Alma Mater Studiorum – Università di Bologna

Ocular Chronic Graft Versus-Host Disease after Hematopoietic Stem Cell Transplantation

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1. Abstract

Graft-versus-host disease (GVHD) is a potentially severe complication that may develop in different tissues including the eye after allogenic hematopoietic stem cell transplantation (HSCT).

The purpose of this research project was to evaluate ocular surface parameters of patients undergoing HSCT before and after transplantation, and to correlate them with clinical and HSCT features.

Data from the charts of ninety-three patients affected by hematological malignances undergoing HSCT were collected. Values of Ocular Surface Disease Index, Schirmer Test type I, Tear Film Break-up Time, ocular surface staining and Meibomian Gland Dysfunction score obtained before HSCT and 3-6 months after were retrieved from charts. Diagnosis and staging of Dry Eye disease (DED) was performed according to Dry Eye WorkShop criteria. GVHD was classified according to the National Institutes of Health (NIH) 2015 Criteria. Odds ratios for DED onset after HSCT were estimated for demographic, ocular, hematological and HSCT features.

Dry eye disease was diagnosed before HSCT in 50 (53%) of the patients, mostly of hyper-evaporative profile. After HSCT all ocular parameters significantly worsened with no change in DE profile. A 51% of incident cases (22 on the 43 non-DE subjects) were reported. Increasing recipient age and female sex, higher CD34+ cells infused, donor-recipient sex mismatch (males receiving from females), related donors, and peripheral blood cells as stem cell source were associated with a significant higher incidence of DED after HSCT. Systemic chronic GVHD was diagnosed in 42% while ocular GVHD in 35.5% of the patients, which decreased to 12% when taking into account only incident cases.

In conclusion, a high DE prevalence was shown already before HSCT. Therefore, an ocular surface assessment should be recommended already before HSCT for early DED diagnosis and treatment. This new protocol also could influence the real prevalence of ocular GVHD after HSCT and its severity.

Key words: Ocular Graft versus-host disease; Dry eye disease; Hematopoietic stem cell transplantation.

2. INTRODUCTION

2.1) The ocular surface

The ocular surface system consists of the cornea, conjunctiva, lachrymal and meibomian glands, nasolacrimal duct, and their associated tear and connective tissue matrices, as well as the eyelids and eyelashes, all integrated by continuous epithelia and interconnected nervous, endocrine, immune, and vascular systems (Figure 1).[1] This functional unit protects the eye from the external environment and provides for an optimal refractive surface of the cornea through the production of an efficient tear film.[2]

The *cornea* has the highest dioptric power of the optical complex; it needs to be avascular in order to be transparent and receive its nutrients through diffusion from the tear film and aqueous humor. The cornea is one of the body structures most densely innervated and the innervations comes from axons of the sympathetic ganglion and trigeminal ganglion. The epithelium is the external layer, underneath is Bowman's layer, stroma, the recently recognized Dua´s layer, Descemet's membrane and at the most inner level the endothelial cells.[3,4] The corneal epithelium is a stratified, non-keratinized squamous layer. It has three types of cells: the most external type of cells are the superficial epithelial cells, in the middle are the wing cells located on top of the inner layer which are the basal epithelial cells. Due to its histological nature, the epithelium has the primary function of providing a barrier to the cornea and to the entire eyeball. Bowman's layer is composed of thin, type I, III, V and VI collagen microfibrils. It is not an independent membrane, but a modification of the most superficial portion of the stroma of the cornea. The stroma represents the main support of the corneal structure and comprises up to 90% of its volume. This compartment is about 450 μm thick and contains nerves, stromal keratocytes with different morphology and type I and V collagen fibers. Dua's layer is a strong acellular layer in the pre-Descemet's cornea, made of 5 to 8 thin lamellae of tightly packed type I, IV, and VI collagen bundles

running in longitudinal, transverse, and oblique directions.[5] The Descemet's membrane represents the basal membrane of the posterior epithelium. It is formed by very thin filaments of type IV collagen, which are arranged in a very regular pattern. The endothelium is a monolayer of cells that aids in keeping the corneal transparency not only by its barrier function, but also by its ionic pump function.

The *conjunctiva* consists of an epithelium and an underlying loose connective tissue, known as the lamina propria; both are separated by the epithelial basement membrane. The epithelial histology is stratified non-squamous and consists of two-to-three cell layers having cuboidal morphology in most parts. The lamina propria is rich in bone marrow-derived cells that form a mucosal immune system known as the conjunctiva-associated lymphoid tissue and of blood vessels of different kinds. Apart from capillaries and lymph vessels, specialized high endothelial venules for the regulated migration of lymphoid cells are present in the conjunctiva.[6] They are a normal component of ocular lymphoid tissue, have a characteristic ultrastructure as in other lymphoid tissues, and express cell adhesion molecules.

