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USE OF INFLAMMADRY® FOR MMP-9 DETECTION IN OCULAR GRAFT VERSUS HOST DISEASE

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ABSTRACT

Background: InflammaDry® is a point-of-care test that can detect matrix metalloproteinase-9 (MMP-9), an inflammatory biomarker that is elevated in ocular surface stress and desiccation, such as in ocular graft-versus-host disease (oGVHD).

Objectives: To characterize MMP-9 levels in patients diagnosed with oGVHD and to assess InflammaDry® ability to detect ocular surface disease in patients diagnosed with oGVHD.

Methods: A retrospective chart review was completed on 74 patients who have undergone a hematopoietic stem cell transplantation (HSCT) and have been diagnosed with oGVHD. Date of chronic GVHD diagnosis; date of ocular symptoms onset; oGVHD NIH grade on day of InflammaDry® testing; InflammaDry® results; and interventions used to treat ocular symptoms were collected on each patient. Percent positivity, positivity rate and accuracy of InflammaDry® was calculated.

Results: The positivity rate of InflammaDry® showed an increasing trend in relation to increasing oGVHD severity as well as an overall percent positivity of 87.16% and accuracy of 88.36%.

Conclusion: InflammaDry® has demonstrated to be a promising tool that may be used as a screening tool to detect the development of oGVHD onset. However, before InflammaDry® can be implemented into Hematology/Oncology clinics, the rate of positivity of InflammaDry® in patients pre-HSCT must first be determined.

Keywords: Matrix Metalloproteinase 9. Graft-versus-host disease.

INTRODUCTION

Graft-versus-host disease (GVHD), which can be defined as either acute or chronic, is a complication following allogeneic hematopoietic stem cell transplant (HSCT) that is a major contributing factor to patient morbidity and mortality[1]. Acute GVHD was defined as symptoms manifesting before day 100 following HSCT, and chronic GVHD was defined as symptoms continuing or manifesting after one hundred days following HSCT[2]. However, new guidelines suggest that the difference between acute GVHD and chronic GVHD diagnosis should be based on the manifesting symptoms of the patient. While some organs, such as the skin, mouth, gastrointestinal tract, liver, and lungs, can be involved in both acute and chronic GVHD; organs such as the eyes, muscles, genitalia, and nails are typically only impacted in chronic GVHD[3]. Specifically, between 40-60% of patients who develop chronic GVHD will develop ocular complications, also known as ocular graft versus host disease (oGVHD)[4]. Symptoms of oGVHD

include photophobia, redness, pain, excessive tearing, blurred vision, grittiness, or foreign-body sensation[5,6]. Common clinical ocular manifestations include the ocular surface, resulting in keratoconjunctivitis sicca, cicatricial conjunctival fibrosis, corneal perforation, and filamentary keratitis[5, 7-9]. However, oGVHD can additionally affect all tissues of the eye, including the retina and optic nerve[10]. Therefore, oGVHD can negatively affect patient quality of life and cause sight-threatening vision loss[5, 7-10]. The severity of oGVHD is graded on a scale developed by the NIH, ranging from 0-3. A grade of zero represents a patient with no symptoms of oGVHD, whereas a grade of three represents a patient with severe symptoms, which significantly affect their activities of daily living (Table 1)[11].

TABLE 1. National Institute of Health grading scale on severity of ocular graft-versus-host disease. Adapted from Inamoto et al. (2012).

Grade	Findings
0	Asymptomatic
1	Mild dry eye symptoms not affecting ADLs (requiring artificial tears ≤ 3x per day) OR asymptomatic + findings of keratoconjunctivitis sicca
2	Moderate dry eye symptoms partially affecting ADLs (requiring eye drops > 3x per day or punctal plugs) without vision impairment
3	Severe dry eye symptoms significantly affecting ADLs (requiring special eyewear to relieve pain) OR unable to work because of ocular symptoms OR loss of vision caused by keratoconjunctivitis sicca

While the pathogenesis of oGVHD is not completely understood, using murine models has allowed for an improved understanding of the disease process[12]. Concepts regarding the disease process of oGVHD include an increase in IL-6 followed by Treg cell suppression and increased host Th17 cells, corneal infiltration of graft and host mature T-cells, and infiltration of donor-derived fibroblasts leading to immune-mediated fibrosis causing lacrimal gland dysfunction[13-15]. Overall, despite the unclear pathogenesis of oGVHD, it is well accepted that oGVHD is caused by autoimmune destruction of corneal and conjunctival epithelium along with lacrimal gland destruction leading to tear film deficiency. This destruction leads to loss of tissue function, vascularization, and fibrosis resulting in impaired vision or blindness and ocular discomfort or pain[10]. The decreasing morbidity of oGVHD is pertinent and can be achieved by earlier diagnosis and earlier treatment.

