

# **Tear Film Cytokine Metalloproteinase-9 (MMP-9) as a Biomarker for Chronic-Graft-Versus-Host Disease (GVHD)**

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# Introduction

- Allogeneic **H**ematological **S**tem **C**ell Transplantation (allo-HSCT) from HLA matched donors → established and potentially curative form of treatment for both malignant and benign hematologic diseases
- However, associated with significant morbidity and mortality, including **GVHD** in **25 to 70% of transplant recipients**

**GVHD** → *Immunocompetent donor T-cells recognize major and/or **minor histocompatibility antigens** present in recipient tissue*

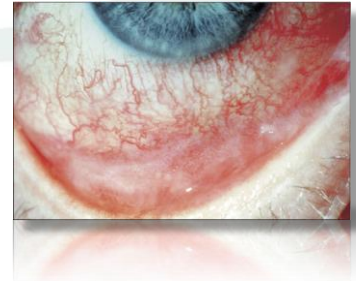
- Inflammation and destruction, most commonly of the skin, liver, gastrointestinal tract and **eyes**

# Introduction - Ocular GVHD (oGVHD)

**Ocular complications** occurs in **60-90%** of **transplant recipients**

Skin and mouth involvement → **risk factors for oGVHD**

- **Dry eye** is the most common ocular manifestation along with chronic blepharitis with **meibomian gland dysfunction**
- Cicatricial conjunctival changes
- Punctate keratopathy, filaments
- Sterile corneal necrosis / perforation



*Can occur in the absence of systemic GVHD*

# Introduction – Dry Eye Syndrome

- **Dry eye** → “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and **inflammation** of the ocular surface” The Definition and Classification of Dry Eye Disease: Report of the Definition and Classification Subcommittee of the International Dry Eye Work Shop (2007). Ocul Surf. 2007 Apr;5(2):93-107
- **Ocular surface inflammation** is both a **marker for dry eye** and is also **involved in its pathophysiology**
- **Matrix Metalloproteinase-9 (MMP-9)** is one of the main mediators identified in the inflammatory cascade associated with dry eye

# Purpose

- To investigate the presence of **tear film cytokine matrix metalloproteinase-9 (MMP-9)** and other ocular surface parameters in patients with **Graft-Versus-Host Disease (GVHD)** as compared to Dry Eye (DE) controls

# Methods

- **242 patients recruited at Bascom Palmer Eye Institute**
- For each patient, demographic information, past ocular and medical history, and topical and systemic medication information was collected
- A comprehensive ophthalmic examination was performed on all patients, including visual acuity, tear osmolarity, MMP-9 testing, tear film break-up time (TFBUT), corneal and conjunctival staining, Schirmer's test, lid margin staining and tear film interferometry

The control group was compromised of patients with DES but without autoimmune disease

# Methods – Staining / MMP9

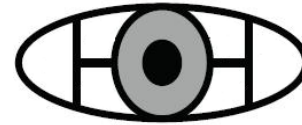
## Corneal Fluorescein Staining

- 10 ul 1 % Fluorescein
- Scored from 0-3 (per quad) at 2'



## Conjunctival Staining

- 15 ul 1% Lissamine Green
- Each sector scored from 0-3 at 2'



- The commercially available MMP-9 kit comes calibrated to indicate a **positive response when levels are > 40 ng/ml**. The sampling fleece is saturated in the patient's tear from the inferior fornix and then left in the test cassette for 10 minutes



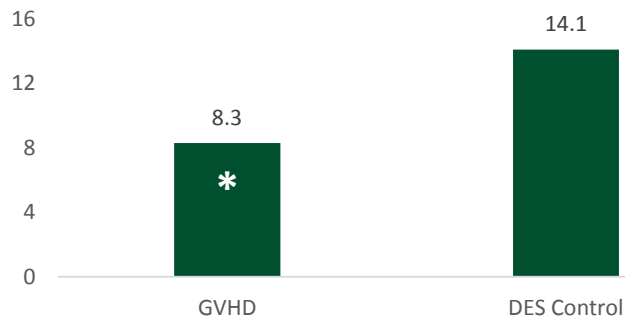
## Results 1. MMP-9 positivity and other Ocular Surface parameters in GVHD patients vs DES Control