The *tear film* covers the ocular surface, and provides major refractive power of the visual system, nutrition, lubrication and protection.[7] It forms a thin film layer of 8 μm thick. Although typically considered as formed of three layers (namely the external lipid layer, the central aqueous layer and the inner mucin layer), it is now recognized that the tear film is more a lipid boundary layer with aqueous phases incorporating differing concentrations of mucins throughout. Meibomian and Moll glands produce the lipid component, mainly wax esters, triglycerides, free fatty acids, as well as neutral diesters. Lachrymal glands produce the aqueous component and goblet cells which are located in the conjunctiva, secrets the mucin and contains membrane associated glycoproteins. Other components of the tear film are metabolites, proteins and electrolytes. Interestingly, the proteins contained in the tear film take part in other processes, for instance, they work as antimicrobials, anti-inflammatories and also help in healing processes after trauma, as well as mechanical protection to the surface of the cornea.
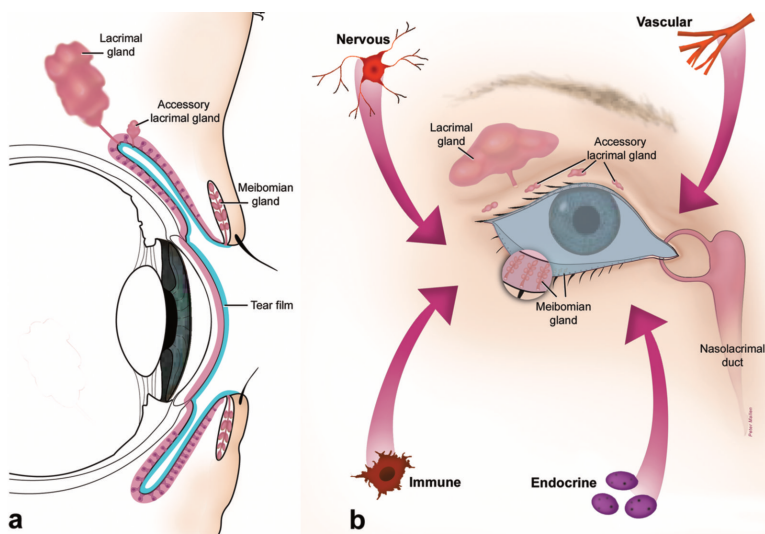
*Figure 1 - Diagrams depicting the Ocular Surface System from Gipson et al. (**A**) Sagittal section through the Ocular Surface System showing that the ocular surface epithelium is continuous (in pink) with regional specializations on/in the cornea, conjunctiva, lacrimal and accessory lacrimal glands, and meibomian gland. Each specialized region of this ocular surface epithelium contributes components of the tear film (in blue). (**B**) Frontal view of the Ocular Surface System, which includes the surface and glandular epithelia of the cornea, conjunctiva, lacrimal gland, accessory lacrimal glands, and meibomian gland (note enlarged lower lid segment) and their apical (tears) and basal connective tissue matrices, the eye lashes, those components of the eyelids responsible for the blink, and the nasolacrimal duct.*

2.2) Ocular surface disease

Factors disturbing the delicate homeostatic balance of the ocular surface system can adversely affect tear film stability and osmolarity, resulting in cellular osmotic, mechanical, and inflammatory damage with Dry Eye Disease (DED) onset.[8] DED is the most frequent disorder in ocular surface system and in Ophthalmological practice in general, with its prevalence ranging from 10.8 % to 57.1 % depending on the population analyzed and on the diagnostic criteria used.[9-11]

The updated Dry Eye WorkShop (DEWS) definition states that *"dry eye is a 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".[8] The major classes of DED are aqueous tear-deficient and evaporative. The first category refers chiefly to a failure of lachrymal gland secretion; the latter has been divided to distinguish those causes that are dependent on intrinsic conditions of the lids and ocular surface and those that arise from extrinsic influences, for example from environmental, occupational and pollution.

There are a lot of tests performed to diagnose DED and to monitor the efficacy of the related therapy.[12] The main tests are summarized below:

- *Schirmer Test type I* is obtained by putting paper strips over the lower lid margin, midway between the middle and outer third and by evaluating the measurement of the wet paper after 5 minutes. It is an estimation of tear flow production stimulated reflexly by insertion of a filter paper into the conjunctival sac. Values higher than 10 sec/5 min are considered normal.

- *Tear Film Break-Up time (TBUT)* is defined as the interval in seconds between the last complete blink and the first appearance of a dry spot, or disruption in the tear film. It is an index of tear stability. Values higher than 10 sec are considered normal.

- *Ocular surface staining* is used to show the damaged areas of cornea and conjunctiva where colorants can deposit. Usually in the clinical practice fluorescein is used to grade the staining of the cornea while lissamine green to grade the staining of the bulbar conjunctiva. Three systems for quantifying staining of the ocular surface are currently used, the van Bijsterveld system,[13] the Oxford system[14] and a standardized version of the National Eye Institute/Industry Workshop system.[15]

- *Tear osmolarity* is obtained by the collection of small nanoliter tear sample by a standard micropipette, then automatically transferred to a chip surface. A precise readout is obtained in seconds after the transfer. Values lower than 290 mOsmol/L are considered normal.

- *Ocular Surface Disease Index (OSDI)* is the most used questionnaire about subjective symptoms

employed in the clinical practice to assess the efficacy of such treatment or to grade disease severity; it consists of 12 questions about 3 major items: visual function (6 questions), ocular symptoms (3), environmental triggers (3). It is validated in dry eye population and used as outcome measure in randomized clinical trials.[16] Scores lower than 12 are considered normal.

2.3) Graft versus-host disease

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is utilized primarily as a curative treatment for both hematological and non-hematological malignancies.[17] In the former disease, the *graft-vs-leukemia or graft-vs-tumor* effect mediated by donor-derived T cells helps to eliminate malignant cells in the transplant recipient.[18] Graft-versus-host disease (GVHD) is a potentially severe complication that may develop after allo-HSCT, with its prevalence ranging from 30% to 70% of transplanted patients. A recent analysis by the Center for International Blood and Marrow Transplant Research of more than 26000 allo-HSCT patients demonstrated that the incidence of GVHD is increasing worldwide.[19] In allo-HSCT patients, GVHD is the most common cause of non-relapse mortality, which refers to mortality not related to the primary malignancy or disease, among patients surviving more than two years.[20] Chronic GVHD manifests as an autoimmune-like inflammatory disease and occurs when donor T cells respond to genetically defined proteins on host cells. The most important proteins are Human Leukocyte Antigens (HLA),[21] which are highly polymorphic and are encoded by the major histocompatibility complex (MHC). Class I HLA (A, B, and C) proteins are expressed on almost all nucleated cells of the body at varying densities. Class II proteins (DR, DQ, and DP) are primarily expressed on hematopoietic cells (B cells, dendritic cells, monocytes), but their expression can be induced on many other cell types following inflammation or injury. Despite HLA identity between a patient and donor, approximately 40% of patients receiving HLA-identical grafts

develop GVHD due to genetic differences that lie outside the HLA loci, or "minor" histocompatibility antigens (HA). Based on an early Seattle experience, *acute GVHD* was defined to occur prior to day 100, whereas *chronic GVHD* occurred after that time.[22-24] This definition is far from satisfactory, and more recently National Institutes of Health (NIH) classification includes late-onset acute GVHD (after day 100) and an overlap syndrome with features of both acute and chronic GVHD.[25,26]

