Subtarsal Fibrosis Is Associated With Ocular Surface Epitheliopathy in Graft-Versus-Host Disease

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• PURPOSE: To evaluate occurrence of subtarsal fibrosis in patients with graft-vs-host disease (GVHD) and to determine its association with ocular surface epitheliopathy.
• DESIGN: Cross-sectional study.
• METHODS: We enrolled 40 patients with moderate or severe dry eye disease, including 20 patients with chronic ocular GVHD and 20 patients without (as the control group). All patients had a comprehensive ophthalmic assessment including evaluation for subtarsal fibrosis, corneal and conjunctival staining, tear break-up time (TBUT), and Schirmer test. Furthermore, meibomian gland drop-out area and densities of epithelial and stromal immune cells were measured using meibography and in vivo confocal microscopy, respectively.
• RESULTS: Subtarsal fibrosis was not seen in any eye of the non-GVHD group. However, 16 eyes (40%) of 10 patients (50%) in the GVHD group had subtarsal fibrosis ($P < .001$) with an average involvement of 28.9% ± 13.7% of the tarsal area. Fibrosis was more frequent in the upper lids (35%) than in the lower lids (5%). Regression analyses showed that corneal fluorescein staining was significantly associated with the extent of fibrosis ($P < .001$, $\beta = 0.14$) and TBUT ($P < .001$, $\beta = -0.53$) but not with other clinical or imaging parameters. Conjunctival lissamine green staining also had a statistically significant association with the extent of fibrosis ($P = .04$, $\beta = 0.12$) but not other clinical or imaging parameters. Eyes with subtarsal fibrosis had a more severe ocular surface epitheliopathy compared with eyes without fibrosis.
• CONCLUSIONS: Subtarsal fibrosis is present in a significant percentage of patients with chronic ocular GVHD, likely contributing to the ocular surface damage in these patients. (Am J Ophthalmol 2018;189:102–110.

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ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSplantation (HSCT) is often followed by graft-vs-host disease (GVHD), which is a significant cause of morbidity and mortality in these patients. This complication, which occurs in 30%–70% of patients after HSCT, is caused by recognition of recipient antigens as foreign by donor cells.[1–3] This results in inflammatory involvement of many organ systems in the body, including the skin, the mouth, and the gastrointestinal and respiratory tracts, with variable clinical features resembling autoimmune disorders.[1–3]

Ocular involvement occurs in a majority of patients with chronic GVHD; it has been reported that 60%–90% of patients with GVHD may have involvement of the eye.[4–6] Ocular manifestations of chronic GVHD include dry eye disease, meibomian gland dysfunction, corneal epitheliopathy, ocular surface inflammation, cataract, eyelid inflammation and scarring, uveitis, scleritis, retinal microvasculopathy, and infectious retinitis. Among these, the most common form of ocular involvement is dry eye disease, which develops in 40%–76% of patients.[4–7] Another finding in ocular GVHD is conjunctival fibrosis. In addition to symblepharon formation and fornix shortening, chronic ocular GVHD may be associated with subtarsal fibrosis.[8–12] Although subtarsal fibrosis has been reported in patients with GVHD,[8–12] it remains unknown how common this manifestation is. Moreover, any association between such fibrosis and ocular surface damage in these patients has not been evaluated.

As it has been shown that a significant correlation exists between corneal complications and lid/tarsal scarring in patients with Stevens-Johnson syndrome,[13] we hypothesized that such correlation may also be present in ocular GVHD. Therefore, this study was designed to evaluate the occurrence of subtarsal fibrosis in patients with chronic GVHD and to investigate the associations of various clinical and imaging parameters, including subtarsal fibrosis, with ocular surface epitheliopathy.

METHODS

IN THIS CROSS-SECTIONAL STUDY, WE ENROLLED 40 PAtients with dry eye disease, including 20 patients with chronic ocular GVHD (the GVHD group) and 20 patients

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with dry eye disease without GVHD (the control group). Patients were recruited from the Cornea Clinic at Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. The protocol of the study was approved by the institutional Human Studies Committee and the study was in adherence to the tenets of the Declaration of Helsinki and the requirements of the Health Insurance Portability and Accountability Act. All patients provided written informed consent before participation in the study.

