Longitudinal Analysis of Infrared Meibography in Patients Undergoing Hematopoietic Stem Cell Transplantation

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Purpose: To evaluate meibomian gland (MG) changes in patients undergoing hematopoietic stem cell transplantation (HSCT) by infrared meibography and to further investigate possible correlations with hematological characteristics.

Methods: Thirty-three patients were included: infrared meibography of the lower eyelid, Schirmer test, tear break-up time, ocular surface staining, and Ocular Surface Disease Index questionnaire were conducted before (V0) and 4 months after HSCT (V1). A paired samples *t* test was used to compare parameters before and after HSCT. A mixed analysis of variance was used to assess the effect of hematological characteristics on changes of MG loss (MGL) after HSCT.

Results: MGL and corneal staining significantly increased after HSCT (respectively, from 24.3% ± 10.1% to 32.2 ± 15.0 and from 1.2 ± 1.5 to 2.0 ± 1.7; always $P < 0.011$), whereas tear break-up time significantly decreased (from 6.6 ± 4.2 seconds to 3.2 ± 2.2; $P < 0.001$). At V1, 19 patients (57.6%) belonged to ocular graft-versus-host disease severity grade 0, 8 (24.2%) to grade I, and 6 (18.2%) to grade II. The percentage of MGL at V0 and the increase of MGL from V0 to V1 did not differ between patients who developed ocular graft-versus-host disease and those who did not (always $P > 0.05$). At V1, MGs' quality reduced in 16 patients (48.5%), remained unchanged in 14 (42.4%), and improved in 3 (9.1%). The increase of MGL after HSCT was higher in patients receiving myeloablative conditioning regimen ($P = 0.005$).

Conclusions: MG function, loss, and quality significantly worsened after HSCT. Myeloablative conditioning regimen was associated with higher MGL.

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Ocular graft-versus-host disease (oGVHD) is one of the major complications after hematopoietic stem cell transplantation (HSCT), occurring in 40% to 60% of transplant recipients.[1–3] The ocular surface system represents the main target of oGVHD, and all the structures can be impaired at a various degree of severity.[1–5] Meibomian gland dysfunction (MGD) is one of the most frequent complications because of the functional impairment of meibomian glands (MGs) that leads to tear film instability and dry eye disease (DED).[6] Traditionally, MGD has been assessed by slit-lamp examination of the lid margins with the evaluation of secretion quality and gland expressibility. More recently, infrared meibography (IM) has been introduced in the clinical practice for the rapid and noninvasive evaluation of MGs.[7] Nowadays, this technique is widely used to detect various types of gland abnormalities occurring in the setting of MGD, including dropout, shortening, dilation, distortion, and vagueness.[8–10] Conversely, only few studies have used IM to investigate the changes of MGs occurring in patients who underwent HSCT.[11,12]

Recently, our group demonstrated that MG alterations were present in hematological patients already before HSCT, probably as a consequence of a multifactorial process that includes concomitant therapies (ie, chemotherapy/radiotherapy) and/or the underlying malignancy itself.[11,13] Because no information is available about MG features in the same patients before and after HSCT, the aim of this study was to perform a longitudinal analysis of IM in hematological patients undergoing HSCT and to further correlate MG changes with hematological characteristics.

MATERIALS AND METHODS

Design and Population

This study is a retrospective analysis of prospectively collected data. The study was conducted at a single tertiary-referral center (Ophthalmology and Hematology Units, S.Orsola-Malpighi University Hospital, Bologna, Italy). Data of consecutive hematological patients who underwent HSCT

between January 2017 and December 2018 were collected. The study was performed in accordance with the principles of the tenets of the Declaration of Helsinki and was approved by the local institutional review board. Written informed consent was obtained from all subjects included in the study.

