Oral Disease Profiles in Chronic Graft versus Host Disease

Journal of Dental Research
2015, Vol. 94(4) 547–554
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for Dental Research 2015
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DOI: 10.1177/0022034515570942
jdr.sagepub.com

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Abstract

At least half of patients with chronic graft-versus-host-disease (cGVHD), the leading cause of morbidity and non-relapse mortality after allogeneic stem cell transplantation, have oral manifestations: mucosal lesions, salivary dysfunction, and limited mouth-opening. cGVHD may manifest in a single organ or affect multiple organ systems, including the mouth, eyes, and the skin. The interrelationship of the 3 oral manifestations of cGVHD with each other and with the specific manifestations of extraoral cGVHD has not been studied. In this analysis, we explored, in a large group of patients with cGVHD, the potential associations between: (1) oral mucosal disease and erythematous skin disease, (2) salivary gland dysfunction and lacrimal gland dysfunction, and (3) limited mouth-opening and sclerotic skin cGVHD. Study participants, enrolled in a cGVHD Natural History Protocol (NCT00331968, $n = 212$), underwent an oral examination evaluating: (1) mucosal cGVHD [NIH Oral Mucosal Score (OMS)], (2) salivary dysfunction (saliva flow and xerostomia), and (3) maximum mouth-opening measurement. Parameters for dysfunction (OMS > 2, saliva flow ≤ 1 mL/5 min, mouth-opening ≤ 35 mm) were analyzed for association with skin cGVHD involvement (erythema and sclerosis, skin symptoms), lacrimal dysfunction (Schirmer's tear test, xerophthalmia), Lee cGVHD Symptom Scores, and NIH organ scores. Oral mucosal disease (31% prevalence) was associated with skin erythema ($P < 0.001$); salivary dysfunction (11% prevalence) was associated with lacrimal dysfunction ($P = 0.010$) and xerostomia with xerophthalmia ($r = 0.32$, $P = 0.001$); and limited mouth-opening (17% prevalence) was associated with skin sclerosis ($P = 0.008$) and skin symptoms ($P = 0.001$). There was no association found among these 3 oral cGVHD manifestations. This analysis supports the understanding of oral cGVHD as 3 distinct diseases: mucosal lesions, salivary gland dysfunction, and mouth sclerosis. Clear classification of oral cGVHD as 3 separate manifestations will improve clinical diagnosis, observational research data collection, and the definitions of outcome measures in clinical trials.

Keywords: clinical research, oral medicine, autoimmune disease, salivary dysfunction, oral cGVHD, stem cell transplantation

Introduction

Chronic graft-versus-host disease (cGVHD) is a major late complication of allogeneic hematopoietic stem cell transplantation (alloHSCT), which is commonly used to treat high-risk and relapsed hematologic malignancies (Copelan 2006). This clinical syndrome is characterized by complex interactions of both autologous and allogeneic immune dysregulation and is the leading cause of non-relapse-related morbidity and mortality in long-term transplant survivors (Pavletic and Baird 2006). Chronic GVHD can persist for months to years and may affect multiple organ systems including the eyes, mouth, gut, liver, lungs, joints, and genitourinary tract. Although cGVHD can manifest almost anywhere, the oral cavity is commonly involved, affecting 25–80% of cGVHD patients (Schubert and Correa 2008; Fassil et al. 2012). Oral cGVHD can be a source of tremendous pain and discomfort and results in diminished oral health, functional capacity, and quality of life in affected patients (Imanguli et al. 2008; Fall-Dickson et al. 2010; Meier et al. 2011; Fassil et al. 2012; Bassim et al. 2014).

The clinical presentation of oral cGVHD is diverse and can involve any site in the oral cavity. Oral mucosal lesions are

characterized as erythema, lichenoid changes, ulceration, mucocoeles (Filipovich et al. 2005; Pavletic et al. 2006) (Fig. 1A), atrophy, pseudomembrane, hyperkeratosis, and edema/

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Figure 1. Oral chronic Graft-versus-Host Disease (cGVHD). **Row A:** Oral mucosal disease in cGVHD, demonstrating erythema, lichenoid lesions, ulcerations, and mucocelles. **Row B:** Salivary gland dysfunction in cGVHD and dry mouth. **Row C:** Oral sclerosis in cGVHD and limited mouth-opening.

cellulitis (Schubert et al. 1992). Salivary gland cGVHD causes salivary dysfunction and xerostomia (Sale et al. 1981; Imanguli et al. 2010), contributing to oral infections and increased caries risk (Castellarin et al. 2012) (Fig. 1B). Oral or perioral cGVHD, which includes sclerosis affecting the mouth or face, can limit mouth-opening (Imanguli et al. 2008; Mays et al. 2013) (Fig. 1C). Oral cGVHD may be described as 3 distinct pathologies affecting the mouth: oral mucosal disease, salivary dysfunction, and limited mouth-opening. These oral cGVHD pathologies have been described separately in the literature (Treister et al. 2012; Mays et al. 2013), but their interrelated prevalence in a large and well-characterized group of patients with cGVHD has not been reported.

