Eye (2009) 23, 202–208

www.nature.com/eye

CLINICAL STUDY

Tear function and lipid layer alterations in dry eye patients with chronic graft-*vs*-host disease

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Received: 7 February 2008
Accepted in revised form: 3 October 2008
Published online: 14 November 2008

This work was presented at 5th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance. Taormina, Sicily, Italy, September 5th–8th 2007.

Abstract

Purpose **To investigate the changes in the tear film lipid layer in haematopoietic stem cell transplantation (HSCT) patients with dry eye (DE) associated with chronic graft-*vs*-host disease (cGVHD) and compare with HSCT recipients without DE.**

Methods **We performed a prospective study in 10 HSCT patients with DE associated with cGVHD and 11 HSCT recipients without DE. We performed Schirmer's test, tear film break up time examinations, ocular surface dye staining and meibum expressibility test and DR-1 tear film lipid layer interferometry. DR-1 interferometry images of the tear film surface were assigned a 'DR-1 grade' according to the Yokoi severity grading system. The DR-1 grades were analysed according to the presence or absence of DE, conjunctival fibrosis and systemic cGVHD.**

Results **The mean DR-1 severity grade in patients with DE related to cGVHD (DE/cGVHD group; 3.9 ± 0.9) was significantly higher than in patients without DE after HSCT (non-DE/non-cGVHD group; 1.3 ± 0.6; $P < 0.05$). The DR-1 grade for HSCT recipients with conjunctival fibrosis was significantly higher than in patients without conjunctival fibrosis ($P < 0.05$). When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report, our results showed a correlation between the severity of DE and DR-1 grades ($r = 0.8812$, $P < 0.0001$).**

Conclusion **DR-1 interferometry may be applicable to diagnosing DE and evaluating its progression subsequent to HSCT.**

Eye (2009) **23,** 202–208; doi:10.1038/eye.2008.340; published online 14 November 2008

Keywords: tear interference; tear lipid layer; dry eye; conjunctival fibrosis; chronic GVHD; bone marrow transplantation

Introduction

Dry eye (DE) is a major complication of chronic graft-*vs*-host disease (cGVHD) and has a significant impact on the quality of life.[1–6] The precorneal tear lipid layer controls tear evaporation, and DE after haematopoietic stem cell transplantation (HSCT) may be associated with changes in the tear lipid layer, like other types of DE or other ocular surface disorders.[7,8] The Schirmer's test is the standard method for diagnosing DE associated with cGVHD, but is invasive, may cause irritation and reflex tearing, and may produce false-negative or false-positive results.[9] Noninvasive tear lipid layer interferometry is a useful method for evaluating the severity of DE.[7–12] However, no report on the tear lipid layer changes with cases of DE associated with cGVHD has been published to date.

In this study, we used DR-1 tear lipid layer interferometry to investigate and classify the tear film lipid layer interference patterns in patients with DE because of cGVHD, and compared the results with those from patients who did not develop DE after HSCT.

Materials and methods

In this prospective and comparative study, we analysed 18 eyes from 10 patients who had DE associated with cGVHD (DE/cGVHD group; median age: 48.0 years; range: 30–63 years; three men, seven women) and 19 eyes from 11 age- and gender-matched patients who did not develop DE after HSCT (non-DE/non-cGVHD

group; median age: 43.8 years; range: 24–64 years; three men, eight women). We excluded two eyes from patients in the DE/cGVHD group that did not fulfil the criteria for DE and three eyes with blepharitis from patients in the non-DE/non-cGVHD group. The median follow-up time after HSCT was 31.9 months for the DE/cGVHD group, and 26.8 months for the non-DE/non-cGVHD group. Topical eye drops including artificial tears, vitamin A, and autologous sera eye drops were instilled five times a day immediately after the diagnosis of DE following HSCT. The median follow-up time from the diagnosis of DE to the first examination was 15.2 ± 12.0 months (range: 3–38 months) for the DE/cGVHD group. Primary diseases and disease stages were also well balanced between the two groups. We used the global diagnostic criteria for DE, which is based on the recommendation of the 2007 International Dry Eye Workshop Report.[13] Patients who had a history of surgical or spontaneous lacrimal punctal occlusion, allergies, simple meibomian gland dysfunction (MGD), glaucoma medications, contact lens use, or other ocular surgery including refractive surgery or radiation to the eyes were excluded, as were patients with infectious blepharitis, blink disorders, disorders of the lid aperture or lid/globe congruity, or other ocular surface disorders. In addition, patients with trachoma and ocular cicatricial pemphigoid were also excluded. The research followed the tenets of the Declaration of Helsinki Principles, and informed consent was obtained from all subjects. IRB/Ethics Committee approval for the examination procedure was obtained for this study.

