excessive release from inflammatory cells. Conversely, pathologies that tend to spare the ocular surface epithelium had the lowest levels of MMP-8 and MMP-9. Interestingly, the one patient with aniridia had low tear MMP levels, despite the presence of limbal stem cell deficiency and significant corneal melting in one eye (Figure 4B). It has been postulated that

the corneal opacification that arises from repeated epithelial erosions and ulcerations in aniridia may be caused by a deficiency in MMP-9 that results in fibrin accumulation and influx of inflammatory cells into the wound bed via chemotactic stimulus and blood vessel extravasation into the stroma.¹⁷ Therefore, it is possible that corneal melting may be the result of collagenolytic enzymes released primarily from stroma-infiltrating inflammatory cells, which are undetectable through tear analysis.

Arafat and colleagues previously reported an association between tear levels of MMP-8, MMP-9 and MPO.⁵ Similarly, we demonstrated a strong correlation between tear levels of MMP-8 and MMP-9 across all diagnostic categories while correlation with MPO was moderate. MMP-8 and MMP-9 levels were also correlated with total MMP activity. MPO, while being responsible for MMP activation through the formation of reactive oxygen species, was not associated with total MMP activity.¹⁸ The relationship between MMP-8 and MPO, which are both produced by neutrophils, and MMP-9, which may be produced by both epithelium and inflammatory cells, suggest that neutrophils are the predominant and common source of these enzymes.⁴¹⁹ This supports prior studies demonstrating that MMPs accumulated in tears were produced by granulocytes.²⁰ Indeed, neutrophils migrate from the limbal vasculature toward the sites of epithelial defect.²¹ As degranulation products of these activated neutrophils, MMP-8, MMP-9 and MPO are thus likely to accumulate in the tear film and be recovered using the tear wash method. In contrast, epithelial production of MMP-9 is much more localized and found at the front of epithelium migrating to cover the wound bed.²²

After B-KPro implantation, a “free” epithelial edge generally persists along the periphery of the front plate. This epithelium may either grow above or below the surface of the B-KPro front plate.²³ The potential impact of these peri-prosthetic epithelial growth patterns on wound healing mechanisms and MMP-9 expression remain unknown. On the other hand, the presence of stromal inflammation appears to be increased in the presence of a keratoprosthesis. Indeed, implantation of a miniature keratoprosthesis (m-KPro) in the murine model has shown increased expression of tumor necrosis factor α (TNF- α) and interleukin-1 β (IL-1 β) as well as increased graft-infiltrating immune cells.²⁴

MMP, MPO and TIMP-1 levels were similar between eyes of patients with bilateral disease and unilateral B-KPro suggesting that these analytes are more influenced by the underlying disease than by the presence of a B-KPro. While the risk of corneal melt after B-KPro is tightly associated with the underlying diagnosis, the relationship between tear MMP level and the presence of corneal melt remains unclear. The variability of MMP levels in the setting of clinically significant melt as well as the unchanged analyte profile in a patient in the presence and absence of melt may be explained by local differences in MMP levels. For example, tear MMP levels measured using the tear wash method may be independent from the MMP levels found in the stroma and along the B-KPro stem, where corneal melting occurs. Furthermore, our study did not account for occult corneal melting that can be visible on anterior segment optical coherence tomography but is undetectable at the slit lamp.

The low tear volume and low tear protein recovery are intrinsic limitations of tear collection methods. The tear wash method achieves higher total protein yield when compared to

capillary tear collection.¹⁰ Schirmer strips may also be used to collect tears but collects cell protein and unwanted debris from the conjunctival surface in addition to tears.²⁵ As such, Schirmer strips may not be a valid method of profiling analytes in tears. Further, tears may have a different profile of mediators when compared to the microenvironment of melting corneas for several reasons, including the presence of a protective contact lens and of the B-KPro itself.

In conclusion, eyes with B-KPro may have constitutively high tear levels of MMP-8, MMP-9 and MPO even years after the onset of disease and the B-KPro implantation. These analyte levels correlated more with an underlying diagnosis of ocular surface disease than with the presence of a B-KPro. High tear MMP levels did not clearly associate with sterile keratolysis. Further studies are needed to improve our understanding of the pathophysiology of B-KPro-related melt and expand our armamentarium. This knowledge will be crucial in the development of targeted MMP inhibitors to protect high-risk B-KPro eyes from the devastating consequences of corneal melting.

Acknowledgments

Conflict of Interest: The authors, Robert, Arafat, Chodosh and Dohlman, are full-time academic employees of the Massachusetts Eye and Ear, the manufacturer of the Boston keratoprosthesis.

Financial support: This project was funded by the Boston Keratoprosthesis Foundation, the Centre Hospitalier de l'Université de Montréal Fellowship Fund (MCR) and NIH NEI grant EY 03306 (IKG).