Chronic GVHD, the major cause of late non-relapse death following HSCT, may be progressive (active or acute GVHD merging into chronic), quiescent (acute GVHD that resolves completely but is later followed by chronic GVHD) or it may occur de novo. The best documented risk factors for chronic GVHD are a history of acute GVHD (seen in 40%-60% of cGVHD patients), the use of peripheral blood stem cells (PBSCs) as source of transplantation, a female donor-male recipient combination, older patient age and the use of HLA-mismatched or unrelated donors.[26-28] The manifestations of chronic GVHD are somewhat protean, and are often of an autoimmune nature. Clinical signs often first appear in the buccal mucosa. New consensus criteria for the diagnosis and staging of chronic GVHD have recently been developed.[26]

2.4) GVHD and the eye

Ocular tissues affected by acute and chronic forms of GVHD include the eyelid and periorbital skin, conjunctiva, cornea, lens, lacrimal system, sclera, uvea, and retina. Cataract formation is a common late complication of allo-SCT. It is mainly attributed to irradiation and steroid therapy, and is the most common cause of visual acuity loss among this type of patients.[29,30] Patients receiving total body irradiation are at higher risk of developing cataracts than recipients of fractionated total body irradiation (83% vs. 21% at 6 years); it tends to develop much earlier in the former group as well. Nonetheless, most surviving patients will eventually require cataract surgery. Other ocular

manifestations of GVHD include cutaneous complications such as eyelid dermatitis, lagophthalmos and ectropion, poliosis, madarosis, and vitiligo.[29] Uveitis can occur in up to 8% of cases with chronic GVHD, and it is important to distinguish infectious etiologies or neoplastic masquerade syndrome from noninfectious uveitis. Neuro-ophthalmologic complications such as disc edema are likely secondary to the toxic effects of chemotherapeutic agents such as cyclosporine A and/or coexisting medical conditions, and are usually reversible.[31] The main vitreoretinal complication seen in association with GVHD is retinal microvasculopathy that may occur in 10% of cases. Findings include optic disc edema, cotton-wool spots in the fundus, intraretinal and vitreous hemorrhage, and lipid deposits. Posterior segment complications also include infections such as infectious retinitis from cytomegalovirus (CMV), herpes simplex virus, or varicella zoster virus, central serous chorioretinopathy and posterior scleritis.[29]

2.5) GVHD and the ocular surface

Throughout the NIH Criteria, diagnostic signs and symptoms refer to those manifestations that establish the presence of chronic GVHD without need for further testing or evidence of other organ involvement. Distinctive signs and symptoms of chronic GVHD refer to those manifestations that are not ordinarily found in acute GVHD but are not considered sufficient in isolation to establish an unequivocal diagnosis of chronic GVHD. Additional testing, such as a biopsy documenting histological features of chronic GVHD (or at least "likely" chronic GVHD), is needed to establish the diagnosis of chronic GVHD. Other features or unclassified manifestations of chronic GVHD define the rare, controversial, or nonspecific features of chronic GVHD that cannot be used to establish the diagnosis of chronic GVHD. Signs and symptoms found in both chronic and acute GVHD are referred as common features.

Distinctive manifestations of chronic ocular GVHD include new onset of dry, "gritty," or painful eyes, cicatricial conjunctivitis, keratoconjunctivitis sicca (KCS), and confluent areas of punctate keratopathy. Other features include photophobia, periorbital hyperpigmentation, and blepharitis (erythema and edema of the eye lids and telangiectasia of lid margin). New ocular sicca documented by low Schirmer's test with a mean value of 5 mm at 5 minutes (preferably with confirmation of normal values at an established baseline) or a new onset of KCS by slit lamp examination with mean Schirmer's test values of 6 to 10 mm (preferably with confirmation of normal values at an established baseline) not due to other causes is sufficient for the diagnosis of ocular chronic GVHD for the purpose of treatment and for clinical trials designed specifically for ocular GVHD, but an additional distinctive feature is necessary to establish eligibility for general chronic GVHD trials (Table 1).

Table 1 – Signs and symptoms of chronic ocular GVHD necessary to reach the diagnosis

Signs and Symptoms of chronic GVHD

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive* (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities[†]	Common[†] (Seen with Both Acute and chronic GVHD)
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis KCS Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	

Patients with ocular symptoms before transplantation should be evaluated by an Ophthalmologist for assessment of ocular surface abnormalities, including presence of KCS, conjunctival scarring, and inflammation. Some Experts strongly encourage baseline evaluation after transplantation (approximately day 100).[32,33] The scoring of ocular involvement includes the number of times a patient has to use lubricant eye drops each day. The International Consensus Guidelines on ocular GVHD have proposed a more detailed scoring schema, which involves comprehensive ophthalmological evaluation,

including pre-transplantation evaluation.[33] These remain to be validated and should be considered in clinical trials addressing ocular involvement. Schirmer's test may be useful for diagnosis of ocular GVHD, but the numerical values are not useful for follow-up of ocular GVHD due to poor correlation with symptom change.[34] For this reason, Schirmer's test values have been removed from the scoring form in the current recommendation.

EXPERIMENTAL SECTION

3) Purpose

Several studies described ocular surface impairment in hematological patients after HSCT, and identified risk factors for the development of ocular GVHD.[32,35-39] Conversely, limited information is available about ocular surface changes after HSCT compared to pre-transplant baseline condition in the same subjects.[40]

Indeed, a comprehensive baseline ophthalmologic evaluation before HSCT has been recently recommended by the First International Chronic Ocular GVHD Consensus Group,[33] the German-Austrian-Swiss Consensus Conference[41] and the 2015 updated NIH Consensus Conference,[26] with the aim to classify the onset of ocular symptoms and signs only after HSCT as incident cases. On the contrary, if dry eye disease is already present before HSCT, current criteria to diagnose ocular GVHD may be not fulfilled and the diagnosis should not be reached.