Inclusion criteria were the diagnosis of hematological disorder requiring HSCT and age above 18 years. Exclusion criteria were as follows: history of ocular surgery, allergy, contact lens use, glaucoma medication, ocular infectious/autoimmune disease, onset of acute oGVHD, and no survival after HSCT. Two scheduled ophthalmological visits have been performed, respectively, 7 to 9 days before the beginning of the conditioning regimen (visit 0, V0), and 4 months after HSCT (visit 1, V1). At V0 and V1, the diagnosis of DED was ascertained using the modified Tear Film & Ocular Surface Society Dry Eye Workshop II Criteria: Ocular Surface Disease Index (OSDI) score >13 plus one among tear break-up time (TBUT) <10 seconds, Schirmer test score <10 mm/5', or corneal and conjunctival staining >0.[14] When needed, ocular treatment was prescribed already at V0, according to the DEWS management algorithm driven by DED severity.[15] Briefly, hyaluronic-based tear substitutes and lid hygiene were prescribed in mild–moderate cases (DED severity levels ≤ 2), whereas additional antiinflammatory therapy (steroids, cyclosporine A) was prescribed in severe cases (DED severity levels > 2). At V1, patients were diagnosed as affected by oGVHD if they satisfied the International Consensus Criteria on oGVHD.[16] Briefly, at V1, oGVHD was ascertained according to the International Consensus Criteria that give a score according to the value of the following parameters: Schirmer test, corneal staining, conjunctival injection, and OSDI. The diagnosis of oGVHD is made based on the presence or absence of systemic GVHD and the aggregate scores assessed.

Hematological Parameters

Patients were prepared for HSCT by receiving one of the following types of treatments: 1) myeloablative conditioning (MAC) regimen consisting of busulfan or total body irradiation (unfractioned 800 cGy from Linear Accelerator at low-dose rate), 2) reduced-intensity conditioning (RIC) regimen consisting in tiothepa 10 mg/kg—cyclophosphamide 60 mg/kg—fludarabine 60 mg/sm or melphalan–fludarabine. The type of pre-HSCT conditioning regimen was chosen based on patient characteristics including age and comorbidities and status of malignancy. In particular, the RIC regimen was preferred in elderly, comorbid, or highly pretreated patients, with the aim of reducing organ toxicity and transplant-related mortality. Data including the underlying hematological disorder, the time interval between hematological diagnosis and ophthalmological examination, the source of hematopoietic stem cells, the type of donor, the presence or absence of sex mismatch, and pre-HSCT therapies were collected and recorded.

Ocular Surface Workup

Before each visit, subjective symptoms of ocular discomfort were scored by the OSDI questionnaire.[17] At V0 and V1, tear production and stability were measured by Schirmer test type I and TBUT, respectively. After administration of 2 mL of 2% fluorescein dye, TBUT was performed measuring the interval in seconds between the last complete blink and the first appearance of a dry spot or disruption in the tear film. Slit-lamp examination with ocular surface staining was performed using the blue cobalt filter and a 7503 Boston yellow filter kit to enhance staining details: corneal staining was graded using the National Eye Institute score, whereas conjunctival staining by using the van Bijsterveld score.[18,19].

Infrared Meibography

Noncontact IM (Topcon SL-D701 equipped with the DC-4/BG-5 meibography system; Tokyo, Japan) was performed in the inferior eyelid of both eyes of all patients at V0 and at V1.[7] To standardize captured images, each photograph was obtained by the same examiner (F.M.) using standard values for magnification (×16), lighthouse angle (45 degrees), and luminous intensity. The following parameters were calculated: 1) MG loss (MGL), defined as the percentage of MG dropout area in relation to the total tarsal area of the lid, was calculated by means of a semiautomated analysis using the polygon selection tool of ImageJ (http://imagej.nih.gov/ij).[20] Meibomian glad dropout was defined as an empty space where a gland should have been observed (Fig. 1)[21]; 2) the local distribution of MGL, evaluated by analyzing separately the individual thirds (nasal, central, and temporal) of the tarsal plate, as already described[22]; and 3) MGs' "quality" was defined based on any possible changes in definition (fading and vague glands) and sharpness (tortuous, thinned/thickened glands) of MG margins detected by IM. It was subjectively evaluated between V0 and V1 and graded as 1) decreased, 2) steady, and 3) increased (Fig. 2). Meibography images evaluation was conducted by 3 masked observers (M.P., F.M., and G.G.). For the statistical analysis of MGL, the average of the 3 values obtained was used for the statistical analysis. For the analysis of the changes of MGs' quality, the result was confirmed when the interpretations of at least 2 of the 3 observers were in agreement.