References

1. Yaghouti F, Nouri M, Abad JC, et al. Keratoprosthesis: preoperative prognostic categories. *Cornea*. 2001; 20:19–23. [PubMed: 11188997]
2. Chang H, Luo Z, Chodosh J, et al. Primary implantation of type I Boston keratoprosthesis in nonautoimmune corneal disease. *Cornea*. 2015; 34:264–270. [PubMed: 25611395]
3. Ciolino JB, Belin M, Todani A, et al. Retention of the Boston keratoprosthesis type 1: multicenter study results. *Ophthalmology*. 2013; 120:1195–1200. [PubMed: 23499061]
4. Fini ME, Cook JR, Mohan R. Proteolytic mechanisms in corneal ulceration and repair. *Arch Dermatol Res*. 1998; 290:S12–S23. [PubMed: 9710379]
5. Arafat S, Suelves A, Spurr-Michaud S, et al. Neutrophil collagenase, gelatinase, and myeloperoxidase in tears of patients with Stevens-Johnson syndrome and ocular cicatricial pemphigoid. *Ophthalmology*. 2014; 121:79–87. [PubMed: 23962653]
6. Määttä M, Kari O, Tervahartiala T, et al. Elevated expression and activation of matrix metalloproteinase 8 in tear fluid in atopic blepharoconjunctivitis. *Cornea*. 2008; 27:297–301. [PubMed: 18362656]
7. Sakimoto T, Ohnishi T, Ishimori A. Simultaneous study of matrix metalloproteinases, proinflammatory cytokines, and soluble cytokine receptors in the tears of noninfectious corneal ulcer patients. *Graefes Arch Clin Exp Ophthalmol*. 2014; 252:1451–1456. [PubMed: 25005620]
8. Rohini G, Murugeswari P, Prajna N, et al. Matrix metalloproteinases (MMP-8, MMP-9) and the tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2) in patients with fungal keratitis. *Cornea*. 2007; 26:207–211. [PubMed: 17251814]
9. Barro C, Romanet J, Fdili A, et al. Gelatinase concentration in tears of corneal-grafted patients. *Curr Eye Res*. 1998; 17:174–182. [PubMed: 9523096]
10. Gipson IK, Spurr-Michaud SJ, Senchyna M, et al. Comparison of Mucin Levels at the Ocular Surface of Postmenopausal Women With and Without a History of Dry Eye. *Cornea*. 2011; 30:1346–1352. [PubMed: 22089171]

11. Markoulli M, Papas E, Cole N, et al. The diurnal variation of matrix metalloproteinase-9 and its associated factors in human tears. *Invest Ophthalmol Vis Sci.* 2012; 53:1479–1484. [PubMed: 22323468]
12. Sack R, Sathe S, Beaton A, et al. Changes in the diurnal pattern of the distribution of gelatinases and associated proteins in normal and pathological tear fluids: evidence that the PMN cell is a major source of MMP activity in tear fluid. *Adv Exp Med Biol.* 2002; 506:539–545. [PubMed: 12613958]
13. Li D, Lokeshwar B, Solomon A, et al. Regulation of MMP-9 production by human corneal epithelial cells. *Exp Eye Res.* 2001; 73:449–459. [PubMed: 11825017]
14. Grothusen C, Bley S, Selle T, et al. Combined effects of HMG-CoA-reductase inhibition and renin-angiotensin system blockade on experimental atherosclerosis. *Atherosclerosis.* 2005; 182:57–69. [PubMed: 16115475]
15. Robert M, Arafat S, Ciolino J. Collagen cross-linking of the Boston keratoprosthesis donor carrier to prevent corneal melt in high-risk patients. *Eye Contact Lens.* 2014; 40:376–381. [PubMed: 25320957]
16. Robert M, Dohlman C. A review of corneal melting after Boston Keratoprosthesis. *Semin Ophthalmol.* 2014; 29:349–357. [PubMed: 25325860]
17. Mohan R, Chintala S, Jung J, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. *J Biol Chem.* 2002; 277:2065–2072. [PubMed: 11689563]
18. Henderson B, Tyagi S. Oxidative mechanism and homeostasis of proteinase/antiproteinase in congestive heart failure. *J Mol Cell Cardiol.* 2006; 41:959–962. [PubMed: 17046785]
19. Gordon G, Ledee D, Feuer W, et al. Cytokines and signaling pathways regulating matrix metalloproteinase-9 (MMP-9) expression in corneal epithelial cells. *J Cell Physiology.* 2009; 221:402–411.
20. Smith V, Rishmawi H, Hussein H, et al. Tear film MMP accumulation and corneal disease. *Br J Ophthalmol.* 2001; 85:147–153. [PubMed: 11159476]
21. Wilson S, Mohan R, Mohan R, et al. The corneal wound healing response: cytokine-mediated interaction of the epithelium, stroma and inflammatory cells. *Prog Retin Eye Res.* 2001; 20:625–637. [PubMed: 11470453]
22. Matsubara M, Zieske J, Fini M. Mechanism of basement membrane dissolution preceding corneal ulceration. *Invest Ophthalmol Vis Sci.* 1991; 32(13):3221–3237. [PubMed: 1660857]
23. Shapiro BL, Cortés DE, Chin EK, et al. High-resolution spectral domain anterior segment optical coherence tomography in type 1 Boston keratoprosthesis. *Cornea.* 2013; 32:951–955. [PubMed: 23591146]
24. Crnej A, Omoto M, Dohlman T, et al. Corneal inflammation after miniature keratoprosthesis implantation. *Invest Ophthalmol Vis Sci.* 2014; 56:185–189. [PubMed: 25515579]
25. VanDerMeid K, Su S, Krenzer K, et al. A method to extract cytokines and matrix metalloproteinases from Schirmer strips and analyze using Luminex. *Mol Vis.* 2011; 17:1056–1063. [PubMed: 21552500]