The clinical presentation of several autoimmune conditions is similar to that of oral cGVHD. Lichen planus manifests as oral mucosal disease with a spectrum of changes similar to those seen in oral mucosal cGVHD (Sato et al. 2006; Pimentel et al. 2010). Sjögren's syndrome causes salivary dysfunction, similar to the dry mouth seen in salivary gland cGVHD, though the pathology differs (Lawley et al. 1977; Sale et al. 1981). Scleroderma has been associated with limitation of mouth movement as part of its systemic sclerosis pattern (Vincent et al. 2009; Kobak et al. 2013). These similarities have led us to form hypotheses about the associations of the oral features of cGVHD with other organ manifestations of cGVHD,

suggested by these autoimmune diseases. Hypothesis 1: The extraoral manifestations of lichen planus mainly affect the skin and mucosal tissues; therefore, an association will exist between oral mucosal cGVHD, skin erythematous cGVHD, and genital cGVHD. Hypothesis 2: The extraoral manifestations of Sjögren's syndrome mainly affect the lacrimal glands; therefore, an association will exist between salivary and lacrimal dysfunction in cGVHD. Hypothesis 3: Scleroderma causes skin sclerosis; therefore, limited mouth-opening will be associated with skin sclerosis in patients with cGVHD.

This cross-sectional study examined a large registry of well-characterized cGVHD patients to determine the level of association of the separate components of oral cGVHD (mucosal disease, salivary dysfunction, and limited mouth-opening) with cGVHD manifestations at other body sites, and to determine the association of the 3 components of oral cGVHD with each other.

Materials and Methods

Study Population

Patients in this study were participants in a cGVHD natural history study at the National Institutes of Health (NIH) (clinical trials.gov #NCT00331968), and were referred to NIH and diagnosed with cGVHD according to the definition of the NIH

Consensus Group Criteria (Filipovich et al. 2005). Participants underwent a 4-day, one-time visit evaluation by a multidisciplinary team of clinical experts in dermatology, ophthalmology, dentistry, rehabilitation medicine, gynecology, pain and palliative care, and HCT care. Clinical assessments and patient-reported forms were recorded at the time of each participant's visit by means of predefined data collection instruments. This research project was approved by the NCI Institutional Review Board (IRB).

Variables and Comparisons

Oral evaluation. The main variables of study involved scoring for oral cGVHD: oral mucosal scoring, xerostomia scoring and salivary function tests, and maximum mouth-opening measurements. Oral evaluation was performed by dentists, calibrated for oral cGVHD evaluation, through the NIH, Clinical Center Dental Clinic. Oral mucosal disease was determined according to the NIH Oral Mucosal Score (OMS), with clinical activity judged by the extent and severity of erythema, lichenoid lesions, ulcerations, and mucocoeles in the intraoral cavity, and based on the Oral Mucositis Rating Scale (OMRS) (Pavletic et al. 2006). For this study, cutoff values were assigned for comparative analysis. Patients with an NIH OMS > 2 were assigned as having oral mucosal cGVHD (Fassil et al. 2012). Five-minute unstimulated whole saliva was collected in the morning, when clinically possible, by having patients expectorate every 30 s for 5 min into a sterile 50-mL conical tube held on ice. Low salivary flow rate was defined as ≤ 1 mL/5 min, a threshold set for severe salivary dysfunction (Imanguli et al. 2010). Maximum mouth-opening was measured by means of a TheraBite® measuring scale (Atos Medical AB, Hörby, Sweden), and limited mouth-opening was defined as the inability to open > 35 mm (Burket 2008; Ferreira et al. 2010). Patient-reported outcome measures included the NIH Oral Symptom Scores (mouth dryness and oral pain, each on a 0-10 scale) (Pavletic et al. 2006) and the Oral Health Impact Profile (OHIP) (Slade and Spencer 1994).

Variables in scoring of the 3 components of oral cGVHD (oral mucosal disease, salivary dysfunction, and limited mouth-opening) were used to divide the study group into 3 groups: a group of patients with cGVHD and (1) oral mucosal disease (NIH OMS > 2), (2) salivary dysfunction (saliva volume ≤ 1 mL/5 min), and (3) limited mouth-opening (maximum mouth-opening ≤ 35 mm). These groups were then analyzed with eye, skin, and other organ systems scoring as continuous data, and compared with a group of patients with no specified oral cGVHD disease.

Organ-specific cGVHD scoring. Comparison variables of all organ manifestations of cGVHD were gathered for patients with cGVHD. Transplant clinicians assigned NIH organ-specific scores based on a scale of 0-3 for 8 organ systems for women and 7 for men: skin, eye, mouth, lung, liver, gastrointestinal tract, joint/fascia, and genital (women only). We calculated the NIH average score by dividing the total of NIH

organ-specific scores by the total number of organ systems assessed (Filipovich et al. 2005).

