Association of Day-100 Oral GVHD with subsequent Chronic GVHD Diagnosed by NIH 2005 Consensus Criteria and Treated with Systemic Immunosuppression

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Abstract

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Background: Graft-versus-host disease (GVHD) is a significant cause of morbidity and mortality post hematopoietic transplantation (HCT). The oral cavity is a common site of chronic GVHD and can be one of the first sites of involvement. Clinical and laboratory investigations have been utilized within the allogeneic transplant population to identify or predict chronic GVHD development near the time of departure from the transplant service (approximately Day +70-120 post-HCT). Such investigations routinely include oral examinations to assess for GVHD-related findings; however, there are currently no published studies in the literature that have exclusively examined the specific association between oral GVHD, based solely on clinical findings at departure and the development of future systemic chronic GVHD.

Aim: To examine the association between oral mucosal GVHD at the time of departure evaluation (day+ 70-120) in patients without prior evidence of other chronic GVHD activity and the development of subsequent chronic GVHD requiring treatment with systemic immunosuppression.

Methods: The electronic medical records (EMRs) of 642 consecutive adult patients (≥ 21 years of age) who received their first allogeneic HCT at Seattle Cancer Care Alliance (SCCA; Seattle, WA, USA) between January 1st 2010- June 30th 2014 were reviewed for inclusion in the study.

Oral mucosal GVHD disease status was determined based on review of oral medicine "departure" examination records (departure exams were completed between day +70-120 post-HCT). Demographic data, transplant protocols, and event timeline information were obtained from the Fred Hutchison Cancer Center (FHCRC) optical web library and the Gateway database.

In cases where an official oral GVHD diagnosis was not made or when oral examination findings were non-specific, clinical descriptors were independently reviewed by three oral medicine providers and, a discussion was held until a consensus on oral mucosal GVHD status was reached. The diagnosis of oral mucosal GVHD was based on the NIH diagnostic criteria. The FHCRC optical web library gateway database was reviewed to determine the primary outcome variable "future chronic GVHD treated with systemic immunosuppression". All cases of chronic GVHD were diagnosed based on the 2005 NIH consensus criteria.

Univariate and multivariable logistic regression were used to examine the association between a diagnosis of oral mucosal GVHD at and the development of chronic GVHD. All analyses were performed using SAS (Statistical Analysis Software) v9.4.

Results: Five hundred and thirty-eight patients met the criteria for inclusion in the study. In both the univariate and multivariable analyses, clinical oral mucosal GVHD at departure without other

chronic GVHD activity prior or at departure was associated with an increased risk for subsequent development of chronic GVHD requiring treatment with systemic immunosuppression. In the univariate analysis, those with oral mucosal GVHD at departure were 1.5 times as likely to develop chronic GVHD compared to those without chronic systemic GVHD at departure (odds ratio (OR)=1.5, 95% CI 1.1-2.2, p=0.02). After adjusting for risk factors related to the development of chronic GVHD (specifically recipient age, patient/donor gender, donor relationship, graft source, acute GVHD grades 2-4, conditioning regimen and prednisone treatment at time of departure), the association was similar (OR=1.6, 95% CI 1.2-2.4, p=0.01).

Conclusion: A clinical diagnosis of oral mucosal GVHD at the time of departure was found to be associated with increased risk for subsequent development of systemic chronic GVHD. This study highlights the potential value of examining patients 70-120 days' post HCT to determine the presence of oral GVHD-related changes. With future studies supporting such an association, it would reaffirm the value of diagnosing oral GVHD accurately and in a timely manner to improve not only oral health and patient comfort but perhaps even overall survival. Patients with an oral GVHD diagnosis at departure should be advised of the increased risk of chronic GVHD development and receive appropriate education to allow for early recognition and reporting of chronic GVHD signs and symptoms to their medical team.

TABLE OF CONTENTS

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I am extremely thankful and grateful to have had the opportunity to work on this research project and hopefully this study will help in providing better care for patients struggling through life threatening diseases.

Dedication

To my husband, Vaishnav who has been a pillar of support through the years without whom I could not have achieved this goal

To my parents who have raised me to be the person I am today

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CHAPTER 1: BACKGROUND

Allogenic hematopoietic cell transplantation (allo-HCT) has been used in the treatment of hematopoietic malignancies since the late 1950s (Schubert & Correa, 2008). More recently, indications for allogeneic HCT have been expanded to include other immune and hematopoietic disorders (Antin & Ferrara, 1992; Mahmoud, et al., 2015). In HCT, hematopoietic cells are harvested from the donor and transplanted into a genetically different individual. Antigenic differences between the donor and recipient put the patient at risk for graft-versus-host disease (GVHD), an immune-based disease which can result in significant morbidity and mortality (Schubert & Correa, 2008; Lee & Flowers, 2008). The disease may affect tissues and organ systems throughout the body, which present with characteristic clinical and laboratory findings. GVHD is a complex disease process that is subdivided into acute and chronic form. Previously the disease was empirically classified based on time of onset since transplant (with acute GVHD occurring prior to day 100) (Filipovich, et al., 2005). Currently, it is established that acute and chronic GVHD have distinct clinical and pathophysiologic features which are used as the basis for diagnosis (Lee, 2005). This recognition has led to new diagnostic categories, such as "late acute GVHD, in which manifestations of acute GVHD occur after day 100 (Arora, et al., 2016). Prior terminology, such as "overlap GVHD" (used to describe the co-occurrence of the clinical features of both acute GVHD and chronic GVHD), has been eliminated in the more recent NIH 2014 consensus criteria (Jagasia, et al., 2015).

A. GVHD pathogenesis

Acute GVHD

The pathogenesis of acute GVHD can be described in 3 sequential phases namely: 1) activation of host antigen presenting cells (APCs), 2) donor T cell activation and 3) the cellular and inflammatory effector phase (Choi, Levin, & Ferrara, 2010; Reddy & Ferra, Immunobiology of acute graft-versus-host disease, 2003).

The conditioning regimen delivered just prior to stem cell transfusion results in significant damage to the host tissues (Antin & Ferra, 1992). The injury to the tissues caused by the conditioning regimen and the underlying disease (for which the patient is being transplanted) are identified and presented by host antigen presenting cells (APC) to donor T lymphocytes (Choi, Levin, & Ferrara, 2010). The extent of tissue damage and intensity of the conditioning regimen (i.e. toxicity) can affect the risk of GVHD (Choi, Levin, & Ferrara, 2010). After interaction with the activated host APCs, there is increased donor T cells proliferation, differentiation and migration. This process results in the activation of T cell co-stimulatory molecules amplifying the inflammatory response along with the production of cytokines such as Type 1 T-helper cells (Th1) (interferon-gamma (IFN-γ), tumor necrotic factor-alpha (TNF-α), interleukin (IL-2), Th2 (IL-4, IL-5, IL-10, IL-13) and Th17 which induce acute GVHD (Choi, Levin, & Ferrara, 2010; Antin & Ferrara, 1992). Finally, donor T cells mediate cytotoxicty at target sites in response to tissue antigens and leaked microbial products from the injured tissue linings inducing a greater immune response and therefore development of GVHD (Choi, Levin, & Ferrara, 2010; Couriel, Caldera, Champlin, & Komanduri, 2004).

The immunological response to treatment and consequent resolution of acute GVHD varies among patients. Complete resolution occurs when the inflammatory response is entirely resolved, whereas partial resolution is seen when the inflammatory response is incompletely resolved (Holtan, Pasquini, & Weisdorf, 2014). Acute GVHD impacts chronic GVHD development; however, a subgroup of patients with acute GVHD do not develop chronic GVHD due to specific T cells which suppress recipient alloreactivity (Atkinson, et al., 1990).

Chronic GVHD

The pathogenesis of chronic GVHD is more complex than acute GVHD and involves both cellular and humoral mediators (Shulman, et al., 1980; Martin P. , 2008). The specific physiologic mechanisms in chronic GVHD are less fully understood than those in acute GVHD. Multiple hypotheses of chronic GVHD pathogenesis have been postulated, largely based on the results of animal studies (Martin P. , 2008).

Thymic damage in acute GVHD is thought to result in failure of negative selection during T-cell maturation, resulting in nascent T cells that respond to antigens on both recipient and donor cells. Furthermore, regulatory T cells damaged in acute GVHD results in expansion of Th1 and Th17 cytokines in the first 2-3 weeks' post HCT that can contribute to chronic GVHD development (Chen, et al., 2007).

Another possible mechanism in chronic GVHD development is believed to involve transforming growth factor- beta (TGF- β) signaling, though the pathophysiology of which is not fully understood (Martin P. , 2008; Baron, et al., 2007).

