

Ocular surface characterization after allogeneic stem cell transplantation: A prospective study in a referral center

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Purpose: To characterize anatomical and functional changes in the ocular surface after hematopoietic stem cell transplantation. **Methods:** Three groups of patients were included in the study. Group 1: patients who had undergone allogeneic hematopoietic stem cell transplantation (HSCT) ($n = 26$). Group 2: patients who developed chronic graft versus host disease (GvHD) after HSCT ($n = 14$). Group 3: healthy subjects ($n = 20$). A complete ophthalmological examination was undertaken in all subjects, including Schirmer's test, TBUT (break-up-time) test, Oxford scale, OSDI test, corneal tomography, and conjunctival CD8+ lymphocyte detection. **Results:** In Branch 1 (comparative analysis before and after HSCT in Group 1), statistically significant differences were found in the following variables: best-corrected visual acuity (BCVA) OD ($P = 0.08$), OSDI test ($P = 0.003$), TBUT OU (OD $P = 0$, OS $P = 0.0003$), Oxford test OU (OD $P = 0.01$, OS $P = 0.0049$), and CD8+ lymphocytes OU (OD $P = 0.003$, OS $P = 0.01$). In Branch 2 (comparative analysis between Group 2 and 3), the variables with statistically significant differences ($P < 0.001$) in OU were: BCVA, OSDI test, Schirmer's test OU, TBUT test, Oxford test, and CD8+ lymphocytes. Finally, in Branch 3 (comparative analysis between Group 1 after HSCT and Group 2), statistically significant differences ($P < 0.001$) were found OU: in OSDI test, Schirmer's test, and Oxford test OU; and with $P < 0.005$ in TBUT test OU. **Conclusion:** In our study, statistically significant changes were observed in the OSDI test, TBUT test, Oxford Scale, and the detection of CD8+ lymphocytes in patients who underwent HSCT. Differences were more significant in those patients who had developed GvHD after HSCT compared to those without GvHD.

Key words: Allogeneic stem cell transplantation, conjunctival CD8+ lymphocyte detection, graft-versus-host disease, OSDI test, Schirmer's test

Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used for the treatment of malignant hematological diseases. However, HSCT is not without complications. The mortality rate ranges from 2%–10%, with graft versus host disease (GvHD) being the most common complication. GvHD is reported to occur in 30%–70% of patients who have undergone HLA-identical donor transplantation.^[1,2] The antigen differences between the host and the donor result in an immunological battle, mostly involving T lymphocytes. This process releases a series of mediators causing tissue damage.^[3]

Historically, GvHD has been defined as acute if it appears within 100 days of the HSCT and chronic if it appears more than 100 days after the procedure.^[4] Currently, the criteria are based on clinical and histological rather than chronological findings.^[5]

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In order of importance, the characteristics predisposing to GvHD are^[6,7]: greater disparity of minor HLA, elderly patients, male patients, transplant from a female donor to a male host, peripheral blood stem cell transplantation, lesser time on treatment with conditioning regimen to the HSCT, and baseline hematological disease decompensation.

GvHD diagnosis is normally based on clinical findings. The most frequently affected organs are the skin (81%), digestive tract, and liver.^[5] Depending on the seriousness, 30%–70% of GvHD patients develop ocular complications, especially those with chronic GvHD.^[8,9] Ocular surface is most frequently involved. The most frequent diagnosis is keratoconjunctivitis sicca.^[10,11] Conjunctival scarring changes and superior limbic keratoconjunctivitis are also commonly described. Severe GvHD-associated ophthalmic conditions include neurotrophic

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ulcer, corneal melting, and impending ocular perforation. A comprehensive ophthalmological study is required.

The standard treatment of GvHD is based on systemic immunosuppressants.^[9] Treatment for ocular involvement requires correct ocular lubrication, topical antiinflammatory therapy, and controlling severe complications by means of amniotic membrane transplantation, punctal plugs, tarsorrhaphy, etc.

In the present study, we report the ocular surface characterization after allogeneic hematopoietic stem cell transplantation in patients who have undergone HSCT, with and without GvHD.

Methods

Field of research

Patients were recruited from March 2015 to March 2016. The study subjects were patients with a diagnosed hematological condition who had undergone HSCT in our center, a reference center from the South of Spain that covers a population of 1,500,000 subjects.