Dermatologists evaluated skin cGVHD using the Body Surface Area (BSA) percentage of skin involvement for erythema or sclerosis. Patient symptom intensity self-report profile included a 0 to 10 scale for the most severe skin itching. Ophthalmologists evaluated lacrimal dysfunction using Schirmer's tear test, measuring the amount of tearing in 5 min, done under topical anesthetic, and the peak severity of the chief eye complaint for the past week, based on a 0 to 10 scale. Lung cGVHD was assessed through pulmonary function testing, and liver cGVHD was judged through liver function tests. Gastrointestinal cGVHD was scored by early satiety, anorexia, nausea and vomiting, dysphagia, odynophagia, and diarrhea signs and symptoms. Joint and fascia cGVHD was evaluated by joint contractures, range-of-motion testing, and limitations of activities of daily living. Female vulva/vaginal cGVHD was scored using clinical signs of genital mucosal disease and vulvar or vaginal scarring. These measures correspond to the clinician-assessed and patient-reported cGVHD measures specified in the 2005 NIH Consensus Working Group for Response Measures (Pavletic et al. 2006).

Chronic GVHD symptoms scale. The Lee symptom scale measures the degree to which patients are bothered by symptoms of cGVHD. The Lee symptom subscales consist of grouped questions concerning the skin, the eyes/mouth, breathing, eating/digestion, muscles/joints, energy, and mental/emotional symptoms (Lee et al. 2002). Symptoms were compared with the 3 oral cGVHD entities as correlated with continuous data.

Statistical Analysis

Non-parametric tests were used. Specifically, comparisons of ordered categorical parameters vs. a dichotomous classification variable were evaluated with a Cochran-Armitage trend test (Agresti 1990). Parameters which were both dichotomous were compared by Fisher's exact test. An exact Wilcoxon rank-sum test was used to determine the significance of the difference between 2 groups with respect to a continuous outcome. Spearman rank correlation was used to determine the correlation between 2 continuous parameters. For the purposes of this study, $|r| > 0.50$ indicated a moderate-to-strong correlation, and $0.3 < |r| < 0.5$ indicated a weak-to-moderate correlation.

To maximize the numbers of participants available for analysis and to minimize potential bias in the disregarding of data, a complete dataset was created based on case deletion for maximum mouth-opening missing data and on regression mean imputation for oral mucosal cGVHD and salivary dysfunction missing data. Regression models were fit for missing data-points for salivary dysfunction based on xerostomia score ($r = -0.48$, $P < 0.0001$) and for oral mucosal score based on OMRS ($r = 0.51$, $P < 0.0001$).

All P values were two-tailed and were not formally adjusted to account for multiple comparisons; however, in view of the number of statistical tests performed, only P values < 0.01 were considered to be statistically significant.

Table 1. Patient Characteristics at the Time of Enrollment.

Patient Characteristics	n (%) or (range)
Total number of patients	212
Age (median, range, years old)	48 (18-70)
Gender	
Male	113 (53)
Female	99 (47)
Disease	
ALL/AML/MDS	89 (42)
CML	30 (14)
CLL	15 (7)
HL, NHL	51 (24)
MM	13 (6)
Sarcoma	2 (1)
Aplastic Anemia/PNH	8 (4)
Other non-malignant	4 (2)
Conditioning regimen	
Myeloblastic	110 (52)
Non-myeloblastic	102 (48)
Total Body Irradiation (TBI)	75 (35)
Donor relationship	
Unrelated	72 (34)
Related	140 (66)
Cell source	
Bone marrow	38 (18)
Peripheral blood	172 (81)
Cord blood	2 (1)
HLA match ^a	
Yes	176 (83)
No	32 (15)
Unknown	4 (2)
cGVHD onset type	
Progressive	78 (37)
Quiescent	53 (25)
De novo	78 (37)
Unknown	3 (1)
Activity by therapeutic intent ^b	
Active	102 (48)
Not active: decrease systemic therapy	21 (10)
Not active: cGVHD stable	51 (24)
Unknown (other)	38 (18)
Intensity of immunosuppression ^c	
None/mild	53 (25)
Moderate	78 (37)
Severe	81 (38)
Number of prior treatments	
< 2	23 (11)
2-5	112 (53)
> 5	74 (35)
Unknown	3 (1)
Individual organs involved ^d	
Mouth	147 (69)
Skin	163 (77)
Eyes	171 (81)
Lung	158 (75)
Liver	108 (51)
Joints or fascia	129 (61)
Gastrointestinal tract	88 (42)
Vulva/vagina (of females)	51 (51)
NIH Average Score ^e	1.0 (0.1-2.1)
NIH Global Score ^f	
Mild	4 (2)
Moderate	70 (33)

(continued)

Table 1. (continued)

Patient Characteristics	n (%) or (range)
Severe	138 (65)
Median number of months from transplant to GVHD diagnosis	7 (2-83)
Median number of months from transplant to enrollment	36 (4-258)

For all values in this table, continuous variables are shown as median values, with ranges and categorical variables shown as frequencies with percentages.

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; F, female; HLA, human leukocyte antigen; M, male; MDS, myelodysplastic syndrome; MM, multiple myeloma; PNH, paroxysmal nocturnal hemoglobinuria.

^aHLA match: a minimum of 8/8 allele match in cases of unrelated donors and 6/6 antigen and/or allele match (HLA-A, -B, -DR) in cases of related donors.