Ocular surface vital staining

The fluorescein and Rose Bengal stain scores for the ocular surface were obtained using the double vital staining method[14] Both stains were scored on a scale of 0–9.[14,15] The van Bijsterveld scoring system was used for the Rose Bengal staining. Briefly, the ocular surface was divided into three zones: nasal conjunctival, corneal, and temporal. A score of 0–3 points was used for each zone, with a minimum possible score of 0 and a maximum total score of 9 points. Scarce punctuate staining was given 1 point. Denser staining not covering the entire zone was given 2 points. Rose Bengal staining over the entire zone was given 3 points. For the fluorescein staining, the cornea was divided into three equal upper, middle, and lower zones. Each zone had a staining score ranging from 0 to 3 points, as with the Rose Bengal stain, and the minimum and maximum total staining scores were 0 and 9 points, respectively. The presence of scarce staining in a zone was scored as 1 point; frequent puncta not covering the entire zone was scored as 2 points; and punctuate staining covering the entire zone was scored as 3 points.

Tear function test

Tear film break up time (TBUT) was measured three times and the median value was calculated.[14] The Schirmer's test was performed using standard strips (Alcon, Fort Worth, TX, USA) placed in the lower conjunctival sac for 5 min without anaesthesia.

Meibomian gland secretions

MGD was assessed by careful slit-lamp examination of the glandular orifices, mucocutaneous junction changes, and digital expression of the meibomian lipids. The same physician (YO) pressed gently on the lower eyelids to express the meibomian lipids. Meibum viscosity was graded as described by Shimazaki *et al*.[16] Briefly, to assess obstruction of the meibomian gland orifice, digital pressure was applied on the lower tarsus, and the expression of meibomian secretion (meibum) was scored as follows: grade 0, clear meibum is easily expressed; grade 1, cloudy meibum is expressed with mild pressure; grade 2, cloudy meibum is expressed with more than moderate pressure; and grade 3, meibum cannot be expressed even with the hard pressure.

Diagnosis of dry eye

The diagnosis and classification of DE disease based on the severity was carried out according to the recommendation of the 2007 International Dry Eye Workshop Report.[13]

Diagnosis of cGVHD

All the patients in our study fulfilled the revised consensus criteria for cGVHD.[17] Briefly, diagnosis of cGVHD requires the following: (1) a distinction from acute GVHD, (2) the presence of at least one distinctive manifestation (eg, keratoconjunctive sicca) confirmed by pertinent biopsy or other relevant tests (eg, Schirmer's test) in the same or other organs, and (3) the exclusion of other possible diagnoses.

DR-1® tear film lipid layer interferometry

Noncontact interferometry micrographs of the surface of the tear film were recorded using the DR-1® tear film lipid layer interferometry system (Kowa, Tokyo, Japan). DR-1® interferometry records the specular light from the tear surface. Light from a white-light source is reflected by a half mirror, focussed by a lens, and used to illuminate the tear surface. The specular light from the tear surface returns through the half mirror to a charge-coupled device camera that produces an image on the

device monitor. Two polarizers and a quarter-wave plate help eliminate any unnecessary reflected light from the lens and detect only the specular light reflected from the tear fluid. The camera is focussed on a 2.2×3.0-mm area of the central cornea such that a circular area 8-mm in diameter is observable. Lipid layer interference images were recorded immediately after a complete blink and were printed out using a colour video printer. The lipid layer grading classification was as reported previously: grade 1, somewhat grey colour, uniform distribution; grade 2, somewhat grey colour, nonuniform distribution; grade 3, a few colours, nonuniform distribution; grade 4, many colours, nonuniform distribution; grade 5, corneal surface partially exposed.[7] This grading scale has been demonstrated to correlate well with the degree of DE.[7] The DR-1 images were analysed by four independent investigators (YB, NT, EG, and DM) who did not collect the interference pattern data (MS, MN, and MS) or perform the DE examination (YO). The clinical status of the patients was masked for the analysis. When three or more of the four physicians agreed on the grade classification, we analysed the relationship between grade and score from the DE examination.[7]