Therefore, the purpose of the present study was to comprehensively evaluate ocular surface parameters in the same hematological patients before and after allogeneic hematopoietic stem cell transplantation, and to correlate them with clinical and transplant variables.

4) Materials and methods

Patients and transplant procedure

Data has been prospectively collected over the period March 2007 to March 2014. The study followed the tenets of the Declaration of Helsinki, and was approved by the Ethics Committee of the S.Orsola-Malpighi Teaching Hospital. Informed consent was obtained from all patients when returning for subsequent check-ups. In our routine practice, the two scheduled ophthalmological visits are performed before HSCT and conditioning regimen (V0) and in a time window ranging from 3 to 6 months after HSCT (V1).[42] Data from 203 patients undergoing HSCT at the Hematology Institute "L.A. Seragnoli", University of Bologna, S. Orsola-Malpighi Teaching Hospital, in Bologna (Italy) were retrieved. Only the 113 charts containing ophthalmological data to be included in the study analysis and collected at V0 and V1 were further selected. Cases excluded referred to: twenty-eight patients who had not received an ophthalmological visit in the first 6 months after HSCT due to poor general health conditions or for the occurred death; twenty-five patients whose charts had not been found to be fully completed; thirty-seven patients living out of our area who had been referred to another eye Center in the post-HSCT follow-up. Twenty further cases were excluded for the history of uveitis (n=4), retinitis (n=8), and concomitant use of eye drops for the treatment of glaucoma (n=8).

Ninety-three Caucasian patients fulfilled the criteria and were then finally included in the study. For the statistical analysis, patients were divided into two groups according to the underlying disease: chronic lymphoproliferative disorders (LPDs) including Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and stem cell malignancies (SCMs) including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS) and chronic myeloid leukemia (CML). The disease phase at transplant was classified as early and advanced. Patients with CML in first chronic phase, ALL and AML in the first complete remission and lymphomas in complete remission were considered as early

phase; all the remaining cases were considered as advanced phase. Twenty patients (21.5% of the total) received HSCT from HLA-identical siblings (Related Donors, RD) and seventy-three (78.5%) from voluntary unrelated donors (VUD). Human leukocyte antigen (HLA) matched were defined all the pairs 10/10 loci matched; if the matching was less than 10/10 they were classified as mismatch. Sources of hematopoietic stem cells were bone marrow (BM) in forty-one cases (44.0%), peripheral blood (PB) in 46 cases (49.5%) and cord blood (CB) in 6 cases (6.5%). In the analysis, stem cells from BM and CB were considered in the same group compared to PB. The intensity of conditioning regimen was standard in 64 patients (69% of the total) and reduced-intensity in 29 patients (31%). Conditioning regimens were busulfan-based (54 patients) or total body irradiation (TBI, unfractioned, 800 cGy from Linear Accelerator at low dose rate) -based (10 patients). Reduced intensity regimens were Tiothepa 10 mg/kg - Cyclophosphamide 60 mg/kg - Fludarabine 60 mg/sqm (20 patients) or Melphalan - Fludarabine (9 patients). All patients received GVHD prophylaxis with Cyclosporin-A and short term Methotrexate (days 1,3,6,11, with 15-10-10-10 mg/sm) or Mycophenolate mofetil (15 mg/kg bid from day +1 to day +30). In addition, all transplants from unrelated donors received antylymphocyte globulin (ATG-F, Grafalon, Bad Homburg, Germany) at 15-30 mg/kg total dose while only 11 (55%) patients receiving transplants from their HLA-identical sibling did. Ocular and systemic GVHD were diagnosed by the standard NIH criteria, i.e. the ocular involvement is only a distinctive manifestation and an additional distinctive feature of another organ is necessary to establish diagnosis.[25,26] Patients were allocated in single, air-positive pressure rooms with HEPA-filtered air. Anti infectious prophylaxis was accomplished with levoxacin and fluconazole during the transplant period and acyclovir and cotrimoxazole until the 9th month after transplant. In case of Cytomegalovirus (CMV) DNA positivity, CMV pre-emptive therapy was administered using gancyclovir or foscarnet if gancyclovir was contraindicated. All patients received filtered and irradiated blood products.

Ophthalmological examination

Ophthalmological examinations were always performed before HSCT and conditioning regimen (V0) and 3-6 months later (V1), as described elsewhere.[42] Briefly, subjective discomfort symptoms were graded 0 to 100 with the Ocular Surface Disease Index (OSDI) score. The ophthalmological examination was performed from the least to the most invasive test as it follows: tear stability was measured by Tear Film Break Up Time (TBUT, average of three measurements) using 2 μL sodium fluorescein (Fluoralfa 0.25%, Alfa Intes, Italy); corneal and conjunctival fluorescein stainings were assessed under cobalt blue illumination with the aid of a 7503 Boston yellow filter kit (equivalent to Kodak Wratten 12) to enhance staining details and graded according to the NEI (National Eye Institute) score and van Bijsterveldt score, respectively; tear production was estimated by the 5-minute Schirmer test performed with sterile strips without anesthetic (ContaCare Ophthalmics and Diagnostics, Gujarat, India).

Conjunctival injection was graded as previously described as stage I: hyperemia; stage II: hyperemia with serosanguinous chemosis; stage III: pseudomembranous conjunctivitis; stage IV: membranous/pseudomembranous conjunctivitis plus corneal epithelial sloughing.[43]

Meibomian gland dysfunction (MGD) was assessed to grade the quality, expressibility, and volume of gland secretion, according to the modified MGD scoring system proposed by Foulks and Bron (range 0-27).[44]

Classification of DE patients was based on a series of thresholds according to the Dry Eye WorkShop severity score[8] (DEWS, ranging from the less severe grade 1 to the most severe grade 4) and modified after Sullivan et al.[45] The criteria required evidence of symptoms, with an OSDI score > 5 and in addition, at least one eye had to exceed thresholds on two of the five subset signs, chosen from TFBUT < 8, Schirmer test ≤ 7, corneal staining > 0, conjunctival staining > 0, and MGD score > 5. The DE severity was assigned depending on the highest number of values falling under each grade. Post versus

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pre HSCT changes were defined as worsening if the increase of at least one point in the level grade occurred.