Research subjects

The study involved three groups of patients with different characteristics. Group 1 (HSCT without GvHD) comprised 52 eyes from 26 patients from the whole reference area. These patients had a previously diagnosed hematological condition and had undergone HSCT. They were examined before and 100 days after the procedure. Group 2 comprised 28 eyes from 14 patients from the whole reference area who were previously diagnosed of the hematological condition and had undergone HSCT and had developed chronic GvHD. Group 3 (control group) comprised 40 eyes from 20 subjects who were demographically similar to the previous groups. TBUT did not have any hematological or ophthalmological conditions whatsoever. The total number of eyes examined was 120, belonging to 60 patients. The study was subsequently divided into 3 branches. Branch 1 analyzed patients from Group 1 before and 100 days after HSCT. Branch 2 compared subjects from Group 2 (GvHD) with Group 3 (healthy individuals). Branch 3 compared subjects from Group 2 (GvHD) with patients from Group 1 after procedure (HSCT without GvHD).

Objectives

To analyze the differences in the best-corrected visual acuity (logMAR scale, Optotype ETDRS CHART 2, ZeissMeditec AG, Germany), ocular surface and corneal examination with slit lamp (ZeissMeditec AG, Germany), Schirmer's Test I (mm, Standardized Schirmer's slides, Alcon6201 South Freeway, Texas, USA), ocular surface disease index test (OSDI) (score), corneal staining with fluorescein eye drops (Oxford test score, Alcon Pharma, Friburg, Germany), tear break-up-time (TBUT) test, and sample collection of conjunctival impression cytology for the detection of CD8+ lymphocytes via immunofluorescence (CD8 Antibody, clonac8/144B de Dako, Glostrup, Denmark).

Data processing

All data were recorded on a PC with Windows 10. Texts were processed with Microsoft Word 2007 and the data archived in spreadsheets with Microsoft Excel 2005. The statistical

analysis was conducted with R (programming language) and the following statistical tests were carried out: Student's *t*-test for the continuous quantitative variables (or Wilcoxon tests if normality criteria were not met). For qualitative variables, the χ^2 test was used (or Fisher test if normality criteria were not met).

Ethical considerations

This study was conducted fully respecting the patients' fundamental rights as well as the ethical tenets concerning biomedical research with human beings. The international recommendations included in the Helsinki Declaration, and in its subsequent revisions, were fully observed. The treatment of personal data strictly observed current legislation in RD 223/2004 of the 6th of February and the Organic Law 15/1999 of the 13th of December on personal data protection.

Patient and public involvement

All were patients undergoing allogeneic hematopoietic transplantation at our center. All patients were informed about the study and received and signed a specific consent on it. The patients were included consecutively when they were going to undergo an allogeneic transplant in the case of Group 1 or patients who had already developed GvHD in the case of Group 2. The control group was designated according to demographic characteristics similar to the previous ones. All patients were informed about each of the tests in the study, the time of completion, and the relevant follow-up visits.

Results

Demographics

A total of 120 eyes from 60 patients were finally included. Three patients had to be excluded due to their decease before completing the study (Group 1). Mean patient age was 49.6 years (Group 1: 47; Group 2: 51; Group 3: 49 years). There is no statistically significant difference between the three groups. The majority of patients included were male (36 patients, 60%), (Group 1: 18; Group 2: 8; Group 3:10). Demographics results are presented in Table 1.

Comparison of the three branches

The following analysis is a comparison of the mean for each variable between different groups.

Branch 1 analysis compared the average difference for each variable between examination 1 (before the procedure) and examination 2 (100 days after) in Group 1 patients. BCVA (logMAR scale) before HSCT was 0.1 (OD) and 0.1 (OS); after HSCT, it was 0.2 and 0.2, respectively. The mean Schirmer's test value (mm) before HSCT was 18 (OD) and 18.5 (OS); after HSCT, it was 15.46 (OD) and 15.57 (OS). The mean OSDI test (score) was 11.2 before HSCT and 16.9 after the procedure. The mean Oxford test (score) was 0.11 (OD) and 0.2 (OS) before HSCT and 0.46 (OD) and 0.52 (OS) after HSCT. The mean TBUT test (seconds) was 11.68 (OD) and 11.18 (OS) before the procedure and 8.26 (OD) and 8.3 (OS) afterward. The number of CD8+ lymphocytes (absolute value) in the conjunctival sample was null before HSCT and 13 after the procedure (7 in OD and 6 in OS). The significant variables in the comparative analysis in Branch 1 were: BCVA for OD ($P = 0.05$), OSDI test ($P = 0.003$), TBUT test OU (OD: $P = 0$, OS: $P = 0.0003$), Oxford test OU (OD: $P = 0.01$, OS: $P = 0.0049$),

and CD8+ lymphocyte values OU (OD: $P = 0.003$, OS: $P = 0.01$). OSDI test and lymphocytes detection of Branch 1 results are represented in Fig. 1.