Conjunctival fibrosis

We diagnosed conjunctival fibrosis in patients who had subconjunctival fibrosis, fornix shortening, symblepharon, and/or ankyloblepharon.[18,19] We evaluated these findings by using slit-lamp microscopy during a routine examination.

Statistical analyses

The nonparametric Mann–Whitney test was used to compare the two groups. Spearman's rank sum test was performed for analysis of the correlation between DR-1 grades and DE severity in patients who received HSCT and developed cGVHD with DE disease as well as patients receiving HSCT who did not develop cGVHD or DE disease. A P-value of <0.05 was considered statistically significant.

GraphPad Instat 3 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis.

Results

Tables 1 and 2 summarized the demographic characteristics of DE patients with cGVHD and non-DE subjects without cGVHD. In the DE/cGVHD group, the mean severity grade of the DR-1 images was 3.9 ± 0.9, which was significantly higher than that of the non-DE/non-cGVHD group (1.3 ± 0.6; $P<0.05$; Table 3). All DE patients in this study had cGVHD, and none of the non-DE HSCT recipients did. In some DE patients, with cGVHD the severity score was as high as grade 5, and DR-1 examination showed an irregular tear film, exposed areas of the corneal surface, and dry spots (Figure 1). Figure 2 shows a representative interferometry print from a dry eye with cGVHD patient with grade 3 DR-1 lipid layer change. Figure 3 shows a representative interferometry print from a normal subject. When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report,[13] our results showed a strong correlation between the severity of DE disease and the DR-1 grades ($r=0.8812$, $P<0.0001$; Figure 4).

We also investigated whether the DR-1 score correlated with the severity of cGVHD. Conjunctival involvement in GVHD is a distinct marker for severe systemic GVHD.[19] In HSCT recipients with conjunctival fibrosis, the mean DR-1 grade was 4.7 ± 0.7 ($n=9$ eyes). In contrast, the score was 1.9 ± 1.0 in HSCT recipients without conjunctival fibrosis ($n=28$ eyes). The difference was statistically significant ($P<0.05$; Table 4).

Conclusion

In this study, we found that the grades of the tear film lipid layer interference patterns measured by DR-1 interferometry in the DE/cGVHD group (3.9 ± 0.9) were significantly higher than those of the non-DE/non-cGVHD group (1.3 ± 0.6). In addition, the mean DR-1 grades for age- and gender-matched normal control subjects was 1.2 ± 0.5 (median age: 45.1 years; range 30–69; three men, eight women; Figure 3). Statistically significant differences in the DR-1 grade for patients with conjunctival fibrosis *vs* without it were also observed. Moreover, there was a strong correlation between the severity of DE disease and the DR-1 grades (Figure 4).

DE is a major complication after HSCT.[1,3,18] It has been shown that tear lipid layer interference patterns are highly correlated with DE severity, and the lipid layer becomes thick between grades 2 and 4 in cases of DE.[9,11] Here, grades 3, 4, and 5 were observed only in the DE/cGVHD group, and grades 1 and 2 were noted in the non-DE/non-cGVHD group, as shown by Yokoi *et al* previously. When DE severity was graded according to the recommendation of the 2007 Dry Eye Workshop Report, our results showed a strong correlation between the severity of the DE disease and the DR-1 grade.