Ocular treatment

Ocular treatment was prescribed to patients affected by DED already at baseline according to DEWS guidelines driven by DED severity score. Briefly, hyaluronic-based tear substitutes, nocturnal ointment and lid hygiene were prescribed in mild-moderate cases (DEWS severity grade 1-2) while additional anti-inflammatory therapy (steroids − loteprednol etabonate 0.5% ophthalmic suspension [Lotemax, Bausch and Lomb,Rochester, NY], 4 times/day for 4 weeks),[46] - or cyclosporine - galenic preparation (0.1% in oil, 2 times/day)[47] was prescribed in most severe cases (DEWS severity grade 3-4).[48] The same rationale was used in patients developing or worsening DE condition after HSCT.

5) Statistical analysis

Statistical evaluation was performed by using the MedCalc and IBM SPSS ver. 20.0. Data from both eyes were collected from charts, but only the value from the worst eye was taken into consideration for statistical purposes. All data were expressed as mean ± SD and median (min value; max value) [95% confidence interval for the median].

Pre- and post-HSCT values changes were evaluated by Wilcoxon test for related samples, Mann-Whitney test for independent samples. For each ocular parameter the post versus pre-HSCT values and the differences between post and pre-HSCT values (D) were correlated to pre-HSCT values by Spearman Coefficient Correlation (small correlation strength 0.10 to 0.29; medium 0.30 to 0.49; large 0.50 to 1.00). Univariate (Chi-square test, Odds Ratio) and multivariate logistic forward regression analysis were used to assess the association between demographic, ocular, hematological and transplant related variables and DE post HSCT. Risk for DE was also estimated by odds ratio (OR) with 95% confidence intervals that independently associated the disease. Data were considered to be statistically significant if $p<0.05$.

6) Results

Demographic, hematological and ophthalmological data were summarized in Table 2. The median interval between HSCT and V1 visit was 115 days (97-150) [95-176] (median, 95% CI, min-max value). Patients showed a similar distribution between genders, with a not statistically significant difference between males and females. Stratification by gender is important because the prevalence of DE is significantly higher in females.[49]

Table 2 – Clinical and demographic features of subjects included in the study. Data are expressed as median (min-max values) [95% CI].

	Patients number	% vs total
DEMOGRAPHIC DATA		
Females	48	51.5
Males	45	48.5
Age (yrs)	46 (18-64) [43-48]	
HAEMATOLOGICAL HISTORY		
Disorders		
AML	28	30.0
ALL	19	20.5
HL	9	9.5
CML	9	9.5
NHL	9	9.5
MM	8	8.5
MDS	7	7.5
CLL	4	5.0
Time from diagnosis to HSCT (days)	281 (113-2783) [231-464]	
Disease stage		
Early	34	36.5
Advanced	59	63.5
Previous autograft	9	9.6
Previous chemotherapy		
≤ 3 cycles	40	43.0
>3 cycles	53	57.0
OCULAR HISTORY		
Contact Lens wearers	17	18.5
VDT users	12*	13.0
Previous ocular surgery	7	7.5
HSCT PARAMETER		
Donor characteristics		
Age (yrs)	32 (19-68) [28-33]	

VUD	73	78.5
HLA match	26	28
HLA mismatch	47	50.5
RD	20	21.5
Sex mismatch	45	48
Conditioning regimen		
Reduced	29	31
Myeloablative	64	69
Stem Cell Source		
Bone marrow	41	44
Peripheral blood	46	49.5
Cord blood	6	6.5

Abbreviations: VDT = Video terminal users; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; HL = Hodgkin lymphoma; CML = chronic myeloid leukemia; NHL = non-Hodgkin lymphoma; MM = multiple myeloma; MDS = myelodysplastic syndrome; CLL = chronic lymphocytic leukemia; VUD = Voluntary Unrelated Donors; RD = Related Donors

Pre-HSCT ophthalmologic parameters analysis

Forty-three patients (47%) were classified as non-DE subjects whereas fifty patients (53%) were classified as DE sufferers. Of these, 25 patients were classified as DEWS modified score 1 (50% of the total DE-subjects), 21 patients as score 2 (42% of the total) and 4 patients as score 3 (8% of the total) (Figure 2). Results of ophthalmological parameters for both non-DE (white rows) and DE patients (grey rows) were summarized in the left column of Table 3. The DE patients appeared to be moderately symptomatic according to OSDI, with a normal tear production as median and a hyper evaporative DE type with tear instability and pathological MGD scores.

Hyaluronic-based tear substitutes, nocturnal ointment and lid hygiene were prescribed to 46 patients (DEWS severity levels 1-2) while loteprednol etabonate 0.5% ophthalmic suspension was prescribed to the four most severe patients (DEWS severity level 3).

Post-HSCT parameters analysis

DE disease was present at the V1 visit in 72 patients (77% of the total): 31 patients (33%) were classified as DEWS severity level 1, 32 patients (34.5%) as DEWS level 2 and 7 patients (9.5%) as DEWS level 3 (Fig. 2).

After HSCT, a statistically significant worsening of ocular parameters compared to pre-transplant values was shown for both groups. Only conjunctival surface damage did not worsen in DE patients after HSCT, remaining comparable to values pre transplant. Despite the significant reduction, the Schirmer test as a median after HSCT resulted in the normal range, whereas the TFBUT as a median was found in the pathological range.