^bActive: (1) increase systemic therapy because cGVHD is worse; (2) substitute systemic therapy due to lack of response; and (3) withdrawal of systemic therapy due to lack of response. Not active: (1) decrease systemic therapy because cGVHD is better; (2) no change in current systemic therapy because cGVHD is stable; and (3) alter systemic therapy owing to its toxicity. Other: either did not receive any immunosuppressive therapy or did not meet any of the criteria.

^cIntensity of immunosuppression: Mild, single-agent prednisone < 0.5; Moderate, prednisone ≥ 0.5 mg/kg/day and/or any single agent/modality; High, 2 or more agents/modalities ± prednisone ≥ 0.5 mg/kg/day.

^dNIH Score of 0 not affected versus Score > 0 affected.

^eNIH Average Score: Total of NIH Scores divided by number of organs affected.

^fNIH Global Score: Mild, only 1 or 2 organs (except lung), with max score of 1 in all organs; Moderate, at least 1 organ with max score 2 or 3 OR more organs with max score of 1 OR lung score of 1; Severe, at least 1 organ with score of 3 OR lung score of 2 or more.

Results

Patient Demographics and Transplant Characteristics

Two hundred and eighty-five post-allo HSCT patients, referred for evaluation of cGVHD, were enrolled for a prospective cross-sectional study of cGVHD from 2004 to 2012. Forty-six individuals were excluded from the current analysis: 25 were judged not to have cGVHD and 21 were pediatric patients (age < 18 y). Of the remaining 239 adults with cGVHD, a dataset of 212 was developed based on: (1) oral mucosal cGVHD (NIH OMS score [$n = 197$], OMRS score [$n = 212$]), (2) salivary pathology (saliva production [$n = 109$], xerostomia [$n = 159$]), and (3) limited mouth-opening (maximum mouth-opening [$n = 212$]). Table 1 shows the patients and cGVHD characteristics of these 212 individuals.

Oral Mucosal Disease

Oral mucosal disease (NIH OMS > 2) was found in 31% (66 of 212) of patients with cGVHD. The finding of oral mucosal lesions in cGVHD (NIH OMS > 2) was not associated with salivary dysfunction (salivary flow ≤ 1 mL/5 min), with only a 2% overlap between these findings ($P = 1$). Oral mucosal disease was also not associated with limited mouth-opening (maximum mouth-opening ≤ 35 mm), with only an 8% overlap

between these manifestations ($P = 0.46$). There was a 17% ($P = 0.003$) overlap between oral mucosal disease (NIH OMS > 2) and skin erythema (BSA $> 0\%$) (Fig. 2). Oral mucosal disease (NIH OMS > 2) was significantly associated with higher mouth pain ($P < 0.001$), higher NIH mouth score ($P < 0.001$), and higher skin erythema BSA% ($P < 0.001$) (Table 2). For symptom association analysis, NIH OMS was correlated with oral pain ($r = 0.43$, $P < 0.001$) (Table 3).

Salivary Dysfunction

Salivary dysfunction (salivary flow ≤ 1 mL/5 min) was found in 11% (23 of 112) of patients with cGVHD. The inability to produce more than 1 mL of saliva in 5 min was not associated with the presence of oral mucosal cGVHD (NIH OMS > 2 , 2% overlap, $P = 1.0$) or with limited mouth-opening (maximum mouth-opening ≤ 35 mm, 1% overlap, $P = 0.09$). There was an 8% ($P = 0.17$) overlap between salivary dysfunction (salivary flow ≤ 1 mL/5 min) and lacrimal dysfunction (tears ≤ 5 mL/5 min) (Fig. 2). Low salivary production (salivary flow ≤ 1 mL/5 min) was associated with xerostomia ($P = 0.004$) and with low lacrimal production ($P = 0.010$) (Table 2). Significant associations were found between salivary dysfunction (salivary flow ≤ 1 mL/5 min) and mouth dryness ($P < 0.001$). For symptom association analysis, salivary production was negatively correlated with xerostomia ($r = -0.63$, $P < 0.001$), and xerostomia was positively correlated with eye symptoms ($r = 0.32$, $P < 0.001$) (Table 3).

Limited Mouth-opening

Limited mouth-opening (maximum mouth-opening ≤ 35 mm) was found in 17% (37 of 212) of patients with cGVHD. The inability to open the oral cavity beyond 35 mm was not associated with the presence of oral mucosal cGVHD (NIH OMS > 2 , 8% overlap, $P = 0.46$) or salivary dysfunction (the inability to produce 1 mL of saliva in 5 min, 1% overlap, $P = 0.09$). There was a 13% ($P = 0.006$) overlap between limited mouth-opening (opening ≤ 35 mm) and skin sclerosis (BSA $> 0\%$) (Fig. 2). Maximum mouth-opening ≤ 35 mm was significantly associated with the presence of mouth pain ($P = 0.006$), average NIH score ($P = 0.002$), skin itching ($P = 0.001$), and skin sclerosis BSA% ($P = 0.008$) (Table 2). A very weak correlation was found between maximum mouth-opening and the Lee scale for skin symptoms ($r = -0.24$, $P = 0.001$) (Table 3).