Goto and Tseng *et al*[10,11] reported that the thick lipid layer in eyes with aqueous tear deficiency results from the retardation of lipid spread, which leads to an uneven distribution of the lipid film. Yokoi *et al*[7] previously reported that aqueous tear deficiency is associated with higher DR-1 grades because of the movement of tear lipids into areas lacking the aqueous component of the

Table 1 Demographic data for patients with dry eye associated with chronic GVHD (DE/cGVHD group)

Case no.	*Sex*	*Age*	*Diag*	*HSCT*	*VA*	*F*	*RB*	*TBUT*	*Sch*	*MGD*	*DES*	*DR-1*	*Term*	*CF*	*cGVHD lesions other than eyes*
1	F	60	ALL	PBSCT									12 mo		+
Rt					6/6	6	0	5	0	2	4	3		−	
Lt					6/4.8	6	0	5	5	3	3	3		−	
2	F	30	AML	PBSCT									40 mo		+
Rt					6/4.8	3	5	2	1	3	4	3		−	
Lt					6/4.8	4	5	2	1	3	4	3		−	
3	M	44	ALL	BMT									40 mo		+
Rt					6/4.8	4	2	3	1	3	4	5		+	
Lt					6/4.8	4	3	4	0	3	4	5		+	
4	F	33	ABL	BMT									6 mo		+
Rt					6/4.8	5	5	3	1	1	3	3		−	
5	M	49	ALL	BMT									24 mo		+
Rt					6/4.8	5	5	4	3	3	3	4		−	
Lt					6/4.8	5	5	4	2	3	3	4		−	
6	F	45	AML	BMT									57 mo		+
Rt					6/4.8	4	7	2		2	4	5		+	
Lt					6/4.8	5	7	2		2	4	5		+	
7	M	63	MDS	RIST									64 mo		+
Rt					6/12	9	8	3	3	3	3	5		+	
Lt					6/4.8	3	4	2	2	3	4	4		+	
8	F	31	CML	BMT									8 mo		+
Lt					6/4.8	4	1	5	6	2	2	3		−	
9	F	59	ML	BMT									24 mo		+
Rt					6/60	6	0	2	2	3	4	5		+	
Lt					6/120	6	0	2	3	3	3	5		+	
10	F	50	AML	BMT									19 mo		+
Rt					6/4.8	1	0	5	2	2	3	3		−	
Lt					6/6	3	5	5	1	2	3	3		+	

ABL, acute biophenotypic leukaemia; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplantation; CF, conjunctival fibrosis; CML, chronic myeloid leukaemia; DES, dry eye severity; Diag, diagnosis; DR-1, tear film lipid layer interference patterns; F, fluorescein staining score; HSCT, haematopoietic stem cell transplantation; Lt, left; MDS, myelodysplastic syndrome; MGD, meibomian gland dysfunction; ML, malignant lymphoma; mo, month; PBSCT, peripheral blood stem cell transplantation; RB, Rose Bengal staining score; RIST, reduced intensity stem cell transplantation; Rt, right; Sch, Schirmer's test; TBUT, tear film break up time; Term, the interval between HSCT and the first DR-1 examination; VA, visual acuity.

tear film, which results in a higher interferometry grade and a thicker lipid layer. In our study, 38.9% of the eyes were grade 5 by DR-1 lipid layer interferometry. All but one of the eyes had grade 2 or 3 meibomian gland expressibility with severe aqueous deficiency, which resulted in extensive grade 5 changes with corneal exposure in DR-1 examinations. In DE associated with cGVHD, we noted severe MGD at the time of disease onset in addition to aqueous tear deficiency.

We believe that both aqueous tear deficiency and evaporative type DE coexist in cGVHD. All the subjects had relatively higher ocular surface epithelial damage with higher fluorescein and Rose Bengal scores. Higher DR-1 grades in the severe aqueous tear deficiency DE subjects despite presence of nonexpressible meibomian gland secretions may be explained by the release of cell membrane lipids into the tear film because of the extensive epithelial damage. There were, however, three eyes (16.7%) with moderate grade 3 DR-1 score with low meibum expressibility grade 3 and low Schirmer's test scores. In another case, DR-1 grade was 5, although MGD score was 2. Further studies are necessary to clarify these discrepancies.

