Original Paper

Acta Haematol
DOI: 10.1159/000502405

Received: April 11, 2019
Accepted: July 27, 2019
Published online: September 10, 2019

Cyclosporine A for the Prevention of Ocular Graft versus Host Disease in Allogeneic Hematopoietic Stem Cell Transplant Recipients Is Safe and Feasible

Olga Graciela Cantú-Rodríguez[a] Alberto Vázquez-Mellado[a] Juan Luis González-Treviño[b] David Mauricio Martínez-Garza[a] Andrés Gómez-De León[a] José Angel Hawing-Zarate[a] José Carlos Jaime-Pérez[a] César Homero Gutierrez-Aguirre[a] Andrea Cecilia Garza-Acosta[a] Consuelo Mancías-Guerra[a] Oscar González-Llano[a] Graciela Alejandra González-Cantú[a] Miguel Angel Herrera-Rojas[a] Isabel Sada-Ovalle[a] David Gómez-Almaguer[a]

[a]Universidad Autónoma de Nuevo León, Hospital Universitario "Dr. José Eleuterio González" Hematology Service, Monterrey, Mexico; [b]Régimen Estatal de Protección Social en Salud, Monterrey, Mexico

Keywords
Ocular graft versus host disease · Cyclosporine A · Allogeneic transplantation

Abstract
Purpose: To evaluate the safety and efficacy of ocular cyclosporine in the prevention of the development of ocular graft versus host disease (oGVHD) in patients undergoing allogeneic hematopoietic stem cell transplantation (AHSCT) in comparison with historic data. ***Design:*** We developed a longitudinal, observational, prospective nonrandomized study. We evaluated the feasibility of prophylactic use of topical cyclosporine A (CsA) to prevent or decrease the incidence of oGVHD and compared this with historic data. ***Methods:*** Patients undergoing AHSCT were treated with prophylactic topical CsA for 12 months after engraftment, followed by serial ophthalmic evaluations, including the Schirmer test. ***Results:*** Twenty patients were included. No serious adverse effects were reported. Poor adherence was documented in 15% of patients. In spite of observing extra-ocular GVHD (acute and chronic GVHD incidence of 50 and 45%, respectively), only 1 in 20 patients developed oGVHD over the 20-month follow-up for the entire cohort. No statistically significant difference was observed in the incidence of oGVHD when compared to a historical cohort. ***Conclusions:*** Topical CsA as a prophylactic measure for oGVHD, administered over a period of 1 year after grafting, is safe and feasible and may decrease the incidence of ophthalmic manifestations of GVHD. These findings must be confirmed in a randomized trial.

KARGER

E-Mail karger@karger.com
www.karger.com/aha

Olga G Cantú-Rodríguez, MD
Universidad Autónoma de Nuevo León
Hospital Universitario "Dr. José Eleuterio González" Hematology Service
Madero y Gonzalitos S/N, Colonia Mitras Centro, 64460 Monterrey (México)
E-Mail ogcantur@yahoo.com.mx

Introduction

One of the cornerstones of success in allogeneic hematopoietic stem cell transplantation (AHSCT) in patients with hematological malignancies is a phenomenon known as the graft versus tumor effect, where donor-derived T lymphocytes are directed against minor histocompatibility antigens expressed by malignant cells, leading to their elimination [1]. This beneficial effect is often hampered by the development of graft versus host disease (GVHD), where T lymphocytes attack the patient's healthy cells and potentially causing organ failure [2–5]. Acute (a)GVHD mostly affects the skin, gastrointestinal tract, and liver [6], whereas chronic (c)GVHD is a serious syndrome that affects a single or several organs. It appears at 100 days posttransplant and can manifest with scleroderma-like lesions, sclerosis of the gastrointestinal tract, oral ulcers, and dry eye syndrome [7].

The majority of patients with cGVHD present with ocular involvement with a reported incidence of 40–60% and higher [8–10]. Dry eye is the most common ocular finding of the disease and is present in almost 90% of cases. Ocular (o)GVHD typically appears after the first 3 months posttransplantation, and can persist indefinitely, especially if other forms of the disease co-occur. Documented risk factors for oGVHD include the use of peripheral blood as a source of stem cells, matched unrelated or haploidentical donors, and the development of other forms of GVHD, particularly acute disease in the skin and gastrointestinal mucosae [11–14].