Discussion

This analysis supports the understanding of oral cGVHD as a trio of distinct diseases: mucosal lesions, salivary dysfunction, and limited mouth-opening. These 3 mouth findings are not associated with each other, but are associated with extraoral manifestations of cGVHD. These extraoral sites are similar to those found in the extraoral sites of autoimmune disorders with

Figure 2. White circles show the prevalence and overlap of oral cGVHD as an oral mucosal disease, as salivary dysfunction, or as limited mouth-opening. Gray circles show the prevalence and overlap of the oral cGVHD manifestations with select extraoral manifestations. 2×2 contingency analysis with Fisher's exact test to test the association of the 2 dichotomized manifestations.

oral findings clinically similar to those found in cGVHD, as suggested by lichen planus, Sjögren's syndrome, and scleroderma. Oral lichen planus is a chronic inflammatory autoimmune disease that affects the oral mucosa, with a clinical presentation very similar to that of clinically active oral mucosal cGVHD, and which has been used as a comparative disease entity for cGVHD (Sato et al. 2006; Pimentel et al. 2010). It has a prevalence of 0.1 to 4%, and, like oral cGVHD, may be a risk factor for oral squamous cell carcinoma (Liu et al. 2010). The skin is a primary target in lichen planus, and manifests as either a lichenoid or sclerodermatous variant, with the skin lesions that often resemble those of skin cGVHD disease (Lodi et al. 2005). Our findings support the commonality between these disease entities by showing an association between oral mucosal disease and skin erythematous lesions and, to a lesser extent, female genital mucosa. The association between oral mucosal disease and female genital mucosal disease warrants further study to assess the association between specific genital

Table 2. Patterns of Associations in Oral Mucosal Disease, Salivary Dysfunction, and Limited Mouth-opening in Patients with cGVHD (*n* = 212).

Clinical Category	Variable	Limited Mouth-opening and Skin Sclerosis			Salivary and Lacrimal Dysfunction			Oral Mucosal Disease and Erythematous Skin Disease		
		Limited Mouth-opening (≤ 35 mm)	Normal Range of Mouth-opening	<i>P</i> Value	Limited Salivary Function (≤ 1 mL/5 min)	Normal Salivary Function	<i>P</i> Value	Oral Mucosal Disease (NIH OMS > 2)	No Oral Mucosal cGVHD	<i>P</i> Value
Demographics	Number of Patients	37 (17%)	175 (83%)	–	23 (11%)	189 (89%)	–	66 (31%)	146 (69%)	–
	Age (years)	45 (21-65)	48 (18-70)	0.70	51 (18-70)	48 (18-67)	0.56	51 (21-65)	47 (18-70)	0.11
	Gender (# males)	17 (46%)	96 (55%)	0.37	7 (30%)	106 (56%)	0.026	44 (67%)	69 (47%)	0.011
	Months from HSCT to study entry	29 (5-204)	36 (4-258)	0.15	40 (7-258)	36 (4-258)	0.52	40 (6-152)	35 (4-58)	0.77
Eye cGVHD	Schirmer's tear test (mm)	2.5 (0-27)	3.5 (0-35)	0.70	1.5 (0-17)	3.5 (0-35)	0.010	4.5 (0-35)	3 (0-27)	0.045
Mouth cGVHD	Eye Symptoms (0-10)	5 (1-10)	5 (0-10)	0.53	7 (0-10)	5 (1-10)	0.16	5 (0-10)	6 (0-10)	0.48
	Mouth Pain (0-10)	2 (0-10)	0 (0-10)	0.006	0 (0-10)	1 (0-10)	0.68	4 (0-10)	0 (0-10)	<0.001
	Mouth Dryness (0-10)	3 (0-8)	3 (0-10)	0.87	5 (0-10)	3 (0-10)	0.004	3 (0-10)	3 (0-10)	0.024
Skin cGVHD	Skin Erythema (BSA%)	2% (0-39%)	0.4% (0-80%)	0.09	0.5% (0-76%)	0.6% (0-80%)	0.80	3 (0-76)	0 (0-80)	<0.001
	Skin Sclerosis (BSA%)	21 (0-95%)	0.5% (0-92%)	0.008	14% (0-81%)	13% (0-45%)	0.30	3% (0-95%)	3% (0-92%)	0.72
	Skin Itching (0-10)	4 (0-10)	1 (0-10)	0.001	3 (0-8)	2 (0-10)	0.27	2 (0-10)	2 (0-10)	0.53
NIH cGVHD	Average NIH Score	0.9 (0.1-2.0)	1.3 (0.4-2.1)	0.002	1.1 (0.8-2.0)	1.0 (0.1-2.1)	0.12	1.0 (0.1-2)	1.1 (0.3-2.1)	0.10
Organ Score	Skin	32 (86%)	131 (75%)	0.14	19 (83%)	144 (76%)	0.61	57 (86%)	106 (73%)	0.034
	Mouth	32 (86%)	115 (66%)	0.017	19	128	0.23	59 (89%)	88 (60%)	<0.001
	Eyes	30 (81%)	141 (81%)	1	20	151	0.58	52 (79%)	119 (82%)	0.71
	GI Tract	19 (51%)	69 (35%)	0.20	12	77	0.37	29 (44%)	59 (40%)	0.65
	Liver	20 (54%)	88 (50%)	0.59	10	98	0.51	35 (53%)	73 (50%)	0.76
	Lungs	32 (86%)	126 (72%)	0.09	18	140	1	43 (65%)	115 (79%)	0.32
	Joint	29 (78%)	100 (57%)	0.024	19	110	0.025	40 (61%)	89 (61%)	1
	Vulva/Vaginal (of females)	10 (50%)	41 (52%)	1	6 (37%)	45 (44%)	0.28	16 (73%)	35 (45%)	0.030

Data presented as medians (range of values) or as number affected (percentage). Boldface indicates $P \leq 0.01$.

and oral mucosal findings. This association also suggests that women with oral GVHD should undergo gynecologic assessment for genital GVHD.