Among the post HSCT DE patients, 22 out of 43 non-DE pre HSCT subjects developed DE after: these incident cases showed different DEWS levels of severity as shown in Fig 2.
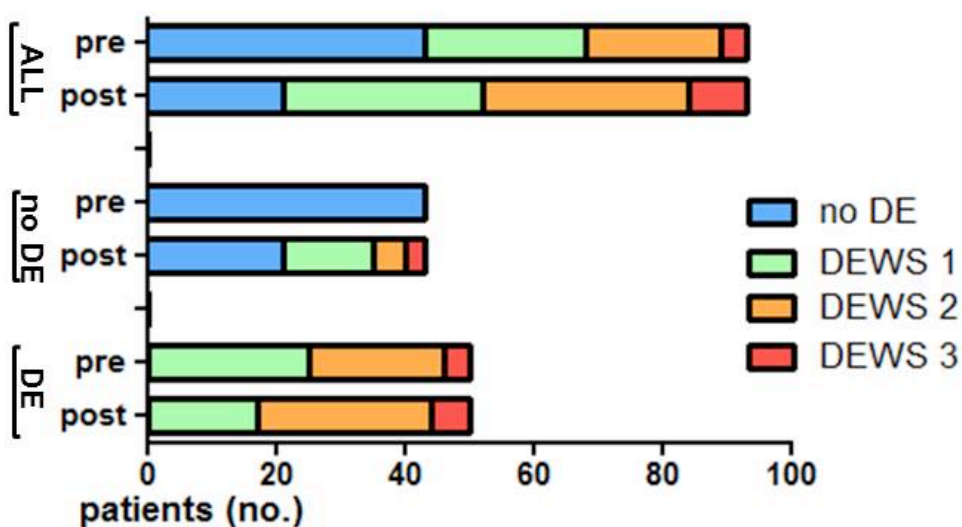

Fig. 2 –Distribution of dry eye disease in patients before and after HSCT. The distribution is shown for the whole population (ALL) in the upper part, for patients who had not been diagnosed as DE pre-HSCT (no-DE) in the middle part, and for patients who had been diagnosed as DE pre-HSCT (DE) in the lower part. Abbreviation: DE= Dry Eye

Also these DE patients could be classified as hyper evaporative DE type (mean values ± SD: Schirmer

test 20.4±12.5 mm/5'; TFBUT 6.3±2.1 sec; MGD score 7.5±4.5).   Results of ophthalmological parameters for non-DE (white rows) and DE patients (grey rows) were summarized in the right column of Table 3. Post HSCT ophthalmological parameters from these two groups of patients were compared; a statistically significant difference was found with worst values shown in the DE pre HSCT group of patients (p always <0.001).

Table 3 - Results from ophthalmological examinations in DE patients before and after HSCT. Values are expressed as mean ±SD and median (minimum value – maximum value) [95% Confidence Interval].

White rows - Results for the group of subjects not diagnosed as suffering from DE before HSCT

Grey rows - Results for the group of subjects diagnosed as suffering from DE before HSCT

Parameters	Pre-HSCT	Post-HSCT	p
OSDI score	6.4±7.4 4 (0-15) [2-6]	15.2±14.7 13 (2-56) [7-19]	<0.0001
TFBUT (sec)	13.1±3.0 15 (4-15) [12-15]	9.2±4.6 8 (1-15) [8-12]	<0.0001
Schirmer test (mm length/5')	30.1±12.1 40 (10-40) [30-40]	23.3±12.2 20 (2-40) [16-27]	<0.001
Conjunctival staining (van Bijsterveldt score)	1.15±1.74 0 (0-5) [0-1]	2.21±2.23 2 (0-8) [1-2]	<0.01
Corneal damage (NEI score)	0±0 0 (0-0) [0-0]	1.7±2.1 0 (0-6) [0-3]	<0.0001
MGD score	1.5±1.1 2.5 (1-4) [2-3]	6.5±5.5 7.5 (4-14) [7-12]	<0.0001
Conjunctival injection	0.2±0.2 0.4 (0-1) [0-1]	0.7±0.8 0.8 (0-2) [0-2]	<0.01
OSDI score	9.7±11.7 6 (6-59) [6-8]	18.2±17.1 15 (6-73) [12-20]	<0.0001
TFBUT (seconds)	6.8±2.3 7.5 (1-15) [7-8]	6.0±2.1 6.1 (1-12) [5-7]	<0.01
Schirmer test (mm length/5')	17.5±13.2 15 (9-40) [10-20]	15.2±11.2 10 (1-25) [0-12]	<0.01
Conjunctival staining (van Bijsterveldt score)	2.1±2.5 3 (0-9) [0-3]	3.6±3.1 3 (0-9) [0-3]	0.4
Corneal damage (NEI staining score)	1.9±2.0 2 (0-12) [1-2]	2.1±2.4 2 (0-12) [2-4]	<0.01
MGD score	6.5±3.5 7 (3-10) [5-8]	9.1±7.5 9.5 (5-16) [8-13]	<0.01
Conjunctival injection	0.5±0.5 0.6 (0-1) [0-1]	0.9±0.8 0.9 (0-2) [0-2]	<0.01

Abbreviations: OSDI = Ocular Surface Disease Index; TFBUT = Tear Film Break-Up Time; NEI = National Eye Institute; MGD = Meibomian Gland Dysfunction.

Thirty-four out of fifty DE pre HSCT patients did not change severity grade whereas the remaining 16 worsened their pre-HSCT DEWS severity level, mostly shifting from level 1 to level 2. The DE profile did not change after the transplant from hyper evaporative to aqueous deficiency or vice versa. Hyaluronic-based tear substitutes, nocturnal ointment and lid hygiene were prescribed to sixty-five patients (DEWS severity levels 1-2) while loteprednol etabonate 0.5% ophthalmic suspension was prescribed to the seven most severe patients (DEWS severity level 3).

Systemic chronic GVHD was diagnosed in 39 patients (42% of the total) while ocular GVHD in 33 patients (35.5% of the total) regardless the presence of DE pre HSCT, according to NIH Criteria. Conversely, the cases of ocular GVHD decreased to 11 cases (12%) in our population when taking into account only incident cases. Also ocular GVHD patients could be classified as hyper evaporative DE type.