Statistical Analysis

The SPSS statistical software (SPSS Inc, Chicago, IL) was used for the data analysis. Values are expressed as mean $\pm$ SD. Data from both eyes were collected, and the intraclass correlation coefficients between right and left eyes were calculated. The intraclass correlation coefficient of MGL before and after HSCT was, respectively, 0.903 (95% confidence interval: 0.740–0.964) and 0.953 (95% confidence interval: 0.876–0.983); thus, only the data from right eyes were taken into consideration for statistical purposes. The Shapiro–Wilk's test was used to assess the normality of data. A paired samples t test was used to compare ocular surface parameters before and after HSCT. Subsequently, a mixed analysis of variance was used to assess the effect of the conditioning regimen, source of hematopoietic stem cells, type of donor, and sex mismatch on the changes of MGL after HSCT. $P < 0.05$ was considered statistically significant.

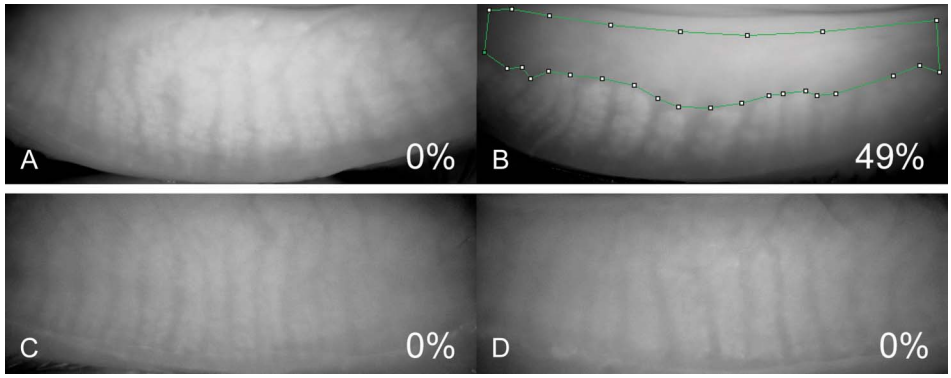

FIGURE 1. IM obtained from the lower eyelid of 2 representative patients before (V0) and 4 months after HSCT (V1). The images were analyzed using ImageJ software for the calculation of the percentage of MGL. In patient 1, the MG dropout was null at V0 (A) and increased to 49% at V1 (B). In patient 2, the MG dropout was null at V0 (C) and remained unchanged at V1 (D).

RESULTS

We screened a total of 54 patients who underwent HSCT during the study period. Of these, 33 patients fulfilled the inclusion criteria and were finally enrolled in the study. The remaining 21 patients were excluded from the analysis because of poor image quality (n = 13) or impossibility to attend the scheduled follow-up visits because of poor systemic conditions, recurrent malignancy, or exitus (n = 8). The demographic and clinical parameters of patients, as well as the characteristics of HSCT, are reported in Table 1. The mean time interval between HSCT and V1 was 4.3 ± 1.1 months (range 3–6). DED was diagnosed in 13 patients (39.3% of the total) at V0 (in all cases DED severity levels ≤ 2), whereas in 17 (51.6%) at V1 (in 11 cases DED severity levels ≤ 2; in 6 cases DED severity level 3). The ocular surface parameters before and after HSCT are reported in Table 2. In particular, MGL significantly increased after HSCT (from 24.3% ± 10.1% at V0 to 32.2 ± 15.0 at V1; $P < 0.001$) and TBUT significantly decreased (from 6.6 ± 4.2 seconds at V0 to 3.2 ± 2.2 at V1; $P < 0.001$), whereas corneal staining significantly increased (from 1.2 ± 1.5 to 2.0 ± 1.7, $P = 0.011$). Conversely, no significant changes were observed for Schirmer test, conjunctival staining, and OSDI score (always $P > 0.05$).