Even though T cells have long been recognized as the principle mediators in chronic GHVD, there is growing evidence that B cells are also involved in pathogenesis. For example, the presence of B cell activation factor (BAFF) and various B cell biomarkers have been identified in higher levels in the setting of chronic GVHD. Sarantopoulos and colleagues detected higher levels of BAFF in patients with active chronic GVHD and a significant drop in levels following corticosteroid therapy (Sarantopoulos, et al., 2007). Additionally, rituximab, a monoclonal antibody against CD20 (a marker on the surface of B cells), has been shown to decrease blood titers of antibodies directed against minor histocompatibility antigens in the Y chromosome (Cutler, et al., 2006). These antibodies are thought to play a role in the increased risk of chronic GVHD in males receiving stem cells from female donors (Cutler, et al., 2006).

B. Epidemiology of GVHD

Acute GVHD

In allogeneic HCT, donor cells are harvested from one of three sources: bone marrow, peripheral blood, or umbilical cord blood (Copelan, 2006; Cornetta, et al., 2005). Historically, bone marrow was identified as the first viable source of donor cells (Copelan, 2006). Though marrow is still utilized in certain circumstances, peripheral blood stem cells (PBSCs) are now the most widely used source. More recently, protocols have also utilized stem cells obtained from umbilical cord blood (CB). GVHD risk varies depending on the cell source, based on the immune properties of the engrafted cells (Copelan, 2006). For example, PBSC transplants (PBSCT) carry the highest risk due to the presence of a greater number of T-lymphocytes which have the capacity to mount an immune response against host tissue antigens. Auberger et al. report that 54% of individuals

receiving PBSCs develop acute GVHD of grade II or greater versus 51% of those receiving bone marrow transplantation (BMT) (Auberger, et al., 2011). The incidence of acute GVHD tends to be lowest in patients who receive cord blood transplants (CBT) compared to other cell sources (Brunstein, et al., 2010). MacMillan in their study found 39% of patients receiving single unit CBT (n=80) developed acute GVHD compared to 58% of those receiving two units of cord blood from two different donors (MacMillan, et al., 2009).

Donors for the purpose of HCT are selected based on the degree of human leukocyte antigen (HLA) match with the patient. HLA are polymorphic genes, with each individual carrying 10-12 genes encoding for HLA-A, -B,-C, DR, DP and DQ (Park & Seo, 2012). Acute GVHD develops in 35-40% of patients with fully matched HLA identical donors and 60-70% of patients with one antigen mismatched donors (Reddy & Ferra, Immunobiology of acute graft-versus-host disease, 2003).

Pre-transplant conditioning regimens also impact the incidence of GVHD with non myeloablative (NMA) regimens having lower incidence than myeloablative (MA) protocols (Couriel, et al., 2004). In a study of 137 patients, Couriel et al, found incidence of acute GVHD to be 12% in patients who received NMA (n=63) conditioning versus 36% amongst those treated with MA regimens (Couriel, et al., 2004).

Chronic GVHD

Chronic GVHD occurs in 30-70% of patients following allo- HCT, with the median time of diagnosis ranging from 4-6 months following HCT (Lee & Flowers, 2008). In the United States,

3000 individuals are diagnosed with new onset chronic GVHD each year. Only 5-10% of patients develop new onset chronic GVHD greater than 1 year following HCT (Lee & Flowers, 2008). The overall chronic GVHD prevalence in the United States is approximately 10,000 cases (when including patients with disease resolution, deaths and relapsed malignancies) (Martin, et al., 2015; Lee & Flowers, 2008).

Chronic GVHD is sub-divided into 3 classes based on its temporal relationship to acute GVHD. "De novo" chronic GVHD is defined as new onset chronic GVHD in a patient with no past history of acute GVHD. Chronic GVHD is termed "quiescent" if it developed after resolution of prior acute GVHD. "Progressive" chronic GVHD is defined as GHVD which develops as a direct extension from active acute GVHD (Sullivan, et al., 1988). In a prospective study by Pidala et al, a cohort of 242 allogeneic HCT patients were evaluated at the time of enrollment and every 6 months between August 2007- December 2009 (Pidala J. , et al., 2011). Of the 242 patients, 181 (74.7%) were diagnosed with chronic GVHD (Pidala J. , et al., 2011). Among those with chronic GVHD, 14% developed de novo onset GVHD", 79% had quiescent onset, and 7% had progressive onset GVHD (Pidala J. , et al., 2011).

The incidence of chronic GVHD is highest amongst patients receiving PBSCT. Auberger and colleagues reported 48% incidence of chronic GVHD in patients receiving PBSCT (n=152) versus 24% among those receiving BMT (n=177). The incidence of chronic GVHD is reported to be lower in both CBT compared to PBSCT and BMT. In a study, by MacMillan et al. the cumulative incidence of chronic GVHD was found to be 17% amongst 265 patients who received CBT (MacMillan, et al., 2009). No difference was found in the incidence of chronic

GVHD in those receiving a single versus double unit CBT, unless the patient also had a history of grade II or higher acute GVHD (MacMillan, et al., 2009).

In addition to graft type, the relationship of stem-cell donor to recipient has also been associated with differences in the incidence of chronic GVHD (Wingard J. , et al., 1989) . Lee et al. reported a 63% incidence of chronic GVHD in those receiving grafts from unrelated donors (matched at HLA-A, -B and -DR) at one-year post transplant, compared to 42% in transplants from sibling donors (Lee, et al., 2002).

The association between conditioning and chronic GVHD is less clear. A retrospective study by Mielcarek et al. found no significant difference in the incidence of chronic GVHD among patients with MA and NMA conditioning regimens. Couriel et al. reported 40% incidence in those receiving MA conditioning (n= 74) versus only 14% in those treated with NMA regimens (n= 63) (Couriel, et al., 2004; Mielcarek, et al., 2003). In contrast, Pérez-Simón et al. reported incidence of chronic GVHD to be higher in patients receiving reduced intensity conditioning when compared to MA protocols (71% versus 63%) (Pérez-Simón, et al., 2005) (Pérez-Simón, et al., 2005).

C. Risk factors for GVHD

Multiple factors have been identified as significant risk factors in the development of both acute and chronic GVHD. Prior to transplant, donor and recipient characteristics evaluated to identify these risk factors to decrease morbidity and mortality associated with GVHD (Flowers, et al., 2011).

Degree of patient-donor HLA match and donor alloimmunization are the strongest risk factors for acute GVHD (Flowers, et al., 2011; Weisdorf, et al., 1991). Alloimmunization primarily occurs in association with pregnancy (with increasing risk in multiparous females) due to maternal exposure to fetal antigens. Additionally, donor alloimmunization is associated with medical therapies, such as blood transfusions, administered prior to stem cell collection (Loren A. W., et al., 2006). Similarly, myeloablative conditioning protocols also carry increased risk for GVHD when compared to reduced-intensity protocols, presumably due to greater cell damage and consequent generation of host "allo-antigens" (Jagasia, et al., 2012).

Jagasia and colleagues identified male recipients with female donors and transplant recipients with advanced primary disease to have the highest risk of developing acute GVHD. Furthermore, patients in these groups who developed acute GVHD were more likely to be diagnosed with higher grade of disease. In contrast, a study by Weisdorf et al. found the lowest risk of acute GVHD in female patients with female donors (Weisdorf, et al., 1991).

Donor lymphocyte infusion (DLI) is a medical therapy utilized to induce the graft-versus-leukemia effect (GVL) in patients with hematologic relapse following myeloablative HCT. (Mapara, et al., 2002). DLI is associated with an increased for acute GVHD especially when administered less than 200 days' post HCT (He, et al., 2014).

Though acute and chronic GVHD share some risk factors, there are important differences in the risk profile between the two conditions. HLA mismatch and transplants from unrelated donors

have a greater influence on the risk of acute GVHD than chronic GVHD (Flowers, et al., 2011). Conversely, older patients and those receiving PBSC are more likely to develop chronic GVHD (Flowers, et al., 2011; Cutler, et al., 2001). That said, physiologic changes in acute GVHD almost certainly influences chronic GVHD development, evidenced by the fact that the single strongest for the development of chronic GVHD is a history of acute GVHD (Sullivan, et al., 1981; Atkinson, et al., 1990). Furthermore, progression from acute GVHD to chronic GVHD is influenced by GVHD severity (Atkinson, et al., 1990). For example, Atkinson and colleagues determined increasing incidence of chronic GVHD based on acute GVHD severity with chronic GVHD incidence ranging from approximately 28% in the absence of acute GVHD to 85% in those with grade IV (with 49%, 59%, and 80% incidence in grades I-III, respectively) (Atkinson, et al., 1990).

Apart from prior acute GVHD history, Wagner et al. report that corticosteroid use (at day 100 post-HCT is an independent risk factor for chronic GVHD development with a relative risk of 2.1 (regardless of history of acute GHVD) (Wagner, et al., 1998). Corticosteroids are believed to mediate chronic GVHD development through antigen specific T cell suppression and increased risk of infection which can increase GVHD activity via infection mediated tissue damage (Wagner, et al., 1998; Sayer, Longton, Bowden, Pepe, & Storb, 1994).