Branch 2 analysis compared the difference in the means for each variable between Group 2 (patients with ocular GvHD) and Group 3 (control group). For BCVA, the mean visual acuity (logMAR scale) in Group 2 was 0.2 (OD) and 0.1 (OS). In Group 3, the BCVA was 0.95 (OD) and 0.98 (OS). The mean Schirmer's test value (mm) in Group 2 was 9.4 (OD) and 8.64 (OS) and in Group 3, it was 26.4 (OD) and 25.8 (OS). The mean OSDI test (score) was 47.1 in Group 2 and 10.4 in Group 3. The mean Oxford test was 2.2 (OD) and 2.07 (OS) in Group 2 and 0 OU in Group 3. The mean TBUT test (seconds) was 6 (OD) and 5.7 (OS) in Group 2 and 12.1 (OD) and 12.3 (OS) in Group 3. The number of CD8+ lymphocytes (absolute value) in the conjunctival sample was 17 in Group 2 (9 OD and 8 OS) and null in Group 3. The significant variables in the comparative analysis in Branch 2 were: BCVA OU (OD: $P = 0.00001$, OS: $P = 0.0001$), OSDI test ($P = 0$), Schirmer's test OU (OD: $P = 0$, OS: $P = 0.0005$), TBUT test OU (OD: $P = 0$, OS: $P = 0$), Oxford test OU ($P = 0$), and CD8+ lymphocytes OU (OD: $P = 0.0005$, OS: $P = 0.0001$). Oxford scale, TBUT test, and lymphocytes detection results from Branch 2 are represented in Fig. 2.

Branch 3 analysis compared the difference for each variable between Group 2 (patients with ocular GvHD) and the second examination (100 days post-transplantation) in Group 1. The BCVA (logMAR scale) in Group 2 was 0.1 (OD) and 0.1 (OS) and in Group 1, it was 0.2 (OU). The mean Schirmer's test value (mm) in Group 2 was 9.4 (OD) and 8.64 (OS), whereas in Group 1, it was 15.46 (OD) and 15.57 (OS). The mean OSDI test (score) was 47.1 in Group 2 and 16.9 in Group 1. The mean TBUT test (seconds) values in Group 2 were 6 (OD) and 5.7 (OS), and in Group 1, they were 8.26 (OD) and 6.3 (OS). The number of CD8+ lymphocytes (absolute value) in the conjunctival samples was 17 in Group 2 (9 for OD and 8 for OS) and 13 in Group 1 (7 for OD and 6 for OS). The variables with statistical significance in the comparative analysis in Branch 3 were OSDI test ($P = 0.0005$), Schirmer's test OU (OD: $P = 0.0001$, OS: $P = 0.00015$), TBUT test OU (OD: $P = 0.00019$, OS: $P = 0.014$), and Oxford test OU (OD: $P = 0.0004$, OS: $P = 0.0003$). TBUT test results from Branch 3 are represented in Fig. 3.

Statistically significant results of the three branches are presented in Table 2.

Discussion

In the present study, a total of 120 eyes from 60 patients were examined: 56 eyes belonging to patients examined before

Table 1: Demographic results

Variables	GROUP 1 (26 patients)	GROUP 2 (14 patients)	GROUP 3 (20 healthy people)
Age (years old average)	47	51	49
Gender, male (%)	18 (69)	8 (27)	10 (50)
Hematologic condition (n/%)			
AML	7 (26.9)	7 (50)	
CML	1 (3.8)	1 (7.1)	
Myelofibrosis	1 (3.8)	1 (7.1)	
ALL	7 (3.8)	3 (21.4)	
Myelodysplasia	2 (7.6)	1 (7.1)	
HL	4 (15.3)	1 (7.1)	
NHL	3 (11.5)	-	
Type of donor HSCT (n/%)			
HLA identical donor	20 (77)	12 (85.8)	
Unrelated donor	6 (23)	2 (14.2)	