Salivary gland pathology is an important component of oral cGVHD, leading to xerostomia, oral infections, and a high caries risk (Castellarin et al. 2012; Mays et al. 2013). The comparable salivary and lacrimal hypofunction of cGVHD and Sjögren's syndrome has been recognized for decades (Lawley et al. 1977; Sale et al. 1981). Sjögren's syndrome is a chronic autoimmune disease that generally affects middle-aged women, with a prevalence of 0.5 to 1% in the population (Mavragani and Moutsopoulos 2013). Imanguli et al., using a subset of our current cGVHD database, found that 27% (22 of 81) of patients with cGVHD had very low salivary flow (<1 mL/5 min) and that 77% of patients had xerostomia (Imanguli et al. 2010). This 2010 study reported a significant association between xerostomia and xerophthalmia, and a lack of association between low salivary flow and oral cGVHD mucosal disease. The authors concluded that salivary gland involvement is a common and clinically distinct manifestation of cGVHD, and that it develops independently of mucosal pathology. The current findings support these conclusions (11% of patients with cGVHD with salivary dysfunction and a significant association of xerostomia and xerophthalmia symptoms with a weak-to-moderate correlation [$r = 0.32$], and little indication of association between salivary dysfunction, oral

mucosal disease, and limited mouth-opening [Fig. 2]). The current analysis also expands this association to include that between low saliva production and lacrimal dysfunction, as measured by Schirmer's tear test.

Sclerotic fibrosis of the perioral tissue as a consequence of chronic inflammation can result in restricted oral range-of-motion in patients with oral cGVHD; this resembles the tissue changes observed in patients with scleroderma (Filipovich et al. 2005; Schubert and Correa 2008). Scleroderma is a rare autoimmune connective and vascular disorder, affecting less than 0.1% of the population, with significant negative impacts on oral and mandibular function (Ferreira et al. 2010; Schouffoer et al. 2013). Scleroderma-like cGVHD can involve any of the orofacial tissues, resulting in fibrosis and limited mouth-opening (Woo et al. 1997). Limitations of mouth-opening could lead to problems with oral hygiene and eating, potentially contributing to infection and malnutrition (Imanguli et al. 2008; Schubert and Correa 2008). The current analysis shows a similarity between oral cGVHD limitation of mouth-opening and scleroderma with limited mouth-opening associated with skin sclerosis, as well as with skin symptoms.

The 3 oral cGVHD types, mucosal disease, salivary gland disease, and limitation of mouth-opening, appear to be separate from each other, with little overlap among their clinical manifestations and little interrelationship. More overlap is seen with specific extraoral manifestations, suggesting underlying tissue-specific

Table 3. Correlation Table Showing the Strengths of Interactions between Oral cGVHD Measures and Symptom Scales for Oral, Skin, and Eye cGVHD.

	Salivary Flow in 5 min	Maximum Mouth-opening	Oral Mucosal Score	Skin Itching, 0-10	Eye Symptoms, 0-10	Mouth Dryness, 0-10	Oral Pain, 0-10	Lee Skin	Lee Eyes/Mouth	Lee Eating/Digestion	OHIP
Salivary flow in 5 min	I					-0.60			-0.27		
Maximum mouth-opening		I	-0.20					-0.24		-0.22	
Oral Mucosal Score		-0.20	I			0.22	0.43				
Skin itching, 0-10				I		0.22		0.53			
Eye symptoms, 0-10					I	0.32			0.61		
Mouth dryness, 0-10	-0.63		0.22	0.22	0.32	I	0.48		0.40	0.41	0.31
Oral pain, 0-10			0.43			0.48	I	0.22	0.51	0.29	0.28
Lee Skin		-0.24		0.53			0.22	I			
Lee Eyes/Mouth	-0.27				0.61	0.49	0.51		I	0.28	0.24
Lee Eating/Digestion		-0.22				0.41	0.29		0.28	I	
OHIP						0.35	0.28		0.24		I

OHIP, Oral Health Impact Profile.