InflammaDry® is a point-of-care test that can detect matrix metalloproteinase-9 (MMP-9), an inflammatory biomarker that is elevated in ocular surface stress and desiccation, such as symptoms seen in oGVHD[16-19]. InflammaDry® has an 85% sensitivity and 94% specificity in detecting MMP-9 at a threshold of 40 ng/ml[20]. MMP-9 is a protease that plays a role in many biological processes, such as wound healing. MMP-9 functions via the degradation of many extracellular matrix proteins, altering cell-to-cell interactions and basement membrane degradation[21]. While overexpression of MMP-9 in ocular surface disease may be necessary for wound healing, this molecular marker may also cause collagenous filaments, tissue remodeling and vascular proliferation[19]. Additionally, MMP-9 has been shown to induce proinflammatory molecules including IL-1, TNF-alpha and NF-kB[19]. Given MMP-9 overexpression to allow for wound healing, this marker has provided a diagnostic marker of ocular surface disease[19], and has been shown to be significantly elevated in oGVHD as compared to other dry eye disease causes[22]. The purpose of this study is to determine if InflammaDry® is able to detect MMP-9 in the tear film of patients diagnosed with oGVHD.

METHODS

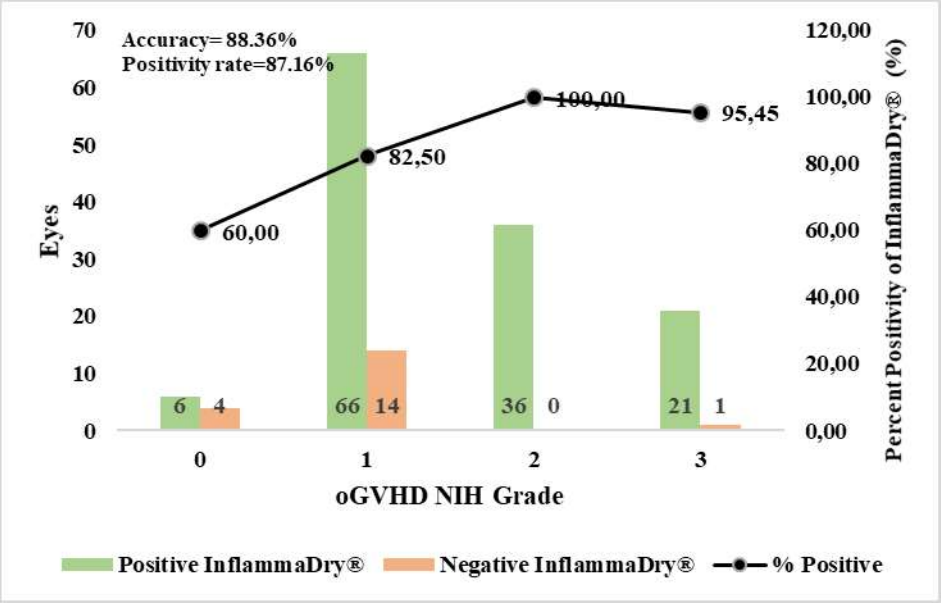
A retrospective chart review was completed on 74 patients who have undergone a hematopoietic stem cell transplantation (HSCT) and have been diagnosed with oGVHD. Demographic data (MRN, age, gender, ethnicity); oncologic diagnosis; date of HSCT; date of chronic GVHD diagnosis; date of ocular symptoms onset; date of referral to ocular surface disease (OSD) clinic; date of