Other well established risk factors for chronic GVHD includes female donor: male recipient pairings and female donor parity (Flowers, et al., 2011; Kollman, et al., 2001). Risk is increased in female donor: male recipient pairs due to formation of cytotoxic T cells which react against H-Y antigens found on the Y-chromosome (Loren A. W., et al., 2006). Additionally, female parity

increases the risk of chronic GVHD (regardless of recipient sex) with a relative risk of 1.40 (95% CI, 1.25-1.57) if the donor has had two or more pregnancies (Kollman, et al., 2001).

Risk factors for acute and chronic GVHD development are included in table 2.

Table 1: Risk factors*

Acute GVHD	Intensity of conditioning regimen
	PBSC > BM >CB
	Degree of HLA donor/patient match and unrelated donor
	Donor lymphocyte infusion (DLI)
	Increasing donor age
	Increasing patient age
	Female donor: male recipient pair
	Female-donor parity
Late acute GVHD	Grade 3-4 early onset acute GVHD
Chronic GVHD	Increasing patient age
	Increasing donor age
	Female donor into male patient
	Degree of HLA donor/patient match and unrelated donor
	Female donor parity
	PBSC > BM >CB
	Previous acute GVHD
Abbreviations: PBSC- peripheral blood stem cell, BM- bone marrow, CB- cord blood, HLA- human leukocyte antigens	

*References: (Lee & Flowers, 2008; Wagner, et al., 1998; Jagasia, et al., 2012; Flowers, et al., 2011; He, et al., 2014; Kollman, et al., 2001).

D. Clinical presentation of GVHD

Acute GVHD

The most common sites of acute GVHD are the skin, liver and gastrointestinal tract (GI). The median time of acute GVHD onset post HCT is about 19 days (range 11-150) (Veltri, et al., 2013; Schubert & Sullivan, 1989; Reddy & Ferrara, 2008). The most common clinical presentations of acute GVHD are inflammatory dermatitis, enteritis, and allo-immune hepatitis. Amongst patients diagnosed with acute GVHD, 81% of patients have skin involvement, 54% have GI and 50% develop liver GVHD (Reddy & Ferra, Immunobiology of acute graft-versus-host disease, 2003).

Acute GVHD severity is graded from I-IV based on extent and severity of involvement. Patients who develop Grade III and IV acute GVHD have the worst outcome due to poor response to treatment (Akpek, et al., 2003; MacMillan, et al., 2002).

i) Skin

The skin is often the initial site of presentation of acute GVHD manifesting as a maculopapular rash in the palms and soles followed by trunk, neck, cheek and ears (Zhou, Barnett, & Rivers, 2000). In severe cases, patients present with bullae formation, epithelial desquamation with generalized erythroderma (Ball & Egeler, 2008).

ii) GI tract

Gastrointestinal manifestations include diarrhea with or without symptoms of nausea, vomiting, abdominal pain and anorexia (Couriel, Caldera, Champlin, & Komanduri, 2004).

iii) Liver

Liver GVHD is characterized by transaminitis and elevated liver function tests due to hepatocyte damage (Couriel, Caldera, Champlin, & Komanduri, 2004).

iv) Oral cavity

Oral involvement in acute GVHD appears to be more common in the setting of severe acute systemic GVHD. Ion et al. suggests that oral acute GVHD occurs in association with classical features of severe grades of acute GVHD (Ion, et al., 2014) . The incidence of oral involvement in patients with grades II-IV acute GVHD is about 40-65% (Schubert & Sullivan, 1989).

Acute GVHD involvement of the oral mucosa can be widespread extending from the lips to the soft palate (Ion, et al., 2014). It is predominated mainly by the presence of mucosal erythema, ulceration and occasionally lichenoid hyperkeratosis (Kuten-Shorrer, Woo, & Treister, 2014; Imanguli, Alevizos, Brown, Pavletic, & Atkinson, 2008). At times, oral acute GVHD presents with extensive crusting of the lips similar to the oral manifestation of erythema multiforme (Kuten-Shorrer, Woo, & Treister, 2014). The diagnosis of acute GVHD is complicated by numerous other factors in the early post-transplant period which can give rise to oral mucosal alterations including oral mucositis (regimen-related toxicity) and recurrent herpes simplex infection (especially in cases without anti-viral prophylaxis). Overlap in clinical presentation between these conditions has made it challenging to establish a generally accepted definition of oral acute GVHD (Ion, et al., 2014).

Chronic GVHD

Chronic GVHD lasts longer than acute GVHD and affects a wider range of organ systems (Sullivan, et al., 1981).

The manifestations of the chronic GVHD are similar to many auto-immune disorders and is characterized by oral or ocular sicca, fibrosis of the skin and oral cavity, with likely involvement of organ systems throughout the body (Sullivan, et al., 1981; Mielcarek, et al., 2003).

i) Skin

Early clinical presentation of chronic skin GVHD includes xerosis (skin dryness), ichthyosis (scaling) and papulosquamous and lichen planus like lesions (Martires, et al., 2011). Diagnostic features of chronic skin GVHD includes poikiloderma, lichen planus like eruption and sclerosis (Filipovich, et al., 2005). As the disease progresses, skin sclerosis can occur, resulting in increased tightening and fragility of the skin which can greatly impact patient quality of life. (Filipovich, et al., 2005).

ii) Liver

Chronic liver GVHD is characterized by cholestasis and elevated laboratory values (e.g. bilirubin, alkaline phosphatase, and gamma-glutamyl transferase) which give the clinical picture of acute hepatitis (Filipovich, et al., 2005; Strasser, et al., 2000).

iii) Eyes

Ocular GVHD manifests as irritation, burning, dry eyes and photophobia due to inflammation and destruction of the lacrimal gland (Filipovich, et al., 2005).

iv) Gastrointestinal tract

GI manifestations of chronic GVHD includes various symptoms including reflux, vomiting, dysphagia, bloating, diarrhea and weight loss (Filipovich, et al., 2005). In a retrospective study involving patients with GI GVHD (n=40) Akpek by et al. 74% patients presented with diarrhea, 45% presented with abdominal pain, 33% with nausea and 19% of patients had weight loss (Akpek, et al., 2003). GI symptoms can be attributed to causes other than GI GVHD. Therefore, extensive evaluation is required to rule out other causes of GI symptoms before making a diagnosis of chronic GVHD (Akpek, et al., 2003).

v) Lungs

Bronchiolitis obliterans (BO) is diagnostic for chronic lung GVHD. In the early stages after onset patients maybe asymptomatic or present with mild dyspnea and unproductive cough. As the lung disease progresses, the symptoms increase with loss of pulmonary function (Hildebrandt, et al., 2011; Dudek, Mahaseth, DeFor, & Weisdorf, 2003). BO also increases the risk of life threatening respiratory infection (Dudek, Mahaseth, DeFor, & Weisdorf, 2003). Dudek et al. report that that probability of 5 year-survival among patients who develop BO is only 29% compared to 54% in patients without BO (Dudek, Mahaseth, DeFor, & Weisdorf, 2003).

vi) Genitals

Chronic genital GVHD presents with lichen-like sclerosis in both sexes (Filipovich, et al., 2005). In women vaginal dryness, burning, and amenorrhea are common. In men inflammatory changes may lead to erectile dysfunction (Mueller, et al., 2013; Zantomio, et al., 2006). Genital involvement can have great impact on quality of life in long-term survivors of either sex.

vii) Scalp

Scarring or nonscarring alopecia is a common distinctive feature of chronic GVHD involving the hair (Filipovich, et al., 2005). Graying, increased brittleness and loss of body hairs are other findings seen in chronic GVHD (Filipovich, et al., 2005).

viii) Nails

Nail changes in chronic GVHD are irreversible and are akin to those seen in lichen planus (Sanli, Arat, Oskay, & Gürman, 2004). Manifestations include as ridging, roughness, fragility, dystrophy and ulceration of the lunula (Sanli, Arat, Oskay, & Gürman, 2004). In a cohort study by Sanli et al. involving 28 patients with chronic cutaneous GVHD, 50% presented with nail changes (Sanli, Arat, Oskay, & Gürman, 2004). Longitudinal ridging of the finger and toe nails was found to be the most common manifestation (8% and 75% respectively) followed by roughness of the nail plates (Sanli, Arat, Oskay, & Gürman, 2004).

ix) Muscles and joints

Chronic GVHD of the muscles and joints present with muscle weakness, fever, induration of the skin over the area of involvement (peau d' orange appearance) with limited range of motion and

joint contractures (Oda, et al., 2009). Fasciitis is a diagnostic feature while myositis is a distinctive manifestation of the disease (Oda, et al., 2009). Oda et al. report that PBSCT is the chief risk factor for development of fasciitis (Oda, et al., 2009).

x) Hematology

Chronic GVHD incidence is increased in patients with eosinophilia (Ahmad, et al., 2011). Patients with chronic GVHD may also develop cytopenia due to stromal damage and other autoimmune disease processes. Thrombocytopenia may occur due to production of platelet autoantibodies is an indicator for high risk of mortality (Anasetti, Rybka, Sullivan, Banaji, & Slichter, 1989).

xi) Oral cavity

Oral mucosal GVHD

Oral GVHD is a common manifestation of chronic GVHD with 72-83% patients developing mucosal involvement post-transplant (Schubert & Correa, 2008; Lee & Flowers, 2008). In many cases, oral GVHD continues to persist even after resolution of GVHD at other sites (Schubert & Sullivan, 1989). The clinical appearance of oral chronic GVHD bares semblance to the oral presentation of autoimmune diseases most notably lichen planus, Sjögren syndrome, and scleroderma.