Spearman's ρ value, $|\rho| > 0.3$, indicates weak to moderate correlation, $|\rho| > 0.5$ indicates moderate to strong correlation, and only significant correlations are shown.

predisposition to a particular cGVHD manifestation type, with tissue-specificity among mucosal area, secretory glands, and sclerosis sites. The clinical similarity of these oral and extraoral manifestations of cGVHD does not necessarily show commonality of etiology or pathophysiology. Oral lesions can be caused by diseases other than autoimmune disorders, and drug reaction and infection should be ruled out prior to a diagnosis of oral cGVHD (Mays et al. 2013). Salivary dysfunction and xerostomia are common drug side-effects (Imanguli et al. 2010). Limited mouth-opening can be secondary to hyperkeratotic mucosal disease, severe mouth dryness, and temporomandibular disorders, and may not be sclerotic in nature (Ferreira et al. 2010). However, the clinical similarities and clinical groupings seen in this analysis between common tissue types in disparate organ systems may indicate some commonality in the onset or progression of cGVHD.

An attempt was made to create cutoff values for oral cGVHD that were clinically relevant but not too restrictive, to categorize patients into the 3 groups: those with oral mucosal disease, those with salivary gland dysfunction, and those with limited mouth-opening. This allowed for broad association analysis of group cGVHD manifestations, and also provided some clinically relevant points to be assessed for a clinician evaluating a post-HSCT patient. The clinical evaluation of NIH OMS for oral mucosal scoring, 5-minute saliva test for salivary dysfunction, and maximum mouth-opening measurement may form the basis of an oral cGVHD examination.

The limitations of this study arise largely from its cross-sectional design. Prospective clinical data would strengthen our association findings, allow for temporal clinical manifestation analysis, and allow for more targeted and less discovery-based hypothesis testing. Tracking these oral manifestations separately should be considered for prospective transplant studies. Additionally, because of the retrospective, cross-sectional design of the current analysis, missing data were addressed by strategies which may introduce bias and skew associations. Also, patients in this study were not medically managed in a uniform manner with respect to systemic and/or topical

therapies, and this could influence cGVHD symptoms and manifestations. Finally, due to the referral-based nature of the cohort, the patients included in this analysis were predominantly those with severe and therapy-refractory cGVHD, which limits the generalizability of our results to a more general population.

Despite these confounding variables, this dataset clearly showed the hypothesized associations to segregate out within the confines of the current analysis. Negative findings also reinforce the main associations, and argue for an underlying commonality in clinical presentation that outweighs these biasing factors.

This analysis supports the conclusion of oral cGVHD as 3 separate manifestations, oral mucosal disease, salivary dysfunction, and oral sclerosis, which has potential effects on the etiology, diagnosis, and treatment of oral cGVHD. Clear classification of oral cGVHD as 3 separate manifestations will improve clinical diagnosis, observational research data collection, and the definitions of outcome measures in clinical trials. The increased specificity in defining and discussing oral cGVHD will guide and improve the understanding of oral cGVHD pathophysiology and treatment.