initial appointment at the OSD clinic; oGVHD NIH grade on day of InflammaDry® testing; InflammaDry® test date; InflammaDry® results; and interventions used to treat ocular symptoms were collected on each patient. InflammaDry® results were marked as either positive or negative. To analyze these data, InflammaDry® test results were plotted against oGVHD NIH grading (Grade 0, 1, 2 or 3). The percentage of positive InflammaDry® tests per NIH grade was also plotted on the same graph, which was calculated with the equation [% Positivity Rate= # Positive Tests in NIH grade/ Total Tests for NIH grade]. The overall positivity rate of InflammaDry® was calculated with the equation [Total Positive Tests/ Total Tests Collected]. Accuracy of InflammaDry® was analyzed with the equation [Accuracy= (# True Positive tests/ (# True Positive + # True Negative))*100]. The number of days it took for a patient to receive InflammaDry® testing from an eye care provider following cGVHD diagnosis was determined by subtracting the date of InflammaDry® testing from the date of cGVHD diagnosis. The average, median and range of number of days was calculated based off data from all 100 patients from which this study collected data from. Additionally, the number of days it took for a patient to receive InflammaDry® testing from an eye care provider following ocular symptom onset was calculated with the question [Number of days= Date of InflammaDry® testing – Date of ocular symptom onset]. The average, median and range was calculated from all 100 patients from which this study collected data from. Lastly, to analyze which ocular treatments patients were using at the time of InflammaDry® testing, the total number of patients on any given ocular treatment was collected. The number of patients were placed into a table under the category of treatment they were using. Patients may have been using multiple concurrent treatments at the time of InflammaDry® testing. Therefore, some patients had multiple treatments and were duplicated to account for each modality they were using. Treatments used prior to InflammaDry® testing were not collected in this analysis.

RESULTS

The positivity rate for oGVHD NIH Grade 0, 1, 2 and 3 were 60%, 82.50%, 100% and 95.45%, respectively. An example calculation for percent positivity for NIH grade 0 [(%)= (6/10) * 100 = 60% positivity]. The overall percent positivity of InflammaDry® was 87.19% (Figure 1).

FIGURE 1. Positivity rate of InflammaDry® in patients diagnosed with ocular graft versus host. Primary axis: Number of eyes that resulted positive or negative using InflammaDry® at a given diagnosed ocular graft versus house NIH grade (0-3). Secondary axis: Positivity rate of InflammaDry® at each ocular GVHD NIH grade. N= 148 eyes (74 patients).



Overall percent positivity of InflammaDry® was calculated using the equation [(129/ (129 + 19)) * 100 = 87.16% positivity]. The positivity rate showed an overall increasing trend that correlated with increasing oGVHD symptom severity. However, while 100% of InflammaDry® tests were positive at oGVHD Grade 2, only 95.45% of InflammaDry® tests were positive at oGVHD Grade 3 (Figure 1). The singular InflammaDry® test that resulted negative in a patient with oGVHD Grade 3 was a false negative, because a repeat InflammaDry® test resulted positive in both eyes. Upon further analysis of data, it was determined that there was another false negative in the category of oGVHD Grade 1. Again, the rationale behind this is because a repeat InflammaDry® test resulted positive in both eyes. False negatives could have been due to inadequate tear collection using InflammaDry®. Given the two false negatives, accuracy of InflammaDry® was calculated to be 88.36% (Figure 1). Accuracy was calculated using the follow equation: (129/ (129 + 17)) *100 = 88.36% accuracy.

The impact of the length of time the patient had oGVHD and treatments for ocular symptoms on InflammaDry® testing was also analyzed. The median number of days from the time a patient was diagnosed with cGVHD to when the InflammaDry ® test was completed was 953 days (Table 2).

TABLE 2. Analysis of the number of days between the date of chronic graft versus host disease diagnosis and date of InflammaDry® testing. Number of days= Date of InflammaDry® testing- Date of chronic GVHD diagnosis. Number of days was collected from 74 patients. Average number of days, median number of days and range of number of days were determined.

Average (Days)	Median (Days)	Range (Days)
1255.87	953	-64-5903

Additionally, the median number of days between the time a patient presented with ocular symptoms, as documented at their oncology appointments, from when an InflammaDry ® test was completed was 834 days (Table 3).

TABLE 3. Analysis of the number of days between the date of ocular symptom onset and date of InflammaDry® testing. Number of days= Date of InflammaDry® testing- Date of ocular symptom onset. Number of days was collected from 74 patients. Average number of days, median number of days and range of number of days were determined.

Average (Days)	Median (Days)	Range (Days)
1166.03	834	7-5079

Furthermore, 78.38% of patients were on symptomatic treatment for oGVHD on the same day of InflammaDry® testing using artificial tears. Additionally, 22.97% of patients were using warm compress, 18.92% were using Cyclosporine and 16.22% were using topical steroid drops (Table 4).

TABLE 4. Therapies used on the day of InflammaDry ® testing. Categories of therapies which patients were using on the same day of InflammaDry ® testing were collected. For each category of treatment, the total number of patients on that given treatment was determined from a population of N=74 patients. The relative percentage of patients on each given treatment was calculated [% of patients on X therapy= (Number of patients on X therapy/ Total number of patients) *100]. N= 74 patients.