At V1, 19 patients (57.6%) belonged to the oGVHD severity grade 0, 8 (24.2%) to grade I, 6 (18.2%) to grade II. The percentage of MGL at V0 and the increase of MGL from V0 to V1 did not differ significantly between patients who developed oGVHD (severity grade I and II) and those did not (severity grade 0) (respectively, $P = 0.877$ and $P = 0.957$).

The analysis of the distribution of MGL over the 3 individual thirds of the tarsal plate showed that the dropout was higher in the nasal third (35.4% ± 23.7% at V0 and 52.2 ± 31.3 at V1), followed by the temporal one (30.3% ± 26.4% at V0 and 36.4 ± 29.8 at V1), and by the central one (17.8% ± 22.4% at V0 and 32.5 ± 21.8 at V1); however, the difference among the sectors was not significant ($P = 0.192$).

After HSCT, the quality of the MGs decreased in 16 patients (48.5%), remained approximately unchanged in 14 (42.4%), and increased in 3 (9.1%).

There was a statistically significant interaction between the type of conditioning regimen and the changes of MGL from V0 to V1. In particular, the increase of MGL after HSCT was higher in patients who underwent MAC compared with those who underwent RIC (respectively, from 25.8% ± 11.1% to 37.7 ± 16.7 and from 22.6 ± 8.8 to 25.7 ± 9.6; $P = 0.005$). Conversely, the source of hematopoietic stem cells, the type of donor, the presence of sex mismatch, and the previous therapies did not have any significant effects on the changes of MGL after HSCT (all $P > 0.05$).

DISCUSSION

The oGVHD is the result of the impairment of various components of the ocular surface system, including meibomian and lacrimal glands, conjunctival and corneal epithelia, and eyelids.[1,23–25] IM allows the rapid and noninvasive evaluation of MGs, detecting various abnormalities including dropout, shortening, dilation, and distortion in patients with MGD owing to different types of DED.[8] Among these alterations, the MG dropout has good diagnostic efficacy, corresponding to the loss of acinar tissue at the histologic level,[26] and being able to predict the response after treatment.[10]

MG dropout has been widely described also in the setting of chronic oGVHD,[11,27] and recent evidence showed that pathological values of MGL and impaired ocular surface parameters have been detected in hematological patients even before transplantation.[11,13,28] This finding is thought to be caused by a combination of factors, such as the infiltration of the glands by tumor cells and/or the effects of chemotherapy/radiotherapy that precede HSCT.

However, to date, limited information is available about the features of MG before and after transplantation in the same hematological patients who underwent HSCT. In fact, the only previous study that evaluated IM findings in patients before and after transplantation suffers from some limitations, such as the small population of study (patients with DED already before HSCT were excluded from the analysis), the short-term follow-up (3 months or less), and the subjective system of analysis of meibography images (meiboscore).[29]

In the present study, we conducted for the first time a longitudinal evaluation of meibography in hematological patients who underwent HSCT, using ImageJ software for the semiautomated computerized grading of MGL. In fact, it has already been showed that intraobserver and interobserver

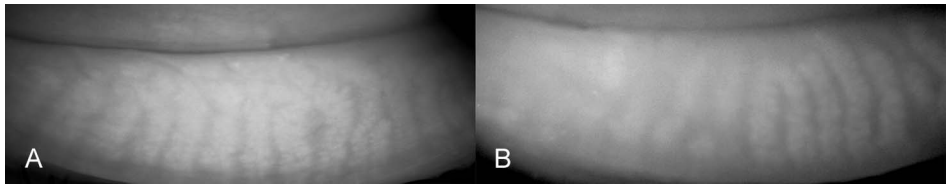

FIGURE 2. IM obtained from the lower eyelid of a representative patient before (A) and 3 months after HSCT (B). Note that MGs became less clear and demarcated after HSCT compared with baseline.