Lichenoid hyperkeratosis is the predominate clinical feature in chronic oral mucosal GVHD which may appear as reticular striae, papules, or patches akin to those seen in lichen planus (Schubert & Correa, 2008; Kuten-Shorrer, Woo, & Treister, 2014; Ion, et al., 2014; Schubert M.

M., et al., 1984). Hyperkeratotic lesions may also present as plaques or sheet-like changes of variable thickness (Lee & Flowers, 2008; Schubert M. M., et al., 1984) .

Gomes et al. report that time post-HCT influences the type of oral manifestation. Lichenoid lesions, hyperkeratotic plaques, erythema, and atrophy are more common findings in patients within the 1st year post HCT (Gomes, et al., 2014). In contrast, Cavalcanti et al. found that ulcers were more common in the early period post HCT (up to 12 months' post HCT) with hyperkeratotic plaques more commonly seen in the intermediate (13-47 months' post HCT) and late (> 47 months' post HCT) periods post-HCT (Cavalcanti, Araújo, Bonfim, & Torres-Pereira, 2015). Other diagnostic features include maxillary anterior gingival atrophy with loss of stippling and vasculitis or telangiectasia like mucosal changes (Schubert & Correa, 2008). Infrequently ulcers may be present, measuring anywhere between 0.5- 3.0 cm. Ulcers generally appear shallow and are covered by yellow-gray pseudomembranes with peripheral erythema (Schubert M. M., et al., 1984) . Multiple mucoceles are often present in patients with chronic GVHD, but are not considered to be diagnostic for oral GVHD (Treister, et al., 2008; Jagasia, et al., 2015). Oral GVHD may involve all oral soft tissues, though the most common sites appear to be the buccal mucosa, labial mucosa, and tongue (with lesser expression in the gingiva, floor of the mouth, and palatal tissues) (Treister, et al., 2008).

Mucosal sensitivity is the chief symptom in patients presenting with oral GVHD. Patients often describe sensitivity to previously tolerated spices, acidic foods and drinks, and mint flavoring (including that in toothpaste) (Schubert & Correa, 2008; Kuten-Shorrer, Woo, & Treister, 2014). Symptoms of pain and sensitivity can occur in the absence of ulceration and be present with only

reticular lesions, erythema and atrophy (Treister, et al., 2008). Oral ulcers in chronic GVHD may cause significant pain, especially when stimulated. Pain and resultant dysphagia can have significant effects on overall quality of life and may compromise nutritional intake and medication compliance (Eckardt, et al., 2004).

In addition to the manifestations described above, chronic GVHD may lead to tissue sclerosis with palpable submucosal banding particularly in the posterior buccal mucosa limiting the range of motion in the jaw (Kuten-Shorrer, Woo, & Treister, 2014; Treister, et al., 2008). Progressive sclerosis results in limited mouth opening which may have negative effects on mastication, nutrition, and delivery of dental care.

Salivary GVHD

Intraoral complications in GVHD may also occur due to alterations structures outside of the oral cavity, most notably the salivary glands. GVHD related salivary gland damage is permanent and persists even after resolution of chronic GVHD. Immune mediators in chronic GVHD may damage salivary gland structures resulting in a decrease in quantitative production of saliva and broad sialochemical changes in saliva that is produced (Izutsu, et al., 1983). Salivary gland dysfunction initially appears to be associated with ductal damage, though acinar destruction may also subsequently occur in severe cases (Izutsu, et al., 1983). Defective ductal resorption and direct acinar damage results in elevated levels of sodium, IgG and albumin within saliva with a concurrent decrease in secretory IgA and inorganic phosphate (Izutsu, et al., 1983; Nagler & Nagler, 1999). These alterations produce negative effects on mucosal protection and defense against dental caries (Nagler & Nagler, 1999). Sialochemical alterations in chronic GVHD may

also serve as effective biomarkers for chronic GVHD, with Izutsu and colleagues reporting that salivary sodium levels can serve as a potential diagnostic test for chronic GVHD when salivary gland biopsies cannot be obtained (Izutsu, et al., 1983).

Patients who develop chronic GVHD of the salivary glands often complain of persistent oral dryness. Oral sicca is highly prevalent in patients with chronic GVHD and symptoms of xerostomia and xerophthalmia are found to be correlated (Imanguli, et al., 2010). The diagnosis of salivary GVHD is confounded by other potential sources of xerostomia including conditioning chemotherapy (especially in protocols that include total body irradiation) and medication effects (Schubert & Correa, 2008; Schubert, Sullivan, & Truelove, 1986). The likelihood of GVHD-related salivary gland dysfunction is increased in the presence of other systemic signs and symptoms of GVHD especially when oral dryness persists past day 100 post-HCT (Schubert & Correa, 2008; Schubert, Sullivan, & Truelove, 1986).

Gustatory GVHD

Oral GVHD may also manifest with changes in taste. This process is felt to be multifactorial, but appears to be atleast partly related to the immune system activity against epithelial-derived taste receptors (Schubert & Correa, 2008). Federmann et al. found 63% of patients with chronic GVHD (79/148 patients) reported changes in taste perception (Federmann B. , et al., 2009). Alterations in taste, including dysguesia and ageusia, may have significant effects on nutritional status, body weight, and overall quality of life. Gustatory GVHD must be distinguished from taste dysfunction related to conditioning therapy, which generally resolves several months' post-

transplant, and the effects of calcineurin inhibitors (which are common prescribed for GVHD prophylaxis) (Schubert & Correa, 2008).

xii) Other findings

Other possible associated chronic GVHD manifestations includes serositis, peripheral neuropathy, myasthenia gravis, Raynaud's phenomenon and cardiac involvement (Filipovich, et al., 2005).

The main diagnostic features for acute and chronic GVHD are further described in table 2 and systemic sites of involvement with frequency of occurrence are provided in table 3

Table 2: Main diagnostic features of acute and chronic GVHD*

Organ/ site	Acute GVHD	Chronic GVHD
Skin	Signs: Erythema, maculopapular rash Symptoms: pruritus	Signs: Poikiloderma, lichen planus like lesions, sclerosis and morphea like features
Liver	Signs: Laboratory studies indicating bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), or aspartate aminotransferase (AST) greater than two times the upper reference limit	
GI	Symptoms: Nausea, anorexia, vomiting, diarrhea, weight loss	Signs: Esophageal webbing, stenosis in the upper and middle 1/3 rd. of esophagus Mucosal sloughing, crypt apoptosis, and edema identified during Endoscopy. Symptoms: Nausea, vomiting, abdominal cramping
Lungs	No recognized manifestations	Signs: Bronchiolitis obliterans associated with dyspnea and unproductive cough
Genitalia	No recognized manifestations	Signs: Lichenoid changes and vaginal scarring
Nails	No recognized manifestations	Signs: Dystrophy, longitudinal ridging, brittle features
Muscles and joint	No recognized manifestations	Signs: Fasciitis and joint stiffness secondary to sclerosis

Oral cavity	Signs: Gingival atrophy, mucositis, erythema, lichenoid hyperkeratosis and pseudomembranous ulcers Symptoms: pain	Signs: Lichenoid changes, hyperkeratotic plaque, pseudomembranous ulcers, atrophy, erythema, restricted mouth opening, mucoceles Symptoms: taste dysfunction, xerostomia, mucosal sensitivity

*References: (Jagasia, et al., 2015; Schubert & Correa, 2008)

Table 3: Sites of chronic GVHD (at initial diagnosis) *

Sites	Frequency (%)
Skin	75%
Oral cavity	72-83%
Liver	29%–51%
Eyes	22%–33%
GI tract	23%–45%
Lungs	4%–19%
Genital tract (female)	1%
Joints	6%

*References: (Lee & Flowers, 2008)

E. Diagnosis of GVHD

Diagnosis of acute and chronic GVHD is primarily made by clinical examination and may be supported by histopathology (Lee, et al., 2002; Akpek, et al., 2003; Hildebrandt, et al., 2011). The National Institute of Health (NIH) diagnostic criteria for chronic GVHD is described in table 4 below (Filipovich, et al., 2005; Espana, Shah, Santhiago, & Singh, 2013).