All DEs in this study had tear instability with low TBUT score, which might be because of the interaction of the several pathophysiological processes. One possibility is the excessive tear evaporation resulting from lipid deficiency, because 61.1% of the meibomian glands could not be expressed in our patients. Although the pathogenesis of MGD in cGVHD is controversial, meibomian gland function was severely damaged in patients with severe DE and cGVHD, leading to tear evaporation and low TBUT.[3] High DR-1 grades in the seven eyes of four cGVHD patients (grade 5 changes) probably resulted because of total obstruction of meibomian ducts by cGVHD fibrosis around the meibomian ducts. As MGD is a risk factor of DE, the severity of DE is more serious in the pathophysiology of cGVHD related DE, which is different from simple aqueous deficiency type of DE. The other explanation for

Table 2 Demographic data for HSCT recipients without dry eye (non-DE/non-cGVHD group)

Case no.	*Sex*	*Age*	*Diag*	*HSCT*	*VA*	*MGD*	*DES*	*DR-1*	*Term*	*CF*	*cGVHD*
1	M	33	NHL	BMT					10 mo		+
Rt					6/4.8	1	0	2		−	
Lt					6/4.8	1	0	1		−	
2	F	36	MDS	BMT					3 mo		+
Rt					6/4.8		0	2		−	
Lt					6/7.5		0	1		−	
3	F	60	MF	BMT					12 mo		−
Rt					6/4.8	2	0	2		−	
4	F	49	Mantle	BMT					23 mo		−
Rt			Lymphoma		6/4.8	0	0	1		−	
Lt					6/4.8	0	0	1		−	
5	F	46	CML	CBT					14 mo		−
Rt					6/4.8	2	0	1		−	
Lt					6/4.8	2	0	1		−	
6	F	42	MDS	BMT					25 mo		−
Rt					6/4.8	0	0	1		−	
Lt					6/4.8	0	0	1		−	
7	M	50	AML	BMT					37 mo		−
Lt					6/4.8	1	0	2		−	
8	F	24	AML	BMT					49 mo		−
Rt					6/4.8	0	0	2		−	
Lt					6/4.8	1	0	2		−	
9	F	38	ML	BMT					46 mo		−
Rt					6/4.8	0	0	1		−	
Lt					6/4.8	1	0	1		−	
10	M	64	ML	PBSCT					25 mo		−
Rt					6/4.8	0	0	1		−	
Lt					6/4.8	0	0	1		−	
11	F	58	MDS	BMT					70 mo		−
Rt					6/4.8	2	0	1		−	

AML, acute myeloid leukaemia; BMT, bone marrow transplantation; CBT, cord blood transplantation; CF, conjunctival fibrosis; CML, chronic myeloid leukaemia; DES, dry eye severity; Diag, diagnosis; DR-1, tear film lipid layer interference patterns; F, fluorescein staining score; HSCT, haematopoietic stem cell transplantation; Lt, left; mo, Month; MDS, myelodysplastic syndrome; MF, myeloid fibrosis; MGD, meibomian gland dysfunction; ML, malignant lymphoma; mo, month; NHL, non-Hodgkin's lymphoma; PBSCT, peripheral blood stem cell transplantation; RB, Rose Bengal staining score; Rt, right; Sch, Schirmer's test; TBUT, tear film break up time; Term, the interval between HSCT and the first DR-1 examination; VA, visual acuity.

Table 3 Comparison of DR-1 grade between DE/cGVHD group and non-DE/non-cGVHD group

	DE/cGVHD group	*Non-DE/ non-cGVHD group*	P-*value*
F (points)	4.6 ± 1.7*	0.05 ± 0.2	$P<0.0001$
RB (points)	3.4 ± 2.7*	0.05 ± 0.2	$P=0.0002$
TBUT (seconds)	3.3 ± 1.3*	9.0 ± 1.9	$P<0.0001$
Schirmer (mm)	2.1 ± 1.7*	9.8 ± 7.4	$P=0.0017$
MGD (points)	2.6 ± 0.6*	0.8 ± 0.8	$P<0.0001$
DR-1 grade (1–5)	3.9 ± 0.9*	1.3 ± 0.6	$P<0.0001$

*$P<0.05$.
F, fluorescein staining score; MGD, meibomian gland dysfunction; RB, Rose Bengal staining score; TBUT, tear film break up time.
Points of MGD indicate the meibum expressibility grade.

the low TBUT scores can be associated with a mucin deficient DE state. Indeed, conjunctival goblet cells and MUC5AC mRNA expression have been reported to decrease in cGVHD previously by us.[20] Problems of interaction between the tear film and ocular surface in cGVHD resulting from irregularities of the cornea and conjunctiva as evidenced by the high fluorescein and Rose Bengal score may explain the perturbation of the tear film stability.