agreement is better in computerized grading compared with subjective scales.[20] In our study, we found that MGL increased significantly after HSCT; in parallel, also MGs function worsened and patients presented lower TBUT after HSCT compared with baseline. A recent work demonstrated that the order or stiffness of meibum is higher in patients with oGVHD compared with controls.[30] It is plausible that more ordered meibum could block the MGs forming aggregated islands of lipid on the tear film surface that hamper the spreading of the tear film lipid layer and contribute to the development or the worsening of tear film instability.[31]

TABLE 1. Demographic and Clinical Characteristics of Hematological Patients Undergoing HSCT

Characteristic	Number	% Versus Total
Patients	33	
Men	15	45.5
Women	18	54.5
Age (yrs)	46.5 ± 14.6	
Diagnosis		
AML	7	21.2
ALL	10	30.3
HL	5	15.1
MDS	6	18.1
MM	1	3.0
AA	2	6.0
PMS	2	6.0
Time from diagnosis to HSCT (mo)	19.4 ± 17.9	
Previous therapies		
Autologous HSCT	9	27.3
Radiotherapy	6	18.1
Chemotherapy	29	87.9
Number of cycles	4.5 ± 3.9	
Conditioning regimen		
MAC	18	54.5
RIC	15	45.5
Source of HSCT		
Peripheral blood	25	75.8
Bone marrow	8	24.2
Type of donor		
Matched unrelated donor	22	66.7
Matched sibling donor	11	33.3
Sex mismatch		
No	19	57.5
Yes	14	42.1

AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; HL, Hodgkin lymphoma; MDS, myelodysplastic syndromes; MM, multiple myeloma; PMS, primary myelofibrosis.

Regarding corneal fluorescein staining, post-HSCT values were significantly higher compared with the pre-HSCT ones. These results are in agreement with Kim and coauthors, who demonstrated the worsening of these parameters already in the early stage of the post-HSCT course.[29] On the contrary, the remaining ocular surface parameters did not change significantly. Surprisingly, the percentage of MGL at baseline and the increase of MGL after HSCT were not higher in patients who developed oGVHD compared with those did not. We believe that this finding could be related to the criteria used for reaching the diagnosis of oGVHD (ie, symptoms, Schirmer test, corneal staining, and conjunctival injection)[16] that do not include neither TBUT nor MGL.[32]

Surprisingly, no significant difference was found in the OSDI score, despite that it was found higher during post-HSCT visits compared with baseline values. We hypothesize that the absence of a significant worsening in OSDI score may be related to a combination of factors including small sample size, short-time interval after HSCT [usually symptoms worsen over time in patients with graft-versus-host disease (GVHD) during the first post-HSCT years] and the detection of pathological values of discomfort symptoms already before HSCT.

To analyze more in detail MGs appearance on infrared images, we calculated not only the percentage of dropout but also its distribution along the individual thirds of the tarsal plate. In fact, it has already been demonstrated that MGL shows an uneven distribution in the setting of conventional DED, with the nasal third more impaired compared with the other tarsal parts.[22,33] In the present study, this typical topographical distribution of MGL was present in our cohort of patients already at baseline and was then maintained over the post-HSCT course. The values of MGL recorded in population of hematological patients are higher if compared with those of patients with conventional MGD.[33] This difference highlights the peculiar picture of GVHD patients who show a more severe impairment of MGs.

TABLE 2. Ocular Surface Parameters in Hematological Patients Before and After HSCT

Parameter	V0	V1	*P*
MGL (%)	24.3 ± 10.1	32.2 ± 15.0	<0.001
TBUT (s)	6.6 ± 4.2	3.2 ± 2.2	<0.001
Schirmer test (mm/5')	21.9 ± 13.0	20.5 ± 12.3	0.553
Corneal staining (NEI score)	1.2 ± 1.5	2.0 ± 1.7	0.011
Conjunctival staining (VB score)	2.5 ± 2.8	2.8 ± 2.2	0.582
OSDI (score)	14.6 ± 14.0	17.5 ± 11.8	0.300

NEI, National Eye Institute; VB, van Bijsterveld.