i) Skin

Histopathological findings of acute skin GVHD tends to be non-specific and therefore diagnosis is usually made in conjunction with clinical exam. The most important findings include basal cell vacuolar degeneration, dyskeratosis and spongiosis (Kohler, Hendrickson, Chao, & Smoller, 1997). The pathological features of chronic skin GVHD overlaps with that of acute GVHD (Shulman, et al., 2015). Chronic skin GVHD diagnosis is made by the presence of

hyperkeratosis, extensive apoptosis, saw tooth rete-ridges and adnexal inflammation (Shulman, et al., 2015).

ii) Liver

Acute and chronic GVHD histopathological manifestations are indistinguishable (Shulman, et al., 2015). Liver biopsies indicate significant changes in the epithelial and ductal structures depending on the duration of GVHD related damage along with attack by cytotoxic lymphocytes (Shulman, et al., 2015).

iii) Eyes

Ocular GVHD is most commonly diagnosed based on patient reports of xerophthalmia and can be confirmed by objective measurement of tearing through use of a schirmer's test. Schirmer's scores of less than ≤5 mm are consistent with ocular GVHD (Jagasia, et al., 2015). Biopsies are seldom utilized, but show features of epithelial necrosis, basal cell vacuolization, and lymphocyte exocytosis in the bulbar conjunctiva (Jagasia, et al., 2015; Shulman, et al., 2015). GVHD affecting the lacrimal glands shows characteristic mononuclear cell infiltration of the ductal system and fibrosis of the lacrimal acini, analogous to salivary GVHD (Shulman, et al., 2015).

iv) GI tracts

Gastrointestinal (GI) GVHD is diagnosed based on the presence of clinical symptoms and a confirmatory biopsy obtained via endoscopy or retrosigmoidoscopy. Histologic features include mucosal sloughing, crypt apoptosis and edema (Akpek, et al., 2003; Shulman, et al., 2015). The

histologic manifestations of gastrointestinal GVHD are comparable between acute and chronic GVHD (Shulman, et al., 2015).

v) Lungs

The lungs are usually evaluated by pulmonary function tests (PFT) and chest imaging (Horwitz & Sullivan, 2006). Lung biopsies are used in cases where PFT and CT scans show features bronchiolitis obliterans in the absence of other sites of chronic GVHD involvement (Shulman, et al., 2015). Biopsy is characterized by distinctive pathological changes of BO which includes obliteration of the terminal bronchioles and perivascular lymphocytic infiltration (Shulman, et al., 2015; Dudek, Mahaseth, DeFor, & Weisdorf, 2003).

vi) Genitals

GVHD related histopathological changes in the genitalia are similar to findings in the oral mucosa characterized by apoptosis, spongiosis, mononuclear cell infiltration and epithelial dyskeratosis (Da Silva Lara, et al., 2010).

vii) Oral cavity

Oral GVHD diagnosis is usually made by history, clinical exam and time of onset of signs and symptoms (Kuten-Shorrer, Woo, & Treister, 2014; Treister N. , Duncan, Cutler, & Lehmann, 2012). While making a diagnosis of oral mucosal GVHD, it is important to rule other conditions such as candidiasis, herpes infection, malignancies and drug reactions using appropriate diagnostic methods (Meier, et al., 2011).

Oral biopsies can help confirm a chronic GVHD diagnosis in patients without apparent clinical features of oral GVHD; however, a positive biopsy alone is insufficient to establish diagnosis oral chronic GVHD (Shulman, et al., 2015). Histopathological changes associated with oral mucosal GVHD are non-specific and there are no distinct differences between acute and chronic GVHD (Schubert & Correa, 2008; Santos, Coracin, Barros, & Gallottini, 2014). Santos el al report that GVHD related changes manifest primarily in the epithelium characterized by the presence of acanthosis, exocytosis, apoptosis, dyskeratosis thickening of the basal cell layer (Santos, Coracin, Barros, & Gallottini, 2014). The most distinct feature is the presence of perivascular inflammation and lymphocytic infiltration in the connective tissue (Imanguli, Alevizos, Brown, Pavletic, & Atkinson, 2008). Minor salivary gland changes are characterized by a patch or diffuse lymphoplasmacytic infiltrate. There is an increase mucopolysaccharides level in the lobular acini and gland fibrosis (Shulman, et al., 1980). Major salivary gland pathology shows the presence of an inflammatory infiltrate with edema mainly periductal areas without the formation of myoepithelial islands (Shulman, et al., 1980).

Immunopathology studies of the oral mucosa and salivary glands confirm the presence of a T cell mediated process in chronic GVHD (Hasseus, Jontell, Brune, Johansson, & Dahlgren, 2001). Basal cell layer of the epithelium shows morphological changes and is infiltrated with CD4 and CD8 cells (Hasseus, Jontell, Brune, Johansson, & Dahlgren, 2001). The oral mucosa and salivary gland are infiltrated by Langerhans cells expressing CD80 and CD86 which are used as targets for GVHD therapy (named as B7 molecules) (Hasseus, Jontell, Brune, Johansson, & Dahlgren, 2001; Greaves & Gribben, 2013).

Table 4: NIH chronic GVHD diagnostic criteria*

2005 NIH diagnostic criteria for chronic GVHD is as follows
Distinct from acute GVHD Presence of a minimum of one clinical manifestation of chronic GVHD (or) Presence of a minimum of one diagnostic manifestation which is confirmed by biopsy or other suitable testing Exclusion of other possible diagnoses

*Reference: (Filipovich, et al., 2005; Espana, Shah, Santhiago, & Singh, 2013)

F. Management of GVHD

Most therapeutic and preventive approaches to GVHD stem from an understanding of the roles of T and B cells in the pathogenies of the disease process.

GVHD prophylaxis

Attempts to prevent GVHD are made with the use of immunosuppressants such as calcineurin inhibitors (e.g. tacrolimus and cyclosporine), methotrexate (MTX), corticosteroids, mycophenolate mofetil (MMF), sirolimus and anti-thymocyte globulin (ATG). Differences in regimen protocols exists across various transplant centers with no definitive acute GVHD prophylactic regimen that can be applied to all patient circumstances (Ram & Storb, 2013).

Table 5: Mechanism of action of pharmaceutical agents in GVHD prevention and treatment*

Mechanism of action
• Depletion of alloreactive T cells • Depletion of B cells • Targeting B cell receptor signaling pathway • Inhibiting B cell development • Inhibition of Cytokine Receptor-Mediated Signaling • T regulatory cells Reconstitution

- Enhancement of CD4Treg
- Adoptive T cell therapy

*References: (Cutler, Koreth, & Ritz, 2016)

GVHD management

The cornerstones of GVHD management include early recognition of disease, prompt intervention, and periodic monitoring, all of which help to limit disease-related morbidity. (Flowers & Martin, 2014; Nakamura, et al., 1996). The extent and severity of GVHD greatly effects response to treatment and duration of systemic therapy. The median duration of therapy is 19-23 months after chronic GVHD diagnosis (Treister, et al., 2008). The majority of patients with prolonged GVHD experience resolution within 7 years of diagnosis; however, 10% remain on systemic therapies indefinitely (Flowers & Martin, 2014; Saliba, et al., 2007).

Steroids and immunosuppressants

Corticosteroids are the most common initial therapy for both acute and chronic GVHD. After reaching a diagnosis, systemic and/or topical corticosteroids are added to the patient's existing prophylactic medication to decrease the potentially damaging effects of GVHD. (Jacobsohn & Vogelsang, Acute graft-versus-host disease, 2007; Goerner, et al., 2002). Other non-steroidal immunosuppressive agents may also be used as initial therapy or second-line therapies (with or without concurrent corticosteroid use). Amongst various systemic and topical agents available for the management of chronic GVHD, Lee et al. conclude that systemic corticosteroid, cyclosporine and MMF offers the greatest success for chronic GVHD management (Furlong, et al., 2009).

Topical therapy

Topical corticosteroids and other immunosuppressants are most effective in the management of oral, ocular, and limited dermal GVHD (Kuten-Shorrer, Woo, & Treister, 2014; Goerner, et al., 2002).

Various oral agents have been employed including topical steroids, cyclosporine, tacrolimus, and azathioprine in various forms including gels, rinses, ointments and creams (Meier, et al., 2011). Topical dexamethasone (0.1mg/mL) is often used as a first line therapy for oral mucosal GVHD with high potency steroids (such as 0.05% clobetasol or fluocinonide) and topical tacrolimus as second line therapies (Treister N. , Duncan, Cutler, & Lehmann, 2012; Noce, et al., 2014). Major drawbacks of topical steroid therapy in the management of oral GVHD are the need for frequent dosing and increased risk for candidiasis (Schubert & Correa, 2008; Treister, Li, Lerman, Lee, & Soiffer, 2015; Treister, Li, Lerman, Lee, & Soiffer, 2015).