Lipids are potential targets of oxidative radicals.[12] Oxidative stress in blood cells in mice is noticeable 3 weeks after HSCT, and is higher in mice receiving allogeneic spleen cells than in those receiving transplanted syngeneic cells, consistent with an association between oxidative stress and GVHD.[21] Given that radiation damage is associated with the production of activated oxygen species,[22] the total body irradiation performed before HSCT may affect the targets of oxidative radicals, including the tear lipid layer.

We found that the DR-1 tear film lipid layer interference patterns were significantly altered in cGVHD patients with conjunctival fibrosis and cGVHD. Consistent with our observations, Danjo and Hamano previously reported the DR-1 grade in patients with

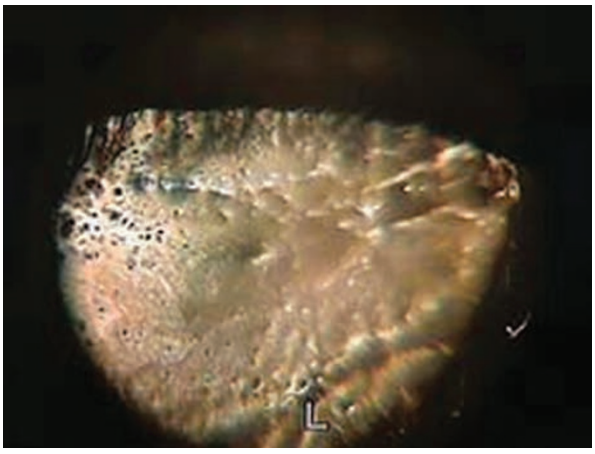

Figure 1 Interferometry pattern from the tear film surface of a 59-year-old woman, 24 months after HSCT. Conjunctival fibrosis (+). Grade 5 DR-1 change with an irregular tear film, areas of corneal surface exposure, and several large dry spots (case 9).

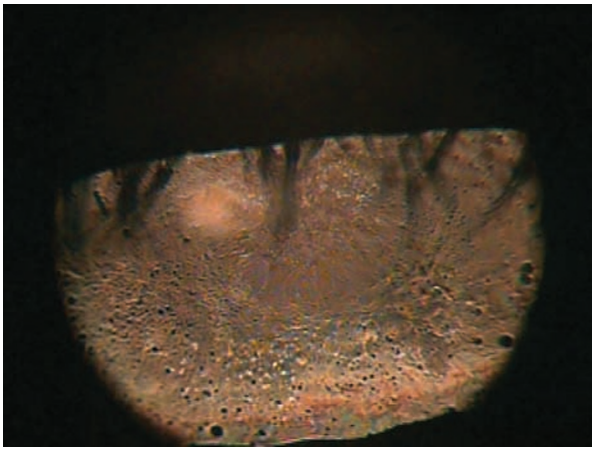

Figure 2 Interferometry pattern from the tear film surface of a 50-year-old woman, 19 months after HSCT. Conjunctival fibrosis (+). DR-1 grade 3 (case 10).