The most common symptoms of oGVHD include ocular irritation, dry and red eye syndrome, intermittent blurring of vision, ocular discharge, photophobia, and ocular pain [15, 16]. Although not in all cases, the disease diminishes quality of life, complicates activities of daily living, and can cause temporary or permanent vision loss, particularly in cases of cGVHD [17, 18].

Due its wide spectrum of severity and the lack of randomized studies, oGVHD therapy is mostly empirical, consisting of artificial tears, corneal support measures, and topical anti-inflammatory and immunosuppressive treatment including steroids, cyclosporine A (CsA), and tacrolimus, all with varying success rates [19, 20]. Developing an effective alternative for preventing the onset of oGVHD and its complications would be ideal. Our center performs peripheral blood HSCT, and for the last 10 years both mismatched and haploidentical, which are both considered as risk factors to develop GVHD. This study aims to elucidate the benefits of topical CsA in patients with oGVHD as an innovative approach to overcome severe ocular morbidity.

Patients and Methods

This was designed as a single-center, prospective study, performed from February 2014 to May 2015.

Patient Selection and Intervention

Consecutive patients >18 years with any hematologic disease and undergoing AHSCT either human leukocyte antigen (HLA)-identical or haploidentical were included. We excluded patients with (1) a history of ophthalmic surgery, (2) a previous diagnosis of dry eye syndrome, (3) any rheumatologic or dermatologic condition requiring systemic treatment, (4) active ocular infection, or (5) documented topical cyclosporine allergy or intolerance. All patients received 0.1% ocular CsA solution (MODUSIK-A OFTENO®, Sophia Lab) as a baseline treatment in addition to our local GVHD prevention protocol (described below). Topical CsA was administered at a dose of 2 drops twice a day in each eye for an uninterrupted period of 12 months, starting after engraftment was confirmed (≥500 neutrophils/μL and ≥20,000 platelets/μL in 2 different complete blood counts >24–48 h apart). Patients were also provided with artificial tears containing 0.5% carboxymethylcellulose, to be used as needed.

Our systemic GVHD prevention protocol for HLA-identical AHSTC included methotrexate, 10 mg intravenously (i.v.) on days 1, 3, and 5. For haploidentical transplants, we used cyclophosphamide 50 mg/kg i.v. on days 3 and 4, and mycophenolate mofetil 500 mg BID for 1 month starting on day 5. All patients received oral CsA at 6 mg/kg/day, immediately after HLA-identical transplant and at day 5 for haploidentical graft recipients. For patients developing systemic GVHD, we used a variety of standard immunosuppressive schemes including corticosteroids, rituximab, alemtuzumab, and tacrolimus [21]. Conditioning regimens employed at our institution varied according to baseline diagnosis and have been reported elsewhere [22–24]).

Before transplantation, baseline ocular characteristics were assessed with a complete ophthalmic evaluation that included: (1) Schirmer test score, (2) tear break-up time, (3) visual acuity, (4) fundoscopy, and (5) corneal fluorescein testing. Complete assessment by an ophthalmologist was repeated at 3, 6, and 12 months post-transplant and monthly with a Schirmer test score during routine visits at the outpatient clinic, when patient adherence was documented by standard interview. If patients exhibited dry eye symptoms or ocular manifestations of disease, an immediate complete ophthalmic evaluation was performed.