|                                  | GVHD<br>(n=49)     | DES<br>(n=193)     | p-value          |
|----------------------------------|--------------------|--------------------|------------------|
| Gender, n (%) female             | <b>21 (43%)</b>    | <b>153 (79%)</b>   | <b>&lt;0.001</b> |
| Age, mean (SD)                   | <b>53.0 (14.4)</b> | <b>61.2 (14.8)</b> | <b>0.0003</b>    |
| Total OSDI, mean (SD)            | 46.9 (29.1)        | 50.3 (24.8)        | 0.2083           |
| Osmolarity†, mOsm/L, mean (SD)   | 328.8 (29.0)       | 329.3 (34.4)       | 0.4667           |
| MMP-9 positive* (%)              | <b>49 (100%)</b>   | <b>86 (45%)</b>    | <b>&lt;0.001</b> |
| Schirmer score†, mm, mean (SD)   | <b>8.3 (9.6)</b>   | <b>14.1 (9.6)</b>  | <b>0.0002</b>    |
| TFBUT†, second, mean (SD)        | 3.6 (2.8)          | 4.3 (3.2)          | 0.0951           |
| Corneal staining score†          | <b>8.8 (5.4)</b>   | <b>5.3 (4.6)</b>   | <b>&lt;0.001</b> |
| Conjunctival staining score†     | <b>11.8 (5.6)</b>  | <b>7.1 (4.7)</b>   | <b>&lt;0.001</b> |
| Lipid layer thickness†, ICU (SD) | <b>48.7 (20.7)</b> | <b>71.8 (22.7)</b> | <b>&lt;0.001</b> |

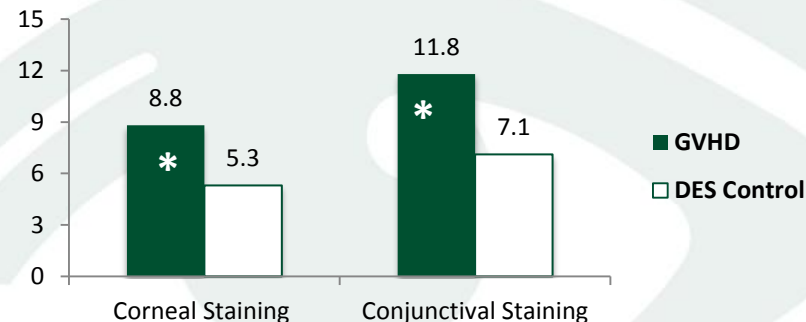
\*Matrix metalloproteinase positive in either eye; †=value in more severely affected eye; GVHD= Graft Versus Host Disease; n=number in group; DES=dry eye syndrome; SD=standard deviation; ICU=interferometry color units

## Results 2. MMP-9 positivity and other ocular surface parameters. GVHD (n=49) vs DES Control (n=193)

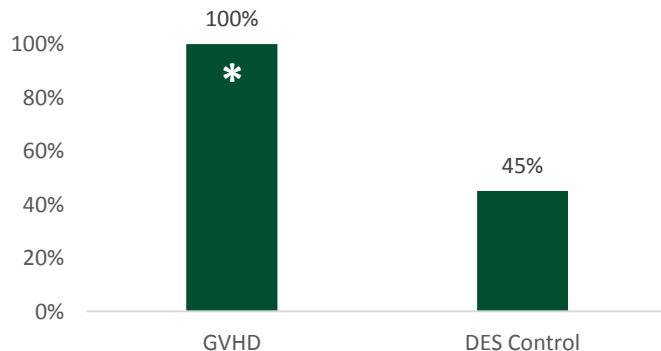
### Schirmer's Test



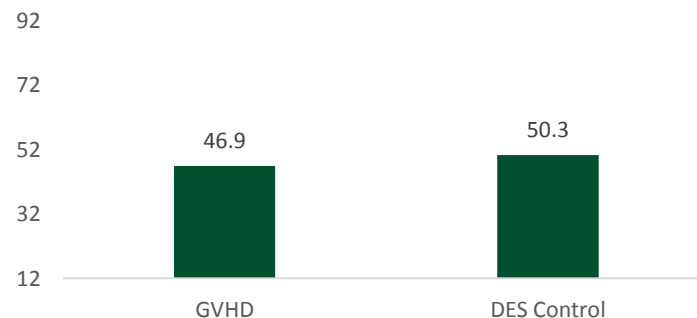
### Corneal and Conjunctival Staining



### MMP-9 positive (%)



### OSDI Score



# Results

- **All 49 (100%) of the GVHD patients tested positive for MMP-9, versus 85 (45%) in the DES control group ( $p<0.001$ ).**
- Total conjunctival and corneal staining was significantly greater in the GVHD group than in the DES control.
- The GVHD group had significantly thinner lipid layer thickness than the DES control group
- No significant differences were noted between the groups in total OSDI, tear film osmolarity and tear film breakup time

# Conclusion

- **100% of patients with GVHD tested positive for MMP-9**, even in the absence of ocular GVHD.
- **Patients with GVHD had more severe signs of DE than patients with DES alone.** Compared to the DES control group, GVHD patients had more corneal and conjunctival staining, lower Schirmer's score, greater MMP-9 positivity, and thinner lipid layer.
- Interestingly, **worse signs of DE did not translate into worse symptoms.** No statistically significant differences between groups were found on the OSDI, a measure of DE symptoms

# Conclusion

- Treatment modalities that **diminish the immune response** responsible for propagating inflammation on the surface of the eye may be particularly useful in patients with GVHD.
- **GVHD** provides a unique study model for DES as the rejection of the graft is a **well-defined cause and start point of disease**.
- Symptoms and signs of DE were collected at a single point in time, and thus we are not able to comment on changes in MMP-9 positivity with treatment.