Correlations

A statistically significant correlation between post versus pre transplant values was only found for Schirmer test (rho=0.512) and OSDI score (rho=0.461). The difference between pre and post-HSCT values (Δ) was calculated and correlated to the corresponding pre-HSCT value. Δ values of all ocular parameters showed a significant inverse correlation with pre-HSCT values: TFBUT (rho= -0.577), Schirmer test (rho= -0.605), OSDI (rho= -0.364), NEI score (rho= -0.525) and van Bijsterveldt score (rho= -0.534) (always $p<0.001$). This finding indicates a greater worsening after HSCT in patients who had already shown impaired parameters before HSCT.

Univariate analysis

In univariate analysis, increasing recipient age, recipient female sex and higher CD34+ cells infused

were associated with a significant higher incidence of DE after HSCT in those subjects not suffering from DE before HSCT (Table 4 A). Advanced disease stage at the time of HSCT, donor-recipient sex mismatch (males receiving from females), related donors, and peripheral blood cells as stem cell source were associated with a significant higher incidence of DE after HSCT in subjects either having or not having DE before HSCT (Table 4 A and B).

Table 4A - Univariate odds ratios (OR) for developing DE after HSCT in the subgroup of patients defined as not suffering from DE before HSCT.

VARIABLE	UNADJUSTED OR (95% CI)	P
Recipient age (years) [§]	1.04 (1.01-1.08)	0.03
Recipient gender (Female vs Male) [§]	1.43 (1.20-4.43)	0.01
Ocular variables		
VDT use	0.27 (0.10-1.11)	0.21
Previous eye surgery	0.21 (0.48-1.23)	0.32
Hematological variables		
Type of disease	0.57 (0.16-2.06)	0.39
Time from diagnosis to HSCT (> 6 months)	0.81 (0.28-2.34)	0.71
Advanced disease stage[§]	1.23 (1.08-3.55)	0.02
Previous chemotherapy medications (no. cycles)	1.06 (0.87-1.30)	0.50
Transplant variables		
Donor-recipient sex mismatch[§]	1.33 (1.12-4.47)	0.03
Donor age	1.07 (0.98-1.16)	0.07
Donor type (related donors) [§]	7.50 (1.56-35.9)	0.03
Stem cell source (PB cells) [§]	2.12 (1.49-5.02)	0.01
Intensity of conditioning regimen	0.71 (0.29-1.74)	0.45
HLA mismatch	0.43 (0.10-1.76)	0.24
CD34+ cells infused [§]	1.11 (1.09-1.92)	0.01
GVHD systemic °	1.23 (1.13-5.38)	0.01

§= factors which showed a positive association in predicting DE post HSCT in those patients without DE pre HSCT.
°= factor positively associated to DE development post HSCT in those patients without DE pre HSCT.

Table 4B - Univariate odds ratios (OR) for worsening DE after HSCT in the subgroup of patients defined as those suffering from DE before HSCT and worsening DE after HSCT.

VARIABLE	UNADJUSTED OR (95% CI)	P
Recipient age (years)	0.98 (0.93-1.03)	0.61
Recipient gender (Female vs Male)	0.98 (0.29-3.26)	0.92
Ocular variables		
CL wear	0.69 (0.21-2.24)	0.53
VDT use	0.25 (0.15-1.08)	0.28
Previous eye surgery	0.31 (0.18-1.23)	0.38
Hematological variables		
Type of disease	0.87 (0.22-3.40)	0.84
Time from diagnosis to HSCT (> 6 months)	0.90 (0.36-2.24)	0.83
Advanced disease stage	1.08 (1.02-4.71)	0.03
Previous chemotherapy medications (no. cycles)	0.94 (0.79-1.13)	0.55
Transplant variables		
Donor-recipient sex mismatch[§]	1.17 (1.11-1.61)	0.02
Donor age	1.02 (0.97-1.09)	0.30
Donor type (related donors) [§]	1.22 (1.05-2.42)	0.02
Stem cell source (PB cells) [§]	1.31 (1.21-1.89)	0.01
Intensity of conditioning regimen	0.80 (0.30-2.07)	0.55
HLA mismatch	1.26 (0.31-5.19)	0.74
CD34+ cells infused [§]	1.03 (0.99-1.04)	0.76
GVHD systemic °	1.51 (1.23-3.53)	0.03

§= factors which showed a positive association in predicting worst DE post HSCT in those patients with preexisting DE pre HSCT.
°= factor positively associated to DE worsening post HSCT in those patients with DE pre HSCT.

Multivariate analysis

Multiple analysis of variables significant in the univariate, or clinically relevant, did not show any statistically significant p value in subjects either having or not having DE before HSCT.

7) Discussion

In this retrospective study data from comprehensive ocular surface evaluation in the same patients before and after allogeneic hematopoietic stem cell transplantation were analyzed. To our knowledge, only another study was performed on 53 patients analyzing prospectively the same population before and after HSCT.[40] The remaining studies analyzed ocular surface involvement only after HSCT without a baseline examination.[32,35-39,50,51]

In our study DE was present in 50 patients (53%) before HSCT, this value being higher as compared to Ogawa et al[40] who reported an incidence of 17%, and at the upper limit of the wide range (5-50%) reported for a general hospital-based population.[52-57]

The explanation could likely to be related to the history of the patients population of this study, who underwent several previous chemotherapy and total body irradiation treatments before allo-HSCT. However, a similar prevalence was shown in a previous study from our group, where a larger population of pre-transplant patients had been analyzed.[42] However no specific previous treatments had been found to be related to DE pre-HSCT. The DE patients showed mild to moderate levels of severity in both signs and symptoms with a hyper evaporative DE profile. To the best of our knowledge, pre HSCT dry eye profile had not been characterized previously.