Outcome Measurements

Our primary outcomes were safety and oGVHD incidence at 1-year follow-up. oGVHD was assessed by the presence of dry, gritty, and painful eyes, keratoconjunctivitis sicca (KCS), cicatricial conjunctivitis, or punctate keratopathy confirmed by a mean Schirmer test ≤5 mm at 5 min or a mean Schirmer test of 6–10 mm plus KCS findings on slit-lamp examination by a GVHD-experienced ophthalmologist, in the absence of other evident causes [25]. The 2015 NIH recommendations were not considered since they were published during the study period, and the International Chronic Ocular Graft-versus-Host Disease Consensus Group diagnostic criteria had yet to be validated [25]. We considered a diagnosis of oGVHD as treatment failure, and patients with the presence of oGVHD were classified according to NIH disease scores as follows: 0 = no symptoms; 1 = mild dry eye symptoms not affecting

daily life activities (requiring lubricant eye drops ≤3 times per day); 2 = moderate dry eye symptoms partially affecting daily life activities (requiring lubricant eye drops >3 times per day) without new vision impairment due to KCS; 3 = severe dry eye symptoms significantly affecting activities of daily life (including requiring special eyewear to relieve pain), or an inability to work because of ocular symptoms or vision loss due to KCS.

After 1 year of follow-up from successful grafting and oGVHD prophylaxis, topical CsA was suspended, and we prospectively recorded any self-reported signs or symptoms related to oGVHD or systemic GVHD according to our standard institutional protocol. Patients who developed oGVHD were allowed to receive alternative treatments or continue with therapeutic CsA as determined by the treating physician. Those who developed asymptomatic ocular sicca documented by a Schirmer test ≤5 mm at 5 min or 6–10 mm with slit-lamp documented KCS in the absence of distinctive signs in other organs, were not considered to have oGVHD [26]. Patients who developed extra-ocular GVHD continued with prophylactic CsA despite receiving alternative systemic treatments. Topical CsA was stopped in the event of secondary graft failure or disease relapse without oGVHD.

Historical Controls

Historical unmatched controls were included in a 2:1 ratio to consecutive retrospective patients allografted in our institution over a period of 1 year before the study period (2013–2014). Both men and women ≥18 years of age undergoing allogeneic or haploidentical transplantation due to any disease were included. Only controls with pre-transplantation ocular surgery, contact lens use, or any ocular pathology were excluded. Standard conditioning and systemic GVHD prophylaxis protocols included neither prophylactic topical CsA nor serial detailed ophthalmic evaluation for asymptomatic patients. Patients who developed ocular manifestations in the post-transplant period were referred to an ophthalmological evaluation to confirm (or not) the presence of oGVHD before starting treatment. However, it is not possible to determine if there were cases with asymptomatic dry eye in the previous or post-transplant period, or another diagnosis in this group, in order to establish a complete comparison.

Statistical Analysis

All variables were evaluated from baseline to each follow-up visit. Baseline characteristics were analyzed using central tendency measures. The χ^2 was used for categorical data and the Mann-Whitney U test for quantitative comparison. For survival and mortality analysis, we used the Kaplan-Meier method. The incidence of oGVHD in the study group and the historical cohort was compared using the log-rank test. A 2-sided p value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS v22 (IBM Inc., Armonk, NY, USA).

Results

Twenty-eight patients were allografted over the study period, and 8 declined to participate. A total of 20 patients were included with a median age of 42 years (range 18–66 years) and 50% were women. The baseline demographics and ocular characteristics of the participants are shown in Table 1. The patients that declined to participate had a median age of 43 years (range 20–60 years), 7 were HLA-identical, and 6 were men.

Most patients underwent AHSCT due to a neoplastic disease; acute lymphoblastic leukemia (ALL) was the most common diagnosis ($n = 8$) followed by acute myeloid leukemia ($n = 6$). Thirteen patients were followed for 12 months and just 1 developed oGVHD in the 4th month. Seven patients were followed <12 months due to death, with a median follow-up of 7 months (range 3–9 months). Baseline ophthalmic evaluation was relevant in 3 patients; 2 were diagnosed with hypertensive retinopathy and 1 with open-angle glaucoma. All had a normal corneal integrity test and none of these patients were eliminated. Additional baseline ophthalmic assessment variables and aGVHD and cGVHD incidences are shown in Table 2. Seven patients received a haploidentical graft, while the remaining 13 had an HLA-identical 6/6 sibling donor. The median CD34+ cell dose infused was 5.8×10^6/kg (range $3.4–9.1 \times 10^6$/kg). No primary or secondary graft failure occurred. Median neutrophil and platelet engraftment occurred on day 18 (range 12–21) and 14 (range 11–21), respectively.