Sjögren's disease.[23] The reported values of DR-1 grades for Sjogren's syndrome (SS) were much lower than the DR-1 grades in our paper, suggesting that the severity of DE associated with cGVHD. In our study, higher grades of DR-1 lipid layer interferometry were observed in DE patients with cGVHD compared with patients with DE and no history of GVHD such as patients with SS and other simple types of DE disease. We believe that the higher DR-1 grades observed in this study may be representative for severe DE associated with GVHD may be related to severe DEs with cicatrising conjunctivits. It is also our observation that patients with other cicatrising conjunctival diseases such as ocular cicatricial pemphigoid have high DR-1 grades

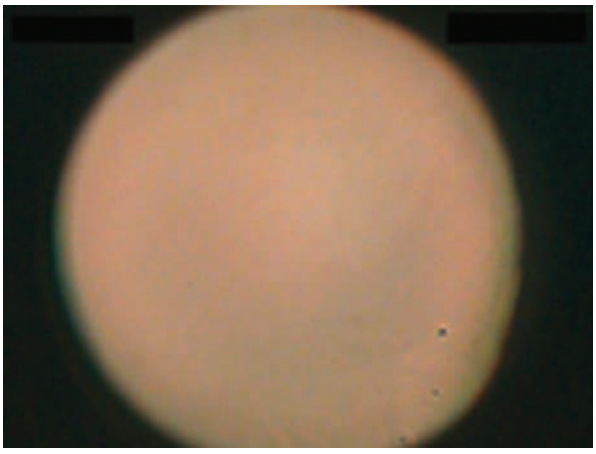

Figure 3 Interferometry pattern from the tear film surface of normal control, a 50-year-old woman. Note the regularity of the tear film and uniform homogenous grey–white lipid interferometry pattern grade 1.

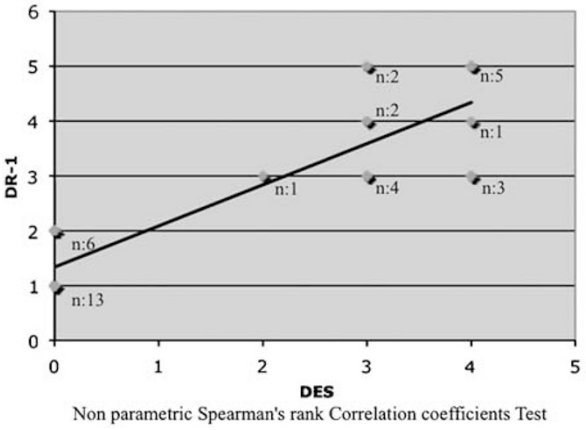

Figure 4 Correlation between DE severity scores and DR-1 tear film lipid layer grades. Nonparametric Spearman's rank test. Correlation coefficients test. $\gamma = 0.8812$, $P < 0.0001$. '*n*' indicates the number of patients.

Table 4 Comparison of the DR-1 grades for HSCT recipients with or without conjunctival fibrosis and with or without dry eye

	CF (+)	*CF (−)*
DE (+)	4.7 ± 0.7 ($n = 9$)	3.2 ± 0.4 ($n = 9$)
DE (−)	($n = 0$)	1.3 ± 0.6 ($n = 19$)
Total	4.7 ± 0.7 ($n = 9$)	1.9 ± 1.0 ($n = 28$)

CF, conjunctival fibrosis; DE, dry eye; HSCT, haematopoietic stem cell transplantation.

similar to the DE disease in cGVHD (unpublished observation).

Conjunctival fibrosis occurs subsequent to conjunctival GVHD with pseudomembrane formation, owing to the

loss of the conjunctival epithelium. Conjunctival involvement in GVHD is a marker for severe systemic GVHD.[19] In our study, cGVHD patients with conjunctival fibrosis had systemic complications and a poor prognosis following HSCT. Thus, the DR-1 grade may be a useful tool for predicting the systemic complications as well.

In conclusion, DR-1 lipid layer interferometry grades in cGVHD patients with severe DE disease were markedly higher compared with patients without DE and no history of cGVHD after HSCT. DR-1 tear lipid interferometry is a noninvasive test, which may be a useful tool for monitoring the onset and progression of DE associated with cGVHD. The DR-1 grade also seems to be a promising severity marker for cGVHD. Further studies analysing the potential association between the DR-1 grades and different therapeutic options will definitely enrich our understanding on the pathological changes of the tear lipid film layer in cGVHD.

Acknowledgements

This study was supported by Grant nos. 17791254 and 20592058 from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (Tokyo, Japan).

Disclosure/Conflict of interest

None.

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