**HSCT (allogeneic hematopoietic stem cell transplantation); AML (acute myeloid leukemia); CML (chronic myeloid leukemia); ALL (acute lymphoblastic leukemia), HL (Hodgkin's lymphoma); NHL (Non-Hodgkin's lymphoma); HLA (human leukocyte antigen)

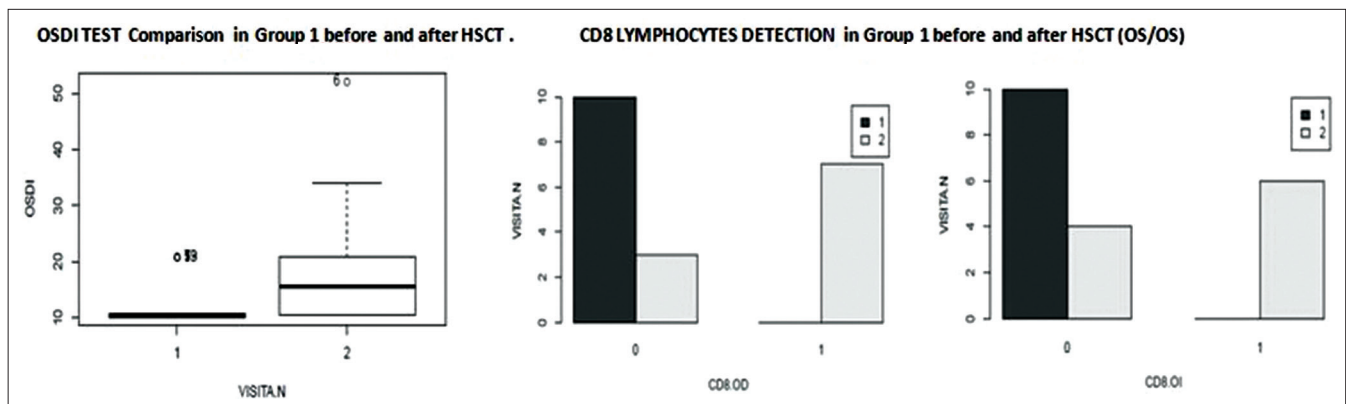


Figure 1: OSDI test and CD8 lymphocytes detection results in Branch 1

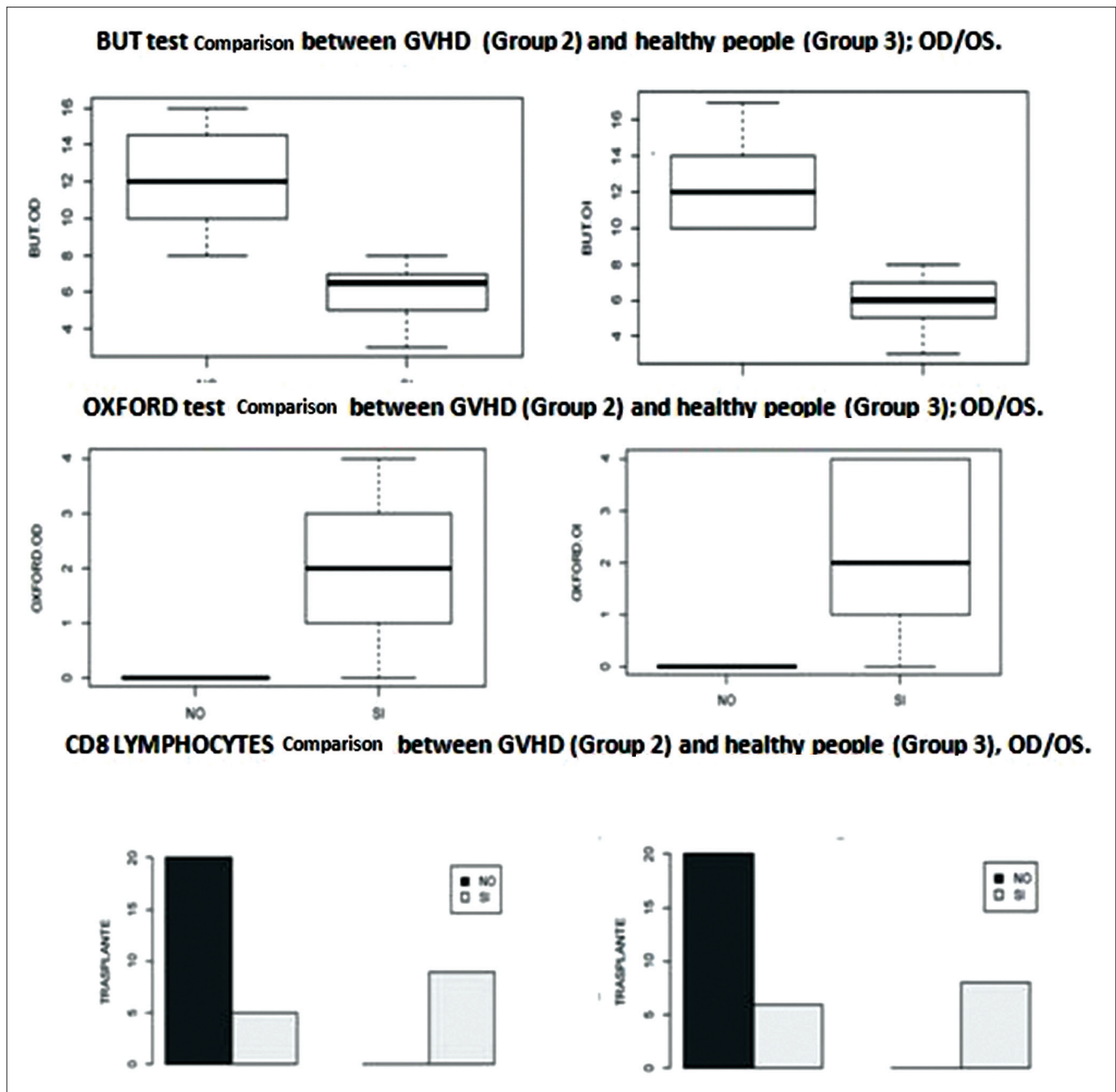


Figure 2: Oxford scale, TBUT test, and CD8 lymphocytes detection results in Branch 2

and 100 days after HSCT (Group 1), 28 eyes from patients with chronic GvHD (Group 2), and 40 eyes from 20 patients demographically similar to the previous groups without any hematological or ophthalmological condition. Concerning patient age, mean value was 49.6 years. There were no statistically significant differences between the three groups. The number of male patients was slightly superior (36, 60%) compared to female patients (24, 40%). This difference is consistent with the higher prevalence of hematologic diseases in men in Spain (21.7/100000 inhabitants).^[12]

Branch 1 (comparison before and after HSCT) showed no statistical significance in BCVA. This agrees with Allan *et al.*^[11] who reported that 96% of patients treated with HSCT (with GvHD) maintained the same BCVA as before, despite

changes in the ocular surface such as pseudomembranous conjunctivitis and fibrosis. The reported decrease in BCVA was only found in patients who developed cataract formation during the process. However, in our study, a statistically significant change in BCVA OU was found in our patients with evident ocular GvHD. This observation could be attributed to extreme values in BCVA in a few patients with associated retinal complications (Purtscher-like retinopathy). Therefore, our study suggests that BCVA is not affected by HSCT, even if the subject presents with GvHD.

The results of the Schirmer's test showed a reduction in secretion values, with significant differences in Branch 2 (comparison between GVHD and healthy individuals) and Branch 3 (comparison between patients who underwent HSCT