Topical tacrolimus 0.1% has been shown to be an effective topical medication in the treatment of oral GVHD without concurrent corticosteroid therapy (Eckardt, Starke, Stadler, Reuter, & Hertenstein, 2004). Topical tacrolimus is found to be more effective than systemic tacrolimus in ameliorating symptoms of chronic oral GVHD (Eckardt, et al., 2004). Similarly, Mawardi et al. examined the benefit of a compounded rinse containing dexamethasone and tacrolimus and found significant improvement in both subjective and objective outcomes (including improvement in clinical appearance and symptom-based NIH scoring). (Mawardi, Stevenson, Gokani, Soiffer, & Treister, 2010). Topical tacrolimus application may be associated with development of local infection (Albert, et al., 2007). A few studies report increased serum

tacrolimus concentration following topical application in severe cases of oral GVHD linked to significant impairment of the mucosal barrier (Conrotto, et al., 2006; Albert, et al., 2007).

Other studies on oral GVHD management have also shown promising results with topical budesonide rinse (3mg/10 ml), which is commonly used in the management of gastrointestinal GVHD (Elad, et al., 2012; Treister N. , Duncan, Cutler, & Lehmann, 2012). Oral GVHD that is refractory to topical therapies may respond to intralesional steroid injections (such as triamcinolone acetonide 40 mg/mL) (Treister N. , Duncan, Cutler, & Lehmann, 2012).

Ophthalmic solutions of prednisone and cyclosporine are commonly utilized in the management of ocular GVHD, while hydrocortisone, triamcinolone, clobetasol, and tacrolimus are mainstays of dermal therapy (Goerner, et al., 2002).

Other treatment modalities

Psoralen and ultraviolet A radiation (PUVA, 320– 400 nm) has been shown to be a safe and effective therapy for patients with refractory skin and chronic oral GVHD (Wolff, et al., 2004). Patient intolerant to psoralen (such as patients with liver dysfunction) may still benefit from the use of shorter wave UVB radiation (280–320 nm) (Elad, Garfunkel, Enk, Galili, & Or, 1999; Treister, Li, Lerman, Lee, & Soiffer, 2015). Caution is needed with use of both UVA and UVB as overuse can result in burns and increase risk for epithelial dysplasia and malignant transformation (Elad, Garfunkel, Enk, Galili, & Or, 1999; Treister, Li, Lerman, Lee, & Soiffer, 2015).

Extracorporeal photochemotherapy (ECP) is another type of photochemotherapy employed in the management of chronic GVHD. In a study of 71 patients with steroid refractory chronic GVHD, Couriel et al, found ECP to be effective in 63% of case (including complete resolution in 14 patients) (Couriel, et al., 2006). GVHD affecting the skin, eyes, liver, and oral mucosa respond most favorably to ECP (Couriel, et al., 2006).

Other devices, including CO_2 lasers have also been utilized in GVHD management. Patients treated with CO_2 lasers do report some measure of pain reduction, though the mechanism is poorly understood (Elad, et al., 2003). Elad et al. report that use of a combined Er: YAG and CO_2 laser offers significant pain relief in patients in addition to improved nutritional uptake (Elad, et al., 2003).

G. Impact of GVHD

Both acute and chronic GVHD provides some antileukemia effect called as graft-versus-leukemia effect (GVL) which decreases the risk of relapse of hematological malignancies (Horowitz, et al., 1990); however, GVHD continues to remain a major cause of post-HCT morbidity and mortality (Levine, et al., 2008; Schubert & Correa, 2008; Mielcarek, et al., 2003; Lee & Flowers, 2008).

Survival amongst patients with acute GVHD directly correlates with severity of skin, liver and GI tract involvement (Sullivan, et al., 1988). The probability of long-term survival amongst patients with Grade C acute GVHD (Glucksberg scale) is roughly 30%. Patients with Grade D

acute GVHD, the most severe grading, have less than 50% probability of long-term survival (Akpek, et al., 2003).

Major systemic complications associated with chronic GVHD include infections, pulmonary and cardiac disease, autoimmune disorders, impairment of growth and development, ocular problems, GI and liver dysfunction and secondary malignancies (Deeg & Flowers, 2005).

Organ failure and infection related to chronic GVHD are the primary causes of mortality (Fraser, et al., 2006). Chronic GVHD-related mortality most often occurs within the first two years of chronic GVHD diagnosis, though death may occur anytime upto 6 years after onset (Wingard J. R., et al., 1989). Wingard et al. reported survival amongst 85 patients diagnosed with chronic GVHD to be approximately 42% at 10 years (Lee, et al., 2002). Patients with de novo chronic GVHD have better chances of survival compared to those with progressive or quiescent onset chronic GVHD (Sullivan, et al., 1988).

Studies assessing quality of life (QOL) have reported significant functional impairment, activity limitation, adverse mental and systemic health effects in patients with chronic GVHD when compared those without GVHD (Fraser, et al., 2006; Pidala J. , et al., 2011). A similar study by Lee et al. that utilized validated self-administered surveys, a short form (SF-12) of the Medical Outcomes Study version and the Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) found that both acute and chronic GVHD have significant adverse impact on QOL (Lee, et al., 2006).

Oral GVHD results in salivary gland dysfunction, taste alteration and can be associated with symptoms of pain and sensitivity and additionally treatment related complications, each impacting health and overall quality of life unfavorably (Eckardt, et al., 2004; Federmann B. , et al., 2009). Studies have identified oral GVHD to independently have an adverse effect on QOL, by not only affecting oral function but also social well-being (DePalo, Chai, Lee, Cutler, & Treister, 2015; Fall-Dickson, et al., 2010). A study by DePalo et al. found no difference in global quality of life measurements (using the SF-36 and FACT-BMT) in patients with oral GVHD, with and without systemic GVHD involvement (despite significant differences in NIH severity score between groups).

Salivary gland GVHD results in diminished salivary anti-cariogenic effect increasing the risk for rampant dental caries mainly in the interproximal surfaces and class V caries (Treister N. , Duncan, Cutler, & Lehmann, 2012). In a retrospective study involving 21 patients with chronic GVHD, it was found that patients examined post HCT had more number of carious lesions requiring treatment compared to the examination prior to the transplant (Castellarin, 2012). Most patients developed significant number of cervical and interproximal carious lesions within two years' post HCT (Castellarin, 2012). Supplement fluoride will be beneficial to reduce risk of caries particularly in a setting of salivary GVHD (Castellarin, 2012).

Mucosal GVHD can be associated with significant sensitivity which may cause decreased compliance to oral hygiene measures leading to increased risk of periodontal disease and caries (Meier, et al., 2011). Secondary infections such as candidiasis due to persistent immunosuppression and steroid therapy are common and can aggravate oral GVHD

manifestations (Schubert & Correa, 2008; Woo, Lee, & Schubert, 1997; Schubert, Epstein, Lloid, & Cooney, 1993; Kuten-Shorrer, Woo, & Treister, 2014). Emphasis is given to maintenance of excellent oral hygiene to prevent oral infection as it can trigger and worsen oral GVHD through the production of pro-inflammatory cytokine (Schubert & Correa, 2008; Loren A. W., et al., 2006). Brushing might difficult due to pain, in such cases chlorhexidine mouth rinses (0.12%) can be used to reduce risk of oral infection (Mays, Fassil, Edwards, Pavletic, & Bassim, 2013).

Rationale for the study

The oral cavity is often the first sites of chronic GVHD involvement (Margaix-Muñoz, Bagán, Jiménez, Sarrión, & Poveda-Roda, 2015; Schubert & Correa, 2008; Lee & Flowers, 2008). Oral exam findings in patients post HCT provides valuable information on the status of chronic GVHD (Resende, de Fátima Correia-Silva, Arão, Bittencourt, & Abreu, 2012). At times, chronic GVHD features may be less distinct or difficult to diagnose. For example, skin and liver signs/symptoms can be non-specific and mimic other conditions. In such circumstances other diagnostic tools maybe beneficial (Hiroki, Nakamura,, Shinohara, & Oka, 1994).

Studies have examined the value of oral exam findings and biopsies in the diagnosis of chronic GVHD (Sale, et al., 1981; Resende, de Fátima Correia-Silva, Arão, Bittencourt, & Abreu, 2012; Nakhleh, Miller, & Snover, 1989; Lee & Flowers, 2008). Resende et al. report that the presence of oral symptoms and salivary gland biopsy consistent with chronic GVHD can assist in GVHD diagnosis (Hiroki, Nakamura,, Shinohara, & Oka, 1994), however, previous studies indicate that there is no value in performing extensive screening tests such as skin biopsies, oral biopsies, oral

examination, platelet count, IgG level, liver function tests and Schirmers test at day 100 to predict subsequent development of chronic GVHD (Wagner, et al., 1998; Loughran, et al., 1990). Wagner's study considered oral exam findings at departure but it is noted that the determination of oral GVHD status is unclear.