Safety and Adherence

Overall, prophylactic ocular CsA therapy was well tolerated. Only 1 patient developed a foreign-body sensation and redness attributed to the use of topical CsA; this appeared after 1 month of use, resolved after discontinuation, and was successfully reinstituted. No other ocular or systemic adverse effects attributed to topical CsA were documented.

Lack of adherence was documented in 3 patients (15%) by standard monthly interview. All received the recommendation to restart treatment and continued the protocol as planned. Patients were followed for a median of 20.2 months (range 3.5–39 months).

Graft versus Host Disease

Overall, 10 patients (50%) developed grade I/II aGVHD with skin involvement, with a higher incidence of grade II (60%), while 2 patients had gastrointestinal manifestations at a median of 41 days (22–69 days) after transplantation (Table 1). No cases of "acute" oGVHD or systemic grade III/IV aGVHD were documented. Furthermore, 9 patients presented with findings consistent with cGVHD. One patient presented with symptomatic distinctive signs of oGVHD in both eyes: a 35-year-old woman with ALL in second complete remission grafted from an HLA-identical donor with self-limited skin grade I aGVHD. She presented

Table 1. Hematologic and ophthalmic characteristics of 20 patients who received oGVHD prophylaxis with topical CsA following engraftment

Sex/age[a]	Diagnosis	CD34+ cells ($\times 10^6$/kg)	aGVHD	cGVHD	oGVHD[b]	Baseline Schirmer, mm	Baseline TBUT, s	12-month Schirmer, mm	12 months TBUT, s	Outcome
F/51	AML	5.04	II	Severe	No	6.0	10	n.a.	n.a.	Relapse, death
M/42	AML	5.8	II	Mild	No[c]	8.0	8	9.0	9	Alive
F/35	ALL	5.8	I	No	No	8.5	9	10.0	9	Relapse, death
M/43	CMML	6.6	II	Mild	No	5.5	8	15.0	9	Alive
M/52	PMF	8	No	Severe	No[c]	6.0	8	3.0	6	Died
M/28	ALL	4.16	No	No	No	6.5	9	n.a.	n.a.	Relapse, death
M/64	ALL	5.9	No	No	No	7.0	10	n.a.	n.a.	Relapse, death
F/28	AML	5.25	No	Severe	No[c]	12.0	8.5	9.0	8	Alive
F/18	ALL	5.7	I	No	No	8.5	10	n.a.	n.a.	Relapse, death
M22	ALL	4.49	II	No	No	8.0	9	9.0	9	Relapse, death
F/61	AA	3.64	No	Severe	No	7.0	9	2.0	10	Alive
F/60	AA	7.21	No	No	No	6.5	9	6.0	9	Died
M/40	AML	8	No	No	No	8.0	9	n.a.	n.a.	Relapse, death
M/66	MDS	3.8	No	No	No	7.5	9	9.0	8	Alive
M/25	ALL	7.2	No	No	No	8.5	9	8.5	9	Alive
M/59	AML	9.1	II	No	No	11.0	11	n.a.	n.a.	Died
F/42	NHL	4.18	II	Mild	No	9.5	8	10.0	7.5	Alive
F/19	ALL	5.8	I	No	No	21.0	10	16.0	10	Relapse, death
F/35	ALL	4.6	I	Mild	1[d]	8.0	8	4.50	2.5	Relapse, death
F/53	AML	3.4	No	Mild	No	7.0	7.5	1.50	8	Alive

n.a., data not available due to the patient's death before the end of the study; (a/c/o)GVHD, (acute/chronic/ocular) graft versus host disease; CsA, cyclosporine A; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; PMF, primary myelofibrosis; AA, aplastic anemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; MRD, matched related donor; TBUT, tear break-up time.

[a] M (male) or F (female)/age in years.

[b] According to the 2005 National Institutes of Health recommendations for chronic ocular GVHD scoring.

[c] Patients with a documented intermittent lack of adherence.