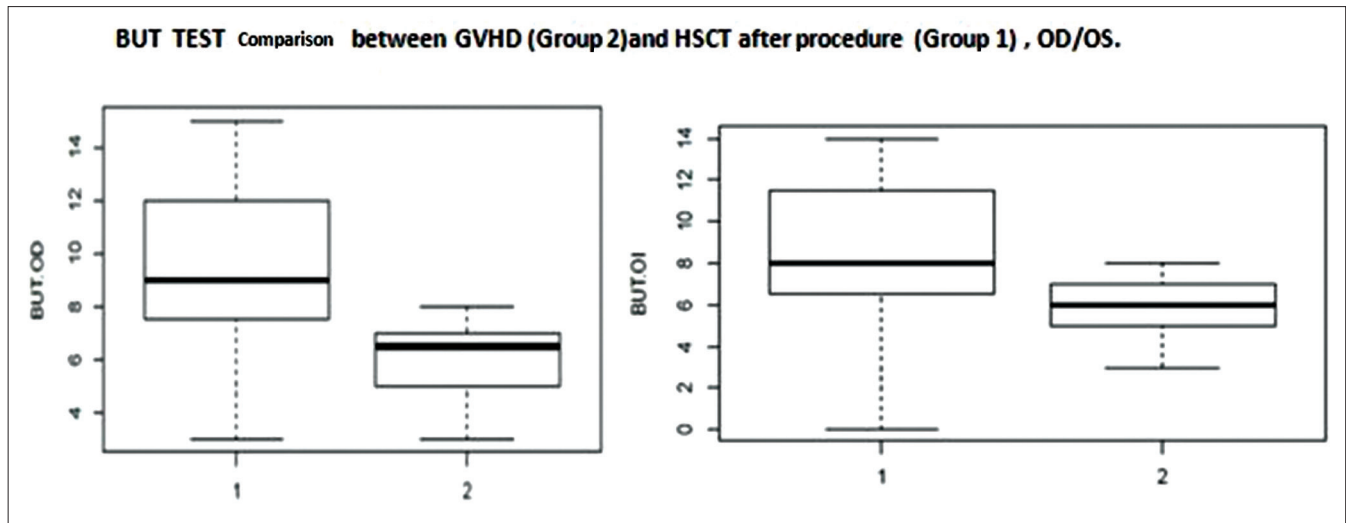


Figure 3: Schirmer's test and TBUT test results in Branch 3

after procedure with those who developed GVHD), TBUT not in Branch 1 (comparison before and after HSCT). Thus, the Schirmer's test results were clearly reduced in patients with ocular GvHD TBUT not in patients treated with HSCT who had not developed ocular GvHD. The Spanish National Health Institute has defined ocular GvHD as a Schirmer's test value < 5 mm associated with clinical repercussions in at least one organ. Wang^[13] suggested that Schirmer's test should be used in the early diagnosis of chronic ocular GvHD. The results of our study agree with this author.

The results of the OSDI test showed a statistically significant difference in the 3 branches, the most notable being Branch 2 (comparison between GvHD and healthy individuals) followed by Branch 3 (comparison between patients who underwent HSCT after procedure with those who developed GvHD). This could mean that treatment with HSCT generates a reduction in eyesight quality, which is more pronounced when there GvHD occurs. Wang^[13] defined a diagnostic consensus based on the OSDI test, which may be consistent with our results. The fact that Group 2 (GvHD) had greater OSDI values indicates a correlation between the clinical severity and the eyesight quality. Nassar *et al.*^[14] established a relationship between the OSDI results and the systemic prognosis. We believe that the relation between OSDI test and quality of life (Karnofsky scale)^[15] is worth studying. Clayton^[16] reported an increment in OSDI values when ocular GvHD is present. Our study suggests that the OSDI test can be altered at a subclinical level in HSCT, especially when GvHD occurs.

The TBUT test results showed a statistically significant decrease in all 3 branches, especially in Branch 2 (GvHD vs healthy individuals). These results matched our presumptive hypothesis: even if the teardrop evaporation was affected in all patients after HSCT, the change would be higher in patients with ocular GvHD (Group 1) compared to those who had not developed ocular GvHD. This is consistent with the results of Wang *et al.*^[13] who suggested that in GvHD, a systematic meibomian gland dysfunction could be found.

Regarding the Oxford Scale, Nassar *et al.*^[14] consider that the degree of punctate keratitis should be evaluated with the Oxford

test and compared to the Schirmer's test in order to establish a GvHD diagnosis if the patient presents clinical repercussions in any other organ. We believe that the clinical criteria proposed by Jack *et al.*^[17] for the classification of acute and chronic ocular GvHD are insufficient, because only the conjunctival involvement is considered, TBUT not the corneal impairment. This is also suggested by Xihui and Cavanagh^[18] who claim the need for monitoring the degree of keratitis of GvHD patients. A greater degree in the Oxford Scale is associated with a risk of severe corneal complications such as corneal ulcers and perforation, as well as a worsening of the life prognosis. In our study, we observed that the Oxford test presented higher results that were statistically significant in the 3 branches, the highest being in Branch 2 (GvHD vs healthy individuals), followed by Branch 3 (GvHD vs HSCT). This could mean that a higher Oxford Scale score could be found in patients who have undergone HSCT, especially those who had already developed ocular GvHD. We observed that in healthy individuals and in those patients with a hematological condition before the HSCT, keratitis was absent; however, almost all patients presented with keratitis after the transplantation.