There are no recent studies in the literature that have focused exclusively on the value of clinical oral mucosal GVHD diagnosis between days 70-120 post HCT and its association of subsequent development of systemic chronic GVHD. Therefore, the aim of this study is to investigate the possibility of such an association.

With proper training, oral examinations are relatively simple to perform and provide a potential source for diagnostic information related to chronic GVHD activity. If oral exam findings at departure can be correlated with subsequent chronic GVHD development, oral examinations could help to identify patients who may benefit from personalized education, more frequent monitoring and/or early therapeutic interventions (Nakhleh, Miller, & Snover, 1989; Boer, Correa, Miranda, & De Souza, 2010; Hiroki, Nakamura,, Shinohara, & Oka, 1994).

Objective and hypothesis

The primary objective of the study is to assess the association of the clinical diagnosis of oral mucosal GVHD between days 70-120 post-HCT (in patients without other manifestations of chronic GVHD) with subsequent development of chronic GVHD (defined by the 2005 NIH consensus criteria) that required treatment with systemic immunosuppression. We hypothesize that individuals diagnosed with clinical oral mucosal GVHD by oral medicine examination

between days 70-120 post-HCT (hereafter referred to as day-100 oral medicine departure examination) will be more likely to develop subsequent chronic GVHD that requires systemic immunosuppressive therapy, than individuals without oral mucosal GVHD at day-100 oral medicine departure examination.

CHAPTER 2: METHODS

A retrospective cohort design was used for this study. Patient demographics, transplant data, acute and chronic GVHD information, and immunosuppressive regimens were obtained through the Fred Hutchison cancer research center (FHCRC) optical web library (OWL) and the Gateway database. Medical information was further supplemented through, and supplemented through review of clinical notes in Electronic Medical Records (EMRs) (ORCA, University of Washington Medical Center, Seattle, WA USA; Powerchart, Cerner Corporation, Kansas City, MO USA). Oral medicine data, including departure examination findings, oral symptoms, and oral GVHD diagnosis, were obtained from Oral Medicine departure examination notes in the EMR (appendix A and B). Informed consent was obtained for all patients enrolled in the study per FHCRC protocols #884 and #1499.

A. Study sample

The study group consisted of a total of 642 consecutive patients who received their 1st allogenic transplant at Seattle Cancer Care Alliance (SCCA) between January 1st 2010- June 30th 2014. Patients with a previous autologous and/or 2nd allogeneic transplants were excluded from the study sample.

B. Inclusion and exclusion criteria

Inclusion criteria
• 21 years and older at the time of transplant • 1st allogenic HCT at SCCA between January 1st 2010 to June 30th 2014 (regardless of graft source) • Consent per FHCRC protocols #1499 and #884
Exclusion criteria

- Prior autologous and/or allogeneic HCT
- Lack of oral examination at departure performed by oral medicine specialists
- NIH chronic GVHD diagnosis at or prior to departure
- Death or relapse of primary disease prior to departure

C. Oral exam data

Oral medicine day-100 departure examinations were performed by trained oral medicine (OM) specialists with attending status in the SCCA oral medicine clinic.

Oral mucosal findings at departure for this study were stratified into 2 main groups

a) Clinical diagnosis of oral GVHD

b) No clinical diagnosis of oral GVHD (either presenting as normal oral mucosa or abnormal/non-specific oral findings that were non-GVHD-related)

In cases where an official oral GVHD diagnosis was not made or when oral examination findings were non-specific, clinical descriptors were independently reviewed by three oral medicine providers, and a discussion held until a consensus on the oral GVHD status was reached.

For all cases, subjective patient-reported evidence of mucosal sensitivity and xerostomia (if documented) were gathered as secondary parameters. Mucosal sensitivity was graded as “mild”, “moderate” or “severe”, based on patient report, or recorded as “unspecified” if not available.

Quality assessment was performed through re-abstracting of a complete data set for a pool of 10 patients. Data verification was completed by the statistician.

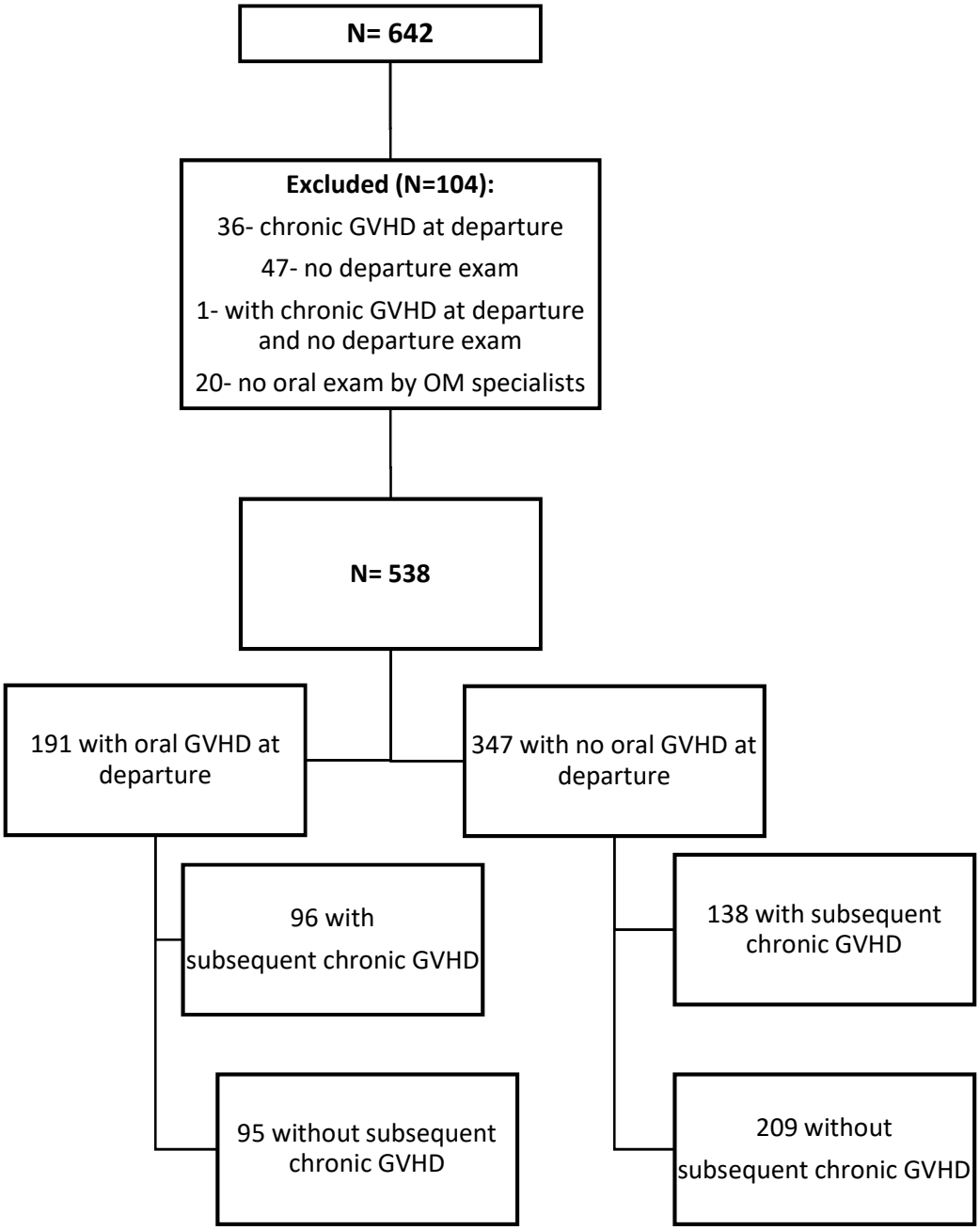
CHAPTER 3: STATISTICAL ANALYSIS

The study population was divided into two groups for analysis, Oral GVHD group (individuals with documented diagnosis of oral mucosal GHVD at post-HCT departure evaluation) and No oral GVHD group (individuals without a diagnosis of oral mucosal GVHD at post-HCT departure evaluation).

Frequency distributions (for categorical factors) or means (for continuous variables) were calculated for demographic and transplant characteristics for the entire study population. Univariate and multivariable logistic regression models were used to examine the relationship between presence/absence of oral GVHD at departure and subsequent development of chronic GVHD. The multivariable analyses were adjusted for factors (specifically recipient age, patient/donor gender, donor relationship, graft source, acute GVHD grades 2-4, conditioning regimen and prednisone treatment at time of departure) known to be associated with chronic GVHD. All analyses were performed using SAS (Statistical Analysis Software, Cary, NC) v9.4.