Under normal conditions, MG structures can be easily observed and recorded with the IM system as grey-scale images: the glands are hypoilluminescent grape-like clusters, whereas ducts and underlying tarsus are hyperilluminescent. Conversely, it has been already demonstrated that MGs become vague and less well demarcated from the surrounding tarsus in the setting of MGD.[9,10,34] Given these findings, we hypothesize that the changes of "quality" of MGs might represent an additional parameter for longitudinal evaluation of patients who underwent HSCT. Because there is currently no validated software for this evaluation, we analyzed the changes of MGs' quality from V0 to V1 in a subjective fashion, showing that this parameter decreased after HSCT in almost half of patients, and MGs became progressively vaguer and less demarcated.

Among the various HSCT-related factors, only the type of conditioning regimen influenced the changes of MGL after transplantation, with higher MGL values at V1 recorded in patients who received the MAC regimen for the preparation of HSCT. This association is investigated, herein, for the first time. On the contrary, conflicting results are available in the literature regarding the effects of different types of conditioning regimens on the risk of developing oGVHD. In fact, some authors reported no association,[3,35] whereas others identified some conditioning regimens as an established risk factor for developing systemic GVHD.[36,37] However, these inconsistencies may be explained, at least partially, by different types of protocols for GVHD prophylaxis. In fact, the intensity of conditioning regimens can vary substantially, and when selecting the optimal conditioning regimen for any given patient, disease-related factors such as diagnosis and remission status, as well as patient-related factors including age, donor availability, and presence of comorbid conditions, need to be considered.[38]

Reversibility of the MG dropout is controversial and previous evaluations showed conflicting results. Some studies showed an improvement of MG dropout after conventional treatment for MGD based on tear substitutes and lid hygiene,[10] or after topical diquafasol.[39] On the contrary, other studies did not show any favorable results of the MG dropout after a thermodynamic treatment for MGD.[40] Because it is reasonable to consider impossible to revitalize atrophic glands, the decrease of MG dropout after such therapy may be related to the improvement of the morphology and quality of ghost glands that become cleared and more distinguishable. In our series, the totality of patients who exhibited DED already before HSCT belonged to the mild–moderate severity level and therefore was treated accordingly with first-line DED therapy. However, although this treatment might have positively influenced MG features, the MG dropout worsened further after HSCT in our cohort of patients, highlighting that the detrimental effects of HSCT have manifested despite treatment.

The main limitations of the study are related to the small sample size and to the analysis MG features only in the lower eyelid to limit/avoid invasive maneuvers. A quantitative objective scale for measuring the intensity of quality of MGs in comparison to the tarsal plate is desirable and would add a new potential index for the interpretation of IM images.[34] Moreover, the study suffers from a short-term period of patient's examination and further studies with larger sample and longer follow-up are needed to confirm the effects HSCT on MG features. In conclusions, IM detected a significant increase of MGL in hematological patients who underwent HSCT. The dropout showed the uneven distribution along the tarsal plate similar to that one of conventional dry eye, with the nasal third more impaired compared with the other parts. Furthermore, also MGs' quality reduced after HSCT in almost half of patients, and the glands appeared vaguer and less visible. Among the various HSCT-related parameters, only the type of conditioning regimen influenced the MG dropout, with higher values of MGL recorded after HSCT in patients who received the MAC regimen for the prophylaxis of GVHD.

Meibography is still a relatively new technique, and grading scales as well as user-friendly digital analysis software are still evolving to better evaluate the features of MGs. Because meibography represents a useful tool to detect MG changes in patients who underwent HSCT, it should be incorporated in the ophthalmological workup of these patients.

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