We intended to use the detection of CD8+ lymphocytes in the conjunctiva via immunohistochemistry as a marker for subclinical inflammatory response. In 13 of the 20 samples analyzed in Branch 1 (before and after HSCT) and 17 of 28 samples in Branch 2 (GvHD vs healthy individuals), CD8+ lymphocytes could be detected in the conjunctiva, whereas none could be found in the control group. In Branch 3 (HSCT without GvHD and HSCT with GvHD), conjunctival CD8+ lymphocytes could be found in both groups, with the Group 2 (HSCT with GvHD) patients having greater positive samples, as they presented greater inflammatory activity. We found statistically significant differences in Branches 1 (patients before and after HSCT) and 2 (HSCT with GvHD patients vs healthy individuals). This finding could suggest that, independently of the existence of ocular GvHD, CD8+ lymphocytes are found in cytology because of HSCT. Weisdorf^[2] described the pathophysiology of acute GvHD. However, in chronic GvHD (associated with ocular disease), the mechanism remains unclear. Nassar *et al.*^[14] suggested that the depletion of regulator lymphocytes could be the cause,

Table 2: Statistically significant results in Branch 1, Branch 2, and Branch 3

Variables Branch 1	Average visit 1 Group 1	Average visit 2 Group 1	Statistical significance
BCVA (LogMAR)			
OD	0.1	0.1	0.05
OXFORD TEST (score)			
OD	0.11	0.46	0.01
OS	0.2	0.52	0.00049
TBUT TEST (seconds)			
OD	11.68	8.26	0
OI	11.18	8.3	0.00003
OSDI TEST (score)	11.2	16.9	0.003
Variables Branch 2	Average group 2	Average group 3	Statistical significance
BCVA (LogMAR)			
OD	0.2	0.0	0.00001
OS	0.2	0.0	0.00001
SCHIRMER (mm)			
OD	9.4	26.4	0
OS	8.64	25.8	0.0005
TBUT TEST (seconds)			
OD	6	12.1	0
OI	5.7	12.3	0
OXFORD TEST (scale)			
OD	2.2	0	0
OS	2.07	0	0
CD8+ (absolute value)			
OD	9	0	0.0005
OI	8	0	0.0001
OSDI TEST (score)	47.1	10.4	0
Variables Branch 3	Average group 2	Average visit 2 Group 1	Estatistical significance
OXFORD test (Scale)			
OD	2.2	0.46	0.0004
OS	2.07	0.44	0.0003
SCHIRMER (mm)			
OD	15.45	9.4	0.0001
OS	15.57	8.64	0.00015
TBUT TEST (seconds)			
OD	8.26	6	0.00019
OI	6.3	5.7	0.014
OSDI TEST (score)	16.9	47.1	0.0005

while Shikari *et al.*^[19] advocated for a mechanism similar to other autoimmune diseases such as Sjögren syndrome, where activated T lymphocytes cause a cascade of tissue fibrosis-inducing markers. Eberwein *et al.*^[20] described the presence of CD8+ lymphocytes in subjects who had undergone HSCT suffering from active ocular disease. However, there is no evidence in the literature of CD8+ lymphocytes after HSCT in asymptomatic patients. Based on the suggestions of Eberwein *et al.*,^[20] we performed cytology to demonstrate the presence of CD8+ lymphocytes in HSCT patients as an early marker of an inflammatory response, before the appearance of symptoms. Considering our goals, we must admit the fact that Branch 1 (patients before and after HSCT) was too small and that the inflammatory response could be attributed to factors other than the HSCT, such as the immunosuppressant treatment.

Future research on the correlation between with the HSCT and conjunctival CD8+ lymphocytes is required.

Conclusion

In the present study, we performed a characterization of the ocular surface in patients who had undergone allogeneic HSCT. In conclusion, in our study sample, statistically significant changes could be observed in the OSDI test, TBUT test, Oxford Scale, and the detection of CD8+ lymphocytes in patients treated with HSCT without GvHD. Schirmer's test and Oxford scores were more affected in those patients who had undergone HSCT and had developed GvHD and subsequently could be used to monitor the activity of GvHD. However, TBUT test, OSDI test, and conjunctival CD8 lymphocytes were altered in

patients treated with HSCT who had not developed GVHD and could be considered preclinical traits of GvHD and/or biomarkers of inflammatory activity. The combination of these tests could be a useful screening tool for GvHD and could be used for the initiation or increase of (intensive) ocular surface treatment/immunosuppressive conditioning.

Herein, we propose a combination of simple, noninvasive, routinely-performed tests for the screening of patients of GvHD that could be used in patients who have undergone allogeneic HSCT.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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