Figure 1.

Bar graph showing the means of A) matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, myeloperoxidase (MPO) and tissue inhibitor of MMP-1 (TIMP-1) and B) total MMP activity in the tears of eyes with Boston keratoprosthesis type I (B-KPro). The eyes are grouped according to the established prognostic hierarchy based on underlying diagnosis leading to corneal blindness and B-KPro implantation. All values were standardized to total protein: A) picograms per microgram of total protein (pg/ μ g) and B) relative fluorescent units per minute per microgram of total protein (RFU/min/ μ g). Levels were compared across all 3 diagnostic groups using the Kruskal-Wallis test ({}) while group pairs were compared using the Mann-Whitney U test (I).

* $P < 0.05$; Error bars represent the standard errors of the mean.

Figure 2.

Scatter graphs illustrating the association between A) matrix metalloproteinase (MMP)-8 and MMP-9; B) MMP-9 and myeloperoxidase (MPO) and C) MMP-8 and MPO. The Spearman rank correlation coefficient (r_s) and p value for all eyes is shown in the upper left corner of each graph while values for each diagnostic category are shown down and to the right ((autoimmune disease (◆); chemical burn (■); non-cicatrizing disease (○)).

Figure 3.

Bar graph showing the means of A) matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, myeloperoxidase (MPO) and tissue inhibitor of metalloproteinase-1 (TIMP-1) and B) total MMP activity in the tears of patients with bilateral disease but unilateral Boston keratoprosthesis type I (B-KPro). All values were standardized to total protein: A) picograms per microgram of total protein (pg/ μ g) and B) relative fluorescent units per minute per microgram of total protein (RFU/min/ μ g). Levels were compared between paired eyes of the same patient using the Wilcoxon matched-pairs signed-rank test. * $P < 0.05$; Error bars represent the standard errors of the mean.

Figure 4.

Slit lamp photographs of eyes with Boston keratoprosthesis type I (B-KPro) and clinically detectable melt on the day of tear collection. The tear level of matrix metalloproteinase (MMP) -8, MMP-9 and myeloperoxidase (MPO) are shown. A) A 43-year old male with Stevens Johnson syndrome (SJS) developed active melting by postoperative 1 month when tear collection was performed. The MMP-9 level was above the detection limit of the assay (at least 1 489 pg/μg total protein). B) A 62-year old male with aniridia and bilateral B-KPro developed corneal melting with extensive exposure of the back plate on the day of first tear collection. Shortly after, the patient underwent repeat B-KPro. Tear collection was performed one year later when the patient had a stable ocular surface with no sign of melt. Tear MMP-8, MMP-9 and MPO levels, at 3, 22 and 131 pg/μg total protein, were very similar to those seen in the presence of active melt. C) A 75-year old female with SJS had mild thinning superiorly and contact lens deposits on the day of tear collection.

Table 1

Preoperative diagnoses leading to corneal blindness and Boston Keratoprosthesis Type 1 surgery, organized by prognostic category.

Preoperative Diagnosis	Total Patients N (%)	Total Eyes N (%)
Autoimmune disease	7 (17.5)	8 (17.0)
SJS/TEN	4	4
Atopic/vernal keratoconjunctivitis	3	4
Chemical burns	2 (5.0)	3 (6.4)
Non-cicatrizing diseases	31 (77.5)	36 (76.6)
Corneal edema (PCE, ACE, FECD, CHED, uveitic)	11	13
Post-infectious scarring		
Bacterial (includes syphilis, trachoma)	3	3
Herpetic	4	4
Keratoconus	4	4
Neurotrophic/exposure keratopathy	2	3
Thermal Burn	2	2
Aniridia	1	2
Salzmann nodular degeneration	1	2
Mechanical trauma	1	1
Topical anesthetic abuse	1	1
Mooren's ulcer	1	1

SJS/TEN - Stevens Johnson syndrome/toxic epidermal necrolysis, PCE – pseudophakic corneal edema, ACE – aphakic corneal edema, FECD – Fuchs endothelial corneal dystrophy, CHED – congenital hereditary endothelial dystrophy.