[d] Patients with adverse effects related to the use of topical CsA.

with mild dry eye symptoms and the need to use lubricant eye drops <3 times a day (NIH score 1), appearing 95 days after transplantation, and was treated successfully with lubricant eye drops (Table 1). She later developed mild oral cGVHD at day 177; her disease relapsed 14 months after transplantation and she died due to related complications at a later date. Five patients presented with mild cGVHD in the skin and mouth, while 4 patients presented with severe disease without ocular manifestations at a median of 226 days (range 126–394 days). No patients were diagnosed with asymptomatic oGVHD (NIH score 0). No relevant conclusions were drawn regarding monthly Schirmer testing evaluations in asymptomatic patients.

Survival and Relapse

Overall survival (OS) for the treated group was 42% at 2 years, while the median OS was 20.4 months (95% CI 10.2–30.5 months). Non-relapse mortality (NRM) was estimated at 18.6% at 2 years (median not reached), while relapse-free survival (RFS) was 40% with a median of 11.1 months (95% CI 2.7–19.5 months). Overall, 11/20 patients died, 8 due to complications associated with progression or relapse after engraftment (including the patient who developed oGVHD), 1 due to severe liver cGVHD, and 2 due to infectious complications leading to sepsis shortly after transplantation (1 had skin grade 2 aGVHD). Nine patients remain alive, 1 without GVHD with T cell ALL relapsed and is in remission following treatment, 5 with cGVHD had no evidence of relapse, and 3 were in remission without GVHD.

Historical Cohort

The historical control group consisted of 44 patients who were allografted in our center between 2013 and 2015. None were excluded, and no cases of pre-trans-

Table 2. Univariate analysis of the CsA prophylaxis group and the historical group

	CsA prophylaxis	Historical control	*p* value[a]
Total number of patients	20	44	
Age, years	42 (18–66)	38 (18–65)	0.31
Female	10 (50)	17 (38.6)	0.43
Male	10 (50)	27 (61.4)	
Diagnosis			
ALL	8 (40)	10 (22.7)	0.37
AA	2 (10)	9 (20.5)	
AML	6 (30)	6 (13.6)	
HL	0 (0)	5 (11.4)	
NHL	1 (5)	3 (6.8)	
MDS	1 (5)	3 (6.8)	
MPNs	2 (10)	4 (9)	
MM	0 (0)	2 (4.5)	
Transplant			0.55
MRD	13	33 (75)	
Haploidentical donor	7	11 (25)	
CD34+ × 10^6/kg cell count	5.8 (3.4–9.1)	6.0 (1.8–16)	0.29
Neutrophil engraftment			0.08
Days	18 (12–21)	16 (10–25)	
Platelet engraftment			0.79
Days	14 (11–21)	14 (10–22)	
aGVHD			0.59
All	10	18	
I/II	10 (100)	16 (88.8)	
III/IV	0 (0)	2 (11.1)	
cGVHD			0.08
All	9	10	
Mild	5 (55.5)	5 (50)	
Moderate	0 (0.0)	3 (30)	
Severe	4 (44.4)	2 (20)	
oGVHD			0.41
All	1	7	
Score 1	1 (100)	3 (42.8)	
Score 2	0 (0)	4 (57.1)	

Values express *n*, *n* (%), or median (range). ALL, acute lymphoblastic leukemia; AA, aplastic anemia; AML, acute myeloid leukemia; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome; MPNs, myeloproliferative neoplasms; MRD, HLA-matched related donor; (a/c/o)GVHD, (acute/chronic/ocular) graft versus host disease.

[a] All comparative analysis was made using nonparametric tests.

plant ocular pathology had been documented. The controls had the following characteristics: a median age of 38 years (range 18–65 years), 61.4% were male, 25% were haploidentical graft recipients with a similar proportion in diagnoses; $CD34^+ \times 10^6$/kg cells infusion, time to neutrophil/platelet engraftment, and GVHD incidence did not show statistically significant differences from the study group (Table 2). Median follow-up was 16 months (range 0.1–51 months). Furthermore, no relevant differences were observed regarding OS, RFS, and NRM in the Kaplan-Meier estimates (data not shown). Seven patients developed oGVHD (15.9%) in contrast to 5% in the study group; 3 had an NIH score of 1 and the other 4 had a score of 2 points. The estimated probability of developing oGVHD was 26% at 24 months versus 5% in the study group, without a statistically significant difference between groups (median not reached; Fig. 1).