We enrolled adult patients who had dry eye disease as defined by an Ocular Surface Disease Index (OSDI) > 22 plus (1) a tear break-up time < 10 seconds and/or (2) a Schirmer test with anesthesia < 10 mm in both eyes. In the GVHD group, all patients had chronic ocular GVHD as defined by the International Chronic Ocular GVHD Consensus Group criteria.[14] For both groups, we excluded patients with any conjunctival cicatricial disease, including chemical or thermal burns, Stevens-Johnson syndrome, or mucous membrane pemphigoid. Additional exclusion criteria consisted of active ocular allergies; contact lens wear or Prosthetic Replacement of the Ocular Surface Ecosystem (PROSE) within 1 month; any intraocular surgery within 3 months; or any ocular infection within 1 month prior to enrollment.

To investigate the possible associations of various clinical (including subtarsal fibrosis) and imaging parameters with ocular surface damage, as evaluated by corneal and conjunctival epitheliopathy, we performed a comprehensive ocular evaluation as detailed below.

• **CLINICAL AND IMAGING EVALUATION:** *Symptoms and Signs*. To assess symptoms and signs of dry eye disease, all patients had an OSDI questionnaire, corneal fluorescein staining (National Eye Institute [NEI] grading, 0–15),[15] conjunctival lissamine green staining (NEI grading, 0–18),[15] TBUT, and Schirmer test with anesthesia.

Slit-Lamp Photography. To evaluate lid margin telangiectasia, tarsal conjunctival hyperemia, and subtarsal fibrosis, all patients underwent slit-lamp photography of the eyelid margin and tarsal conjunctiva of both upper and lower lids of both eyes. The room illumination and the setting for photography were kept the same for all patients. Furthermore, for tarsal conjunctival photography, the lids were everted at the posterior tarsal borders in all patients for maximal exposure of the tarsal area.

Meibography. To assess the extent of meibomian gland drop-out, meibography was performed on the upper lids of both eyes. For this, the diode red light of an optical coherence tomography (OCT) machine (RTVue-100; OptoVue, Fremont, California, USA) was employed as previously described.[16] For imaging, the upper lid was everted along the superior tarsal border to expose the tarsal conjunctiva. While the room light was dimmed, the position of the diode red light relative to the eye was adjusted to obtain a clear infrared image of the meibomian glands as seen on the OCT monitor. Then, the midtarsal area was scanned using the Line mode of OCT. The infrared images of the meibomian glands were used for analysis as detailed below.

In Vivo Confocal Microscopy. In vivo confocal microscopy (IVCM) was performed on the tarsal conjunctiva of the upper lids of both eyes to measure the density of immune cells in the conjunctival epithelium and stroma. For this, a laser-scanning IVCM (Heidelberg Retina Tomograph 3 with the Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany) was used. The technique of IVCM imaging for the tarsal conjunctiva has been detailed before.[17] In summary, after topical anesthesia, we applied hypromellose 0.3% lubricant gel (GenTeal gel; Alcon/Novartis, Fort Worth, Texas, USA) to the eye and then the upper lid was everted. The Cornea Module was then manually advanced to touch the tarsal conjunctiva. Using the Sequence Mode, which acquires 100 images per sequence (with a rate of 3 frames/second), multiple locations in the central part of the tarsal conjunctiva were scanned. A total of 2–3 Sequence scans were obtained for each eyelid. Images were acquired at different depths (0–200 μm) to have both epithelium and stroma (substantia propria) of the conjunctiva.

• **IMAGE ANALYSIS:** Two masked observers performed all image analyses in this study.

Slit-Lamp Photography. Lid margin telangiectasia was graded as none (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). Tarsal conjunctival hyperemia was also scored as none (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). Grading was performed separately for each eyelid, and the averaged values of both lids of each eye were used for the analysis. Furthermore, presence or absence of subtarsal fibrosis was evaluated in the slit-lamp photographs of the tarsal conjunctiva of upper and lower lids. Subtarsal fibrosis was defined as presence of white-colored tissue under the conjunctiva. If fibrosis was present, the ImageJ software (https://imagej.nih.gov/ij/) was used to measure the percentage of visible tarsal conjunctiva in the image that was involved by fibrosis. For this, similar to what has been previously described for meibography (see below), the Polygonal Selection tool was first used to measure the total tarsal conjunctival area in pixels. Then, the same tool was employed to measure the area of fibrosis. The percentage of involvement was then calculated (Figure 1, Top row).

Optical Coherence Tomography Meibography. The infrared images of meibomian glands were analyzed using ImageJ software. From each OCT image of the everted