After HSCT, 72 patients (77%) were found to be affected by DE, mostly presenting tear instability and pathological MGD score whereas the value of tear secretion as a median was found in the normal range. It is difficult to compare our results with the several previous reports in the literature only dealing with DE in post HSCT, and this is due to different time interval from HSCT and ophthalmological examination, concurrent systemic therapy, underlying hematological malignancies (not always specified), heterogeneous criteria for DE diagnosis. However, the ocular surface parameters shown after HSCT in our patients were similar to those found in mild DE populations from another study.[58] In addition, MGD functional impairment found in our study is in agreement with

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others [59] who found meibomian gland morphological alterations and loss after HSCT.

Twenty-two out of the seventy-two DE patients after HSCT were classified as incident cases, i.e. those not diagnosed as DE before HSCT. These patients also showed a mild to moderate hyper evaporative profile.

The influence of ocular, hematological and HSCT-related variables on the ocular GVHD or DE development post-HSCT was investigated previously. The heterogeneity of populations studied and their treatment along with the not univocal classification for GVHD (in particular those preceding the NIH guidelines) in the previous literature make results difficult to be compared. In addition, to date only one study analyzed the same patients before and after HSCT, providing data both from subjects not having a DE pre-HSCT and developing a DE post-HSCT and from those worsening a DE pre-HSCT.[40]

Several factors as donor-recipient sex mismatch, increasing recipient age and peripheral blood as stem cell source had been identified as associated factors either for ocular and systemic GVHD.[32,39,40,50,60] On the contrary, the role of the conditioning regimen, donor-recipient relation and HLA compatibility is still unclear.[32,35,40,50,51]

In the present study, increasing recipient age and chronic systemic GVHD were confirmed as associated with DE post-HSCT [39,40,50]. Recipient female gender, PBSC as stem cell source, related donors, donor-recipient sex mismatch were found to be associated with DE onset post-HSCT, despite previous conflicting results from previous ophthalmological studies.[32,36,39,40,50]

However, some of these (increasing recipient age, PBSC as stem cell source, donor-recipient sex mismatch) are widely recognized as associated factors with GVHD onset in the hematological literature.[60]

Some unexpected results were found for the analysis of conditioning intensity and related donors as associated factors. The intensity of conditioning regimen was not associated with an increased risk of

DE post-HSCT, in disagreement with the hematological literature,[60] but the great variability in protocols and treatment regimen used could have affected more reliable correlations.

Related donors showed a significant association with DE post-HSCT, in agreement with some Authors[35] but not with others.[32,36,40,60] This finding could be attributed to the different GVHD prophylaxis given according to donor type: all unrelated transplant received antylymphocyte globulin in addition to standard prophylaxis (Calcineurin-inhibitor + short term Methotrexate) whereas only roughly half patients received ATG in the HLA identical sibling setting. As recently reported ATG reduces the incidence and severity of cGVHD after allogeneic transplant from HLA identical sibling peripheral blood stem cells; in particular, ocular GVHD appear to be dramatically reduced on the arm with ATG.[61]

We have also analyzed potential risk factors not previously evaluated in the ophthalmological literature; of these, time interval from diagnosis to HSCT and donor age were not found associated with DE post HSCT whereas advanced stages of hematological malignancy and the number of stem cells infused were found associated to DE post HSCT.

In our series, the severity of post-HSCT ocular surface impairment was not as high as reported by other Authors.[32,35-39] No severe corneal complication occurred unlike results from Tabbara et al. in which half GVHD patients suffered from corneal ulcers.[62] Concordantly, no patient developed DE post-HSCT so severe to be classified as DEWS worst severity score 4. A possible explanation is that an ocular therapy had been administered to all those patients diagnosed as DE before HSCT, already before starting the conditioning regimen, and this could have tempered a DE progression post transplant. This hypothesis appears in agreement with Others who suggested the effectiveness of pre-HSCT initiation of therapy before HSCT for the treatment and prophylaxis of DE after transplantation.[63]

According to NIH GVHD criteria, ocular GVHD is diagnosed in the event of a new onset of dry, gritty, painful eyes, cicatricial conjunctivitis, keratoconjunctivitis sicca or confluent areas of punctate

keratopathy observed after HSCT. Ocular involvement represents a distinctive sign and therefore not considered to be sufficient as alone to establish an unequivocal diagnosis for general chronic GVHD trials.[26] Following these guidelines, systemic chronic GVHD was diagnosed in 42% of our patients as a whole whereas ocular chronic GVHD in 35.5%, regardless the pre-HSCT ocular impairment. As a matter of fact, if a patient already suffers from DE disease before HSCT, ocular surface impairment evaluated at the post-HSCT check-up cannot be considered as "incident cases" and cannot be diagnosed as post HSCT ocular GVHD. This finding influenced the real prevalence of ocular GVHD post HSCT, which decreased in the present study to 12%.

As already reported, a poor diagnostic performance in diagnosing DE already before HSCT was found for the Schirmer Test, which has been recently removed in the NIH Guidelines from the markers of severity and from the response criteria.[26,64] In addition, as the Schirmer score does not reflect changes in ocular GVHD activity, it was not recommended for the measurement of the changes in ocular GVHD studies by the Chronic GVHD Consortium.[65]

We recognize that in our study further variables potentially influencing the onset and development of ocular GVHD were lacking as not found in our medical charts. This limitation occurs in any retrospective study based on chart reviews, however this is a major initial study generating data to be verified further by larger cohort prospective studies.

8) Conclusion

This study confirmed that DE is present in high percentages in hematological patients undergoing hematopoietic stem cell transplantation already before HSCT, as already recently demonstrated by our group. This finding demonstrates that comprehensive pre-transplant assessment of ocular surface should be highly recommended, as it has been already recognized for functional respiratory values in lung GVHD.[26,64] This recommendation is not only addressed to an accurate early diagnosis but also to a prompt treatment of patients already suffering from dry eye disease, with positive influences in terms of lower prevalence and severity of ocular GVHD after HSCT compared to traditional ongoing protocols.

However, further larger prospective multicenter studies based on pre and post-transplantation ophthalmic evaluation are needed to identify overall associated factors and the real prevalence of ocular GVHD after HSCT that could be overestimated.

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