Discussion

The main objective of this study was to demonstrate the benefit of topical CsA at 0.1% for the prevention of the development of oGVHD. Our data demonstrated that topical CsA twice a day for 12 months reduced the incidence. Cyclosporine possesses immunosuppressive properties by forming a complex with cyclophilin A, and also by inhibiting the phosphatase activity of calcineurin and blocking the gene expression in activated T cells [27].

The early start of treatment with topical CsA is critical and has been used as a standard option aimed to preserve a functional eye, though most patients begin receiving treatment after permanent damage to ocular tissues has already occurred [20]. Treatment with immunosuppressants may not always be effective for dry eye, among other oGVHD symptoms, especially after they are severe [28, 29]. Damage to the lacrimal gland and corneal epithelium caused by reactive lymphocytes begins once successful grafting is achieved, i.e., 15–20 days after the infusion of hematopoietic stem cells and much earlier than the onset of clinical manifestations. Therefore, to prevent permanent damage to the lacrimal system as a consequence of lymphocyte infiltration [30], a course of topical CsA should be started as soon as grafting is confirmed, or even earlier. Other trials have attempted ocular-directed prophylaxis; for example, a retrospective study demonstrated that pre-AHSCT initiation of topical CsA decreased the incidence and severity of dry eyes among patients undergoing AHSCT [31].

A strong association between systemic GVHD and oGVHD was previously reported in the literature [13], and while we observed severe cases in our study cohort, we did not identify related oGVHD, suggesting a beneficial effect of prophylactic topical CsA for the development of oGVHD (Table 2). Although the Schirmer

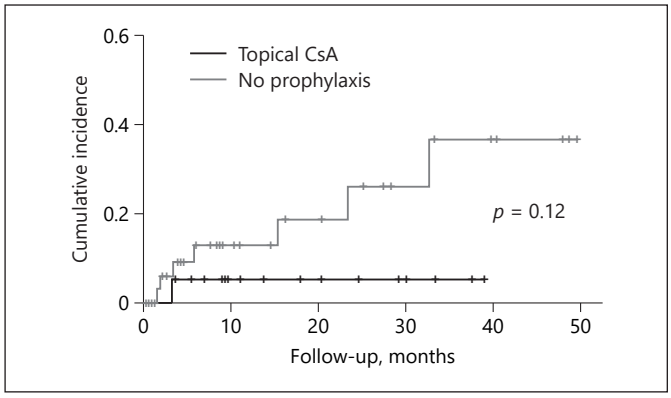

Fig. 1. Cumulative incidence of oGVHD and comparison between populations studied, using a Kaplan-Meier analysis (long-rank test).

test values decreased in some patients, this did not correlate with clinical manifestations or with complete ophthalmologic evaluations where no evidence of oGVHD was observed [26]. The incidence of oGVHD in the historical cohort was 15%, lower than previously reported in the literature [8]. This may be due to the fact that some milder cases in our patients remained undiagnosed due to the lack of more precise diagnostic methods. Interestingly, despite a low incidence of oGVHD in the historical cohort, we observed a tendency for a more reduced incidence in the CsA group (Fig. 1). We are aware of the limitations of historical control comparisons, but we believe this offers interesting insights in this context.

The primary limitations in this study are its limited number of patients and the lack of a randomized controlled design. Nonetheless, the overall results set a precedent for future studies to clarify the potential value of this intervention and account for treatment adherence more robustly.

Given that the OS of patients treated with AHSCT is continually rising [32], it is imperative to guarantee that all patients have a good quality of life, with chronic GVHD being the main obstacle to the endeavor for patients to remain free of relapse [33]. Currently, no standard prophylaxis exists for oGVHD, and the paucity of data regarding this issue represents an unmet need. The results of this study suggest that the administration of topical CsA is safe and feasible in patients receiving an AHSCT. In our study group, the incidence of oGVHD was much lower than in a historical cohort and previous studies. Despite having a normal incidence of other types of systemic GVHD, oGVHD incidence remained considerably low. Given the safety of this preparation and the disabling potential of oGVHD, we suggest that this option is another way to prevent this ocular complication, but our data will need confirmation.