CHAPTER 4: RESULTS

Figure 1: Study flowchart



A. Patient and donor characteristics

Characteristics of the study population (N=538) are described in table 6. A total of 538 patients, ≥ 21 years of age received their first allo-HCT between January 1st 2010- June 30th, 2014 and met all inclusion and exclusion criteria. One-hundred four patients were excluded from the study (36 with chronic GVHD at departure, 47 with no medical departure exam, 20 patients without oral examinations completed by an oral medicine specialist at departure, and 1 with no departure exam and a history of chronic GVHD at departure).

Majority of patients were male (N=318). The mean age of all subjects at the time of transplant was 49.7 years (21.1-77.7). The most common indication for allogenic transplant was acute myeloid leukemia (38.8 %).

The mean donor age was 36.8 years (10.6-77.4). Donors were most likely to be male (53.1%). Nearly half of the donors were HLA-matched unrelated donors (48.2%). Peripheral blood stem cells were the most common graft source at 76%. A total of 152 patients received mini-transplants. Mini-transplants are commonly used in older adults and those with comorbidities and employ non-myeloablative or reduced intensity conditioning regimens (Couriel, et al., 2004) (Veltri, et al., 2013).

Nearly 78% (77.7%) of patients developed acute GVHD prior to departure, most of whom developed grade 2 acute GVHD (61.9%). Prednisone in the early day's post-transplant is associated with risk for chronic GVHD development (Wagner, et al., 1998). In our study, 17.1% remained on prednisone for acute GVHD management at the time of departure.

Table 6: Patient and donor characteristics

	No chronic GVHD diagnosis after departure (n=304)	Chronic GVHD diagnosis after departure n=234)	Total (n=538)
Patient age at transplant (mean, range)	48.6 (21.1- 75)	51.1 (21.1- 77.7)	
Donor age at transplant (mean, range)	37.1 (10.6- 77.4)	36.5 (18- 76.6)	
	N (%)	N (%)	N (%)
Female	132(43.4)	88 (37.6)	220 (40.9)
Male	172 (56.6)	146 (62.4)	318 (59.1)
Donor / Patient gender			
Female/female	66 (21.7)	40 (17.1)	106 (19.7)
Male/male	93 (30.6)	86(36.8)	179 (33.3)
Male/female	52 (17.1)	45 (19.2)	97 (18.0)
Female/male	65 (21.4)	57 (24.4)	122 (22.7)
Donor sex unknown	28 (9.2)	6 (2.6)	34 (6.3)
Donor relationship/histocompatibility			
Related/matched	89 (29.3)	52 (22.2)	141(26.2)
Related/haploidentical/mismatched	14 (4.6)	5 (2.1)	19 (3.5)
Unrelated/matched	123 (40.5)	138 (59)	261 (48.5)
Unrelated/mismatched	25 (8.2)	31(13.2)	56 (10.4)
Unrelated cord/combined	53 (17.4)	8 (3.4)	61 (11.3)
Cell source			
Bone marrow	46 (15.1)	22 (9.4)	68 (12.6)
Peripheral blood stem cell	205 (67.4)	204 (87.2)	409(76.0)
Cord blood	53 (17.4)	8 (3.4)	61 (11.3)
Diagnosis at transplant			
Hematological malignancies*	300(98.6)	232 (99.1)	532 (98.8)
Non- malignant diseases**	4 (1.3)	2 (0.8)	6 (1.1)
Mini-transplants	81 (26.6)	76 (32.5)	157 (29.2)
Died after departure	116 (38.2)	44 (18.8)	160 (29.7)
Relapsed after departure	57(18.8)	20 (8.5)	77 (14.3)
History of acute GVHD	236 (77.6)	182 (77.8)	418 (77.7)
Grades			
0	68 (22.2)	52 (22.3)	120 (22.3)
1	19 (6.3)	14 (6.0)	33 (6.1)
2	184 (60.5)	149 (63.7)	333 (61.9)
3	29 (9.5)	18 (7.7)	47 (8.7)
4	4 (1.3)	1 (0.4)	5 (0.9)

Immunosuppression at the time of post-transplant departure oral examination			
Prednisone	49 (16.1)	43 (18.4)	92 (17.1)
Cyclosporine	51 (16.8)	40 (17.1)	91 (16.9)
Tacrolimus	52 (17.1)	48 (20.5)	100 (18.6)
Mycophenolate mofetil	29 (9.5)	29 (12.4)	58 (10.8)

*Hematological malignancies include: Acute lymphoid leukemia, Acute myeloid leukemia, Chronic myeloid leukemia, Chronic lymphoid leukemia, Non-Hodgkin lymphoma, Hodgkin lymphoma, Polycythemia Vera, and other leukemia
**Non-malignant diseases include autoimmune conditions, immunodeficiency syndromes, and Paroxysmal nocturnal hemoglobinuria

B. Association of oral GVHD with future chronic GVHD development

Amongst 538 patients, 234 (43.4%) patients developed chronic GVHD post departure. A total of 191 (35.5%) patients were diagnosed with oral GVHD at departure. Within this group of patients, 50.3% of patients developed chronic GVHD (based on the NIH 2005 criteria) after departure (Table 7). The median time of chronic GVHD development was found to be 155.9 days' post HCT.

Three hundred and forty-seven patients had no evidence of oral GVHD at departure. Amongst the 347 patients, only 39.8% developed subsequent chronic GVHD.

Table 7: Oral GVHD at departure

	Subsequent diagnosis of chronic GVHD after departure		
Oral GVHD at departure evaluation	**No**	**Yes**	**Total**
No	209 (60.2)	138(39.8)	347
Yes	95(49.7)	96 (50.3)	191
Total	304	234	538

Oral GVHD at departure was found to be associated with an increased risk for subsequent development of chronic GVHD, both in the univariate and multivariable analyses (Table 8). In the univariate analysis, those with oral GVHD at departure were 1.5 times as likely to develop chronic GVHD compared to those without chronic GVHD at departure (OR=1.5, 95% CI 1.1-2.2, p=0.02). After adjusting for risk factors related to the development of chronic GVHD (specifically recipient age, patient/donor gender, donor relationship, graft source, acute GVHD grades 2-4, conditioning regimen and prednisone treatment at time of departure), this association remained, with an odd's ratio of 1.6 (95% CI 1.2-2.4, p=0.01).

Table 8: Univariate and multivariable analysis for chronic GVHD development after departure exam

	Univariate			Multivariable		
Variables	**OR (95% CI)**	**p-value[1]**	**Global p-value[2]**	**OR (95% CI)**	**p-value[1]**	**Global p-value[2]**
Oral GVHD at departure	1.5 (1.1, 2.2)	0.02		1.6 (1.1, 2.4)	0.01	
Age at transplant	1.01 (1.0, 1.02)	0.05		1.0 (0.99, 1.02)	0.42	
Patient/donor gender			0.10			0.68

Male or female donor/female patient	1			1		
Female donor/male patient	1.1 (0.8, 1.7)	0.54		1.2 (0.7,1.8)	0.80	
Unknown donor/patient	0.3 (0.1, 1.0)	0.05		1.7 (0.3, 8.0)	0.58	
Donor relationship			0.04			0.001
Related match	1	--		1	--	
Related mismatch	0.6 (0.2, 1.8)	0.37		0.7 (0.2, 2.0)	0.16	
Unrelated	1.5 (1.0, 2.2)	0.04		2.0 (1.3, 3.1)	0.003	
Graft source			<0.0001			<0.0001
Bone marrow	1	--		1	---	
PBSC	2.1 (1.2, 3.6)	0.01		1.9 (1.1, 3.4)	<0.0001	
Cord	0.3 (0.1, 0.8)	0.01		0.2 (0.1, 0.6)	<0.0001	
Acute GVHD grade 2-4	1.0 (0.7, 1.5)	0.92		0.99 (0.6, 1.5)	0.97	
Prednisone at departure	1.2 (0.7, 1.8)	0.49		1.1 (0.7, 1.8)	0.66	
Mini-transplant	1.3 (0.9, 1.9)	0.14		1.0 (0.6, 1.7)	0.89	

[1]Wald test p-value
[2]Wald test p-value for overall effect of the factor

C. Clinical findings at departure exam

The diagnosis of oral GVHD was made based on the presence of distinct oral mucosal changes at departure (Table 9). The most common manifestation of oral GVHD was the presence of sheet-like hyperkeratosis (78%). Amongst other findings, 73.2% presented with erythema and 60.7% presented with distinct lichenoid hyperkeratosis. Only 8 patients presented with tissue ulceration. Mucoceles were identified in 59 patients (though the presence of mucoceles is not included in the NIH diagnostic criteria for chronic oral mucosal GVHD) (Jagasia, et al., 2015; Treister, et al., 2008).

Table 9: Mucosal findings in patients with oral GVHD at departure

	Subsequent diagnosis of chronic GVHD after departure		
Mucosal findings at departure evaluation	**No (n=95)**	**Yes (n=96)**	**Total (n=191)**
	N (%)	**N (%)**	**N (%)**
-Erythema	63 (45.0)	