Author Contributions

C. Bassim, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; H. Fassil, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript; J. Mays, S. Steinberg, S. Pavletic, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript; D. Edwards, contributed to conception and data acquisition, critically revised the manuscript; K. Baird, contributed to design and data interpretation, critically revised the manuscript; E. Cowen, H. Naik, M. Dantiles, contributed to conception, data acquisition, and interpretation, critically revised the manuscript; R. Gress, contributed to conception, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This study was funded by the intramural programs of the National Cancer Institute (NCI), Center for Cancer Research, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and the National Institute of Dental and Craniofacial Research (NIDCR), as well as by the other intramural programs of the National Institutes of Health (NIH). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- Agresti A. 1990. Categorical data analysis. New York, NY: John Wiley and Sons, Inc.
- Bassim CW, Fassil H, Dobbin M, Steinberg SM, Baird K, Cole K, Joe G, Comis LE, Mitchell SA, Grkovic L, et al. 2014. Malnutrition in patients with chronic GVHD. *Bone Marrow Transplant*. 49:1300–1306.
- Burket L. 2008. *Burket's oral medicine*. 11th ed. Hamilton, Ontario: BC Decker, Inc.
- Castellarin P, Stevenson K, Biasotto M, Yuan A, Woo SB, Treister NS. 2012. Extensive dental caries in patients with oral chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 18(10):1573–1579.
- Coplan EA. 2006. Hematopoietic stem-cell transplantation. *N Engl J Med*. 354(17):1813–1826.
- Fall-Dickson JM, Mitchell SA, Marden S, Ramsay ES, Guadagnini JP, Wu T, St John L, Pavletic SZ; National Institutes of Health Chronic Graft-versus-Host Disease Study Group. 2010. Oral symptom intensity, health-related quality of life, and correlative salivary cytokines in adult survivors of hematopoietic stem cell transplantation with oral chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 16(7):948–956.
- Fassil H, Bassim CW, Mays J, Edwards D, Baird K, Steinberg SM, Williams KM, Cowen EW, Mitchell SA, Hakim FT, et al. 2012. Oral chronic graft-vs.-host disease characterization using the NIH scale. *J Dent Res*. 91(7 Suppl):45S–51S.
- Ferreira E, Christmann R, Borba E, Borges C, Siqueira J, Bonfa E. 2010. Mandibular function is severely impaired in systemic sclerosis patients. *J Orofac Pain*. 24(2):197–202.
- Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, et al. 2005. 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*. 11(12):945–956.
- Grkovic L, Baird K, Steinberg SM, Williams KM, Pulanic D, Cowen EW, Mitchell SA, Hakim FT, Martires KJ, Avila DN, et al. 2011. Clinical laboratory markers of inflammation as determinants of chronic graft-versus-host disease activity and NIH global severity. *Leukemia*. 26(4):633–643.
- Imanguli MM, Alevizos I, Brown R, Pavletic SZ, Atkinson JC. 2008. Oral graft-versus-host disease. *Oral Dis*. 14(5):396–412.
- Imanguli MM, Atkinson JC, Mitchell SA, Avila DN, Bishop RJ, Cowen EW, Datiles MB, Hakim FT, Kleiner DE, Krumlauf MC, et al. 2010. Salivary gland involvement in chronic graft-versus-host disease: prevalence, clinical significance, and recommendations for evaluation. *Biol Blood Marrow Transplant*. 16(10):1362–1369.
- Kobak S, Oksel F, Aksu K, Kabasakal Y. 2013. The frequency of sicca symptoms and Sjögren's syndrome in patients with systemic sclerosis. *Int J Rheum Dis*. 16(1):88–92.
- Lawley T, Peck G, Moutsopoulos H, Gratwohl A, Deisseroth A. 1977. Scleroderma, Sjögren-like syndrome, and chronic graft-versus-host disease. *Ann Intern Med*. 87(6):707–709.
- Lee S, Cook EF, Soiffer R, Antin JH. 2002. Development and validation of a scale to measure symptoms of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 8(8):444–452.
- Liu Y, Messadi DV, Wu H, Hu S. 2010. Oral lichen planus is a unique disease model for studying chronic inflammation and oral cancer. *Med Hypotheses*. 75(6):492–494.
- Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman P, Thongprasom K. 2005. Current controversies in oral lichen planus: report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 100(1):40–51.
- Mavragani C, Moutsopoulos H. 2014. Sjögren's syndrome. *Annu Rev Pathol*. 9:273–285.
- Mays JW, Fassil H, Edward D, Pavletic S, Bassim C. 2013. Oral chronic graft-versus-host disease: current pathogenesis, therapy, and research. *Oral Dis*. 19(4):327–346.
- Meier JK, Wolff D, Pavletic S, Greinix H, Gosau M, Bertz H, Lee SJ, Lawitschka A, Elad S; International Consensus Conference on Clinical Practice in cGVHD. 2011. Oral chronic graft-versus-host disease: report from the International Consensus Conference on clinical practice in cGVHD. *Clin Oral Investig*. 15(2):127–139.
- Pavletic SZ, Baird K. 2006. Chronic graft versus host disease. *Curr Opin Hematol*. 13(6):426–435.
- Pavletic SZ, Martin P, Lee SJ, Mitchell S, Jacobsohn D, Cowen EW, Turner ML, Akpek G, Gilman A, McDonald G, et al. 2006. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 12(3):252–266.
- Pimentel VN, de Matos LS, Soares TC, Adam R, Metzke K, Correa ME, de Souza CA, Cintra ML. 2010. Perforin and granzyme B involvement in oral lesions of lichen planus and chronic GVHD. *J Oral Pathol Med*. 39(10):741–746.
- Sale GE, Shulman HM, Schubert MM, Sullivan KM, Kopecky KJ, Hackman RC, Morton TH, Storb R, Thomas ED. 1981. Oral and ophthalmic pathology of graft versus host disease in man: predictive value of the lip biopsy. *Hum Pathol*. 12(11):1022–1030.
- Sato M, Tokuda N, Fukumoto T, Mano T, Sato T, Ueyama Y. 2006. Immunohistopathological study of the oral lichenoid lesions of chronic GVHD. *J Oral Pathol Med*. 35(1):33–36.
- Schouffoer A, Strijbos E, Schuerwegh A, Mouthon L, Vlieland TV. 2013. Translation, cross-cultural adaptation, and validation of the Mouth Handicap in Systemic Sclerosis questionnaire (MHSS) into the Dutch language. *Clin Rheumatol*. 32(11):1649–1655.
- Schubert MM, Williams BE, Lloid ME, Donaldson G, Chapko MK. 1992. Clinical assessment scale for the rating of oral mucosal changes associated with bone marrow transplantation. Development of an oral mucositis index. *Cancer*. 69(10):2469–2477.
- Schubert MM, Correa ME. 2008. Oral graft-versus-host disease. *Dent Clin North Am*. 52(1):79–109, viii–ix.
- Slade G, Spencer A. 1994. Development and evaluation of the Oral Health Impact Profile. *Community Dent Health*. 11(1):3–11.
- Treister N, Duncan C, Cutler C, Lehmann L. 2012. How we treat oral chronic graft-versus-host disease. *Blood*. 120(17):3407–3418.
- Vincent C, Agard C, Barbarot S, N'Guyen JM, Planchon B, Durant C, Pistorius MA, Dreno B, Ponge T, Stalder JF, et al. 2009. Orofacial manifestations of systemic sclerosis: a study of 30 consecutive patients. *Rev Med Interne*. 30(1):5–11.
- Woo SB, Lee SJ, Schubert MM (1997). Graft-vs.-host disease. *Crit Rev Oral Biol Med*. 8(2):201–216.