Acknowledgements

We thank Sergio Lozano MD PhD for his valuable help with the language evaluation.

Statement of Ethics

This study was approved by the Institutional Review Board of our University Hospital and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. It was also registered in ClinicalTrials.gov (NCT02144025, www.clinicaltrials.gov). Written informed consent was obtained from all participants before enrolment and attached to our database.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research was funded by our institution.

Author Contributions

O.G.C-R.: conception and design of the study. A.V.-M.: major contributions to final analysis. J.L.G.-T.: major contributions to final manuscript and data recovery. DM.M-G.: Data acquisition and statistical analysis. A.G.-D.L., J.C.J.-P., C.H.G.-A., C.M.-G., O.G.-L., G.A.G.-C., D.G.-A.: major contributions to final manuscript. J.A.H.-Z.: data acquisition and statistical analysis. A.C.G.-A.: statistical analysis. M.A.H.-R. and I.S.-O.: data interpretation and analysis.

References

1 Mavroudis D, Barrett J. The graft-versus-leukemia effect. Curr Opin Hematol. 1996 Nov; 3(6):423–9.
2 Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009 May; 373(9674):1550–61.
3 Rojas B, Cuhna R, Zafirakis P, Ramirez JM, Lizan-garciía M, Zhao T, et al. Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation. Exp Eye Res. 2005 Sep;81(3): 313–25.