Current treatment on day of InflammaDry ® test	Number of patients	Percentage of patients on therapy (%)
Cyclosporine	14	18.92
Artificial tears	58	78.38
Lubricating ointment	4	5.41
Warm compress	17	22.97
Cold compress	1	1.35
Punctal plugs	3	4.05
Punctal cautery	1	1.35
Topical steroid	12	16.22
Lifitegrast	7	9.46
Lid scrubs	14	18.92
Autologous serum drops	9	12.61
Topical antibiotics	3	4.05
Oral antibiotics	6	8.11
Albumin drops	2	2.70
Scleral lenses	1	1.35
Fish oil	1	1.35
Red eye drops	4	5.41
Allergy drops	4	5.41
None	8	10.81

DISCUSSION

The positivity rate of InflammaDry® showed an increasing trend in relation to increasing oGVHD severity as well as an overall percent positivity of 87.16% and accuracy of 88.36% (Figure 1). On the day of InflammaDry® testing, patients had been experiencing symptoms of cGVHD for a median of 953 days (Table 2) and symptoms of oGVHD for a median of 834 days (Table 3) prior to InflammaDry® testing. Additionally, 78.38% of patients had been on symptomatic treatment for oGVHD on the day of InflammaDry® testing. This suggests that while patients were being treated for oGHVD, InflammaDry ® testing and therefore MMP-9 production was minimally impacted given an 87.16% overall positivity rate of InflammaDry ® over all oGVHD grades (Figure 1).

Research has shown that treatments such as doxycycline and methylprednisolone lead to a decrease in MMP-9 expression and, furthermore, prevention of recurrent epithelial erosion[23], suggesting that treatment targeting MMP-9 may be beneficial in improving patient's symptoms of ocular surface disease[19]. However, despite patients being on treatment, there was still an 87.16% overall positivity rate of InflammaDry®. On reviewing the types of treatment used by patients, only 16.22% of patients were using topical corticosteroids and only 8.11% were using oral antibiotics, such as doxycycline (Table 4). Treatments with topical corticosteroids and oral antibiotics may have played a role in decreasing MMP-9 production and therefore impacted the overall positivity rate of InflammaDry®. However, given that length of treat-

ment was not considered, nor was InflammaDry® collected before and after initiating treatments, this study cannot draw conclusions on which treatments may have resulted in a decrease in MMP-9 production. Furthermore, it can be inferred that treatment minimally impacted InflammaDry® results, given that patients with more severe oGVHD symptoms (NIH Grade 2 and 3) were likely on a more intensive treatment regimen; however, InflammaDry® positivity rates were improved in these patients as compared NIH Grade 0 and 1.

Given the positivity rate (87.16%) and accuracy (88.36%) of InflammaDry® in detecting MMP-9 in patients with oGVHD, InflammaDry® could be used as a screening tool for oGVHD onset. The importance of a screening tool for oGVHD is to earlier detect the onset of oGVHD prior to symptom onset, allowing for earlier treatment initiation. Research suggests that earlier treatment is necessary to decrease morbidity in patients with oGVHD[5], therefore improving their quality of life. However, further investigation needs to be pursued to determine whether MMP-9 is present in patients prior to undergoing an allogeneic HSCT. It has already been demonstrated that dry eye disease has been detected in patients with hematological diseases before HSCT[24]. Therefore, in order to determine whether InflammaDry® can be used as a screening tool for oGVHD, it needs to first be determined whether MMP-9 is elevated in patients who will be undergoing an allogeneic HSCT. Should MMP-9 be elevated in patients before HSCT, prophylactic treatment for oGVHD, even prior to HSCT, may be warranted.

CONCLUSION

Given the high positivity rate (87.16%) and accuracy (88.36%) in detecting MMP-9 in patients with known oGVHD, InflammaDry® has been demonstrated as a viable screening tool to determine if a patient has oGVHD. Therefore, InflammaDry® can be used: 1) by oncology providers at patient appointments following HSCT to quickly and accurately determine if a patient is developing oGVHD disease, 2) to initiate treatment, and refer a patient to an eye care practitioner sooner. Due to this, implementing the use of InflammaDry® during oncology clinic appointments may help reduce ocular morbidity caused by GVHD. However, before implementing InflammaDry® as a screening tool in Hematology/Oncology clinics, the positivity rate of InflammaDry® pre-hematopoietic stem cell transplant must be determined. If InflammaDry® results positive prior to receiving HSCT, this would suggest candidates have ocular surface inflammation due to a cause other than oGVHD, and therefore InflammaDry® would not be a good screening tool to detect oGVHD onset.

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