4 Bhan AK, Fujikawa LS, Foster CS. T-cell subsets and Langerhans cells in normal and diseased conjunctiva. Am J Ophthalmol. 1982 Aug;94(2):205–12.
5 Takata M, Imai T, Hirone T. Immunoelectron microscopy of acute graft versus host disease of the skin after allogeneic bone marrow transplantation. J Clin Pathol. 1993 Sep;46(9):801–5.
6 Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998 Oct;92(7):2303–14.
7 Bhushan V, Collins RH Jr. Chronic graft-vs-host disease. JAMA. 2003 Nov;290(19):2599–603.
8 Shikari H, Antin JH, Dana R. Ocular graft-versus-host disease: a review. Surv Ophthalmol. 2013 May-Jun;58(3):233–51.
9 Kim SK. Update on ocular graft versus host disease. Curr Opin Ophthalmol. 2006 Aug;17(4):344–8.
10 Ogawa Y, Kuwana M. Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation. Cornea. 2003 Oct;22(7 Suppl):S19–27.
11 Shikari H, Amparo F, Saboo U, Dana R. Onset of ocular graft-versus-host disease symptoms after allogeneic hematopoietic stem cell transplantation. Cornea. 2015 Mar;34(3):243–7.
12 Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea. 2010 Jul;29(7):758–63.
13 Ogawa Y, Okamoto S, Wakui M, Watanabe R, Yamada M, Yoshino M, et al. Dry eye after haematopoietic stem cell transplantation. Br J Ophthalmol. 1999 Oct;83(10):1125–30.
14 Jaime-Pérez JC, Salazar-Cavazos L, Aguilar-Calderón P, Herrera-Garza JL, Gutiérrez-Aguirre CH, Gómez-Almaguer D. Assessing the efficacy of an ambulatory peripheral blood hematopoietic stem cell transplant program using reduced intensity conditioning in a low-middle-income country. Bone Marrow Transplant. 2019 Jun;54(6):828–38.
15 Dietrich-Ntoukas T, Cursiefen C, Westekemper H, Eberwein P, Reinhard T, Bertz H, et al. Diagnosis and treatment of ocular chronic graft-versus-host disease: report from the German-Austrian-Swiss Consensus Conference on Clinical Practice in chronic GVHD. Cornea. 2012 Mar;31(3):299–310.
16 Riemens A, Te Boome LC, Kalinina Ayuso V, Kuiper JJ, Imhof SM, Lokhorst HM, et al. Impact of ocular graft-versus-host disease on visual quality of life in patients after allogeneic stem cell transplantation: questionnaire study. Acta Ophthalmol. 2014 Feb;92(1):82–7.
17 Munir SZ, Aylward J. A Review of Ocular Graft-Versus-Host Disease. Optom Vis Sci. 2017 May;94(5):545–55.
18 Inamoto Y, Valdés-Sanz N, Ogawa Y, Alves M, Berchicci L, Galvin J, et al. Ocular graft-versus-host disease after hematopoietic cell transplantation: Expert review from the Late Effects and Quality of Life Working Committee of the CIBMTR and Transplant Complications Working Party of the EBMT. Bone Marrow Transplant. 2019 May;54(5):662–73.
19 Tung CI. Current approaches to treatment of ocular graft-versus-host disease. Int Ophthalmol Clin. 2017;57(2):65–88.
20 Balaram M, Rashid S, Dana R. Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf. 2005 Oct;3(4):203–11.
21 Gutiérrez-Aguirre CH, Cantú-Rodríguez OG, Borjas-Almaguer OD, González-Llano O, Jaime-Pérez JC, Solano-Genesta M, et al. Effectiveness of subcutaneous low-dose alemtuzumab and rituximab combination therapy for steroid-resistant chronic graft-versus-host disease. Haematologica. 2012 May;97(5):717–22.
22 Jaime-Pérez JC, Heredia-Salazar AC, Cantú-Rodríguez OG, Gutiérrez-Aguirre H, Villarreal-Villarreal CD, Mancías-Guerra C, et al. Cost structure and clinical outcome of a stem cell transplantation program in a developing country: the experience in northeast Mexico. Oncologist. 2015 Apr;20(4):386–92.
23 González-Llano O, González-López EE, Ramírez-Cázares AC, Marcos-Ramírez ER, Ruiz-Argüelles GJ, Gómez-Almaguer D. Haploidentical peripheral blood stem cell transplantation with posttransplant cyclophosphamide in children and adolescents with hematological malignancies. Pediatr Blood Cancer. 2016 Nov;63(11):2033–7.
24 Colunga-Pedraza P, Gomez-De-Leon A, Sotomayor-Duque G, Colunga-Pedraza J, López-García Y, Santana-Hernandez P, et al. Outpatient haploidentical stem cell transplantation using post-transplant cyclophosphamide is feasible. Experience in a single Latin-American center. Blood. 2017;130 Suppl1:1940.
25 Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005 Dec;11(12):945–56.
26 Ogawa Y, Kim SK, Dana R, Clayton J, Jain S, Rosenblatt MI, et al. International Chronic Ocular Graft-vs-Host-Disease (GVHD) consensus group: proposed diagnostic criteria for chronic GVHD (part I). Sci Rep. 2013 Dec;3(1):3419.
27 Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. Immunopharmacology. 2000 May;47(2-3):119–25.
28 Ogawa Y, Okamoto S, Kuwana M, Mori T, Watanabe R, Nakajima T, et al. Successful treatment of dry eye in two patients with chronic graft-versus-host disease with systemic administration of FK506 and corticosteroids. Cornea. 2001 May;20(4):430–4.
29 Boynton GE, Raoof D, Niziol LM, Hussain M, Mian SI. Prospective randomized trial comparing efficacy of topical lotepredonol etabonate 0.5% versus cyclosporine-A 0.05% for the treatment of dry eye syndrome following hematopoietic stem cell transplantation. Cornea. 2015 Jul;34(7):725–32.
30 Kiang E, Tesavibul N, Yee R, Kellaway J, Przepiorka D. The use of topical cyclosporin A in ocular graft-versus-host-disease. Bone Marrow Transplant. 1998 Jul;22(2):147–51.
31 Malta JB, Soong HK, Shtein RM, Musch DC, Rhoades W, Sugar A, et al. Treatment of ocular graft-versus-host disease with topical cyclosporine 0.05%. Cornea. 2010 Dec;29(12):1392–6.
32 Pulte D, Gondos A, Brenner H. Major improvements in survival in hematologic malignancies in the early 2000s. Blood. 2007;110:632.
33 Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant. 2015 Mar;21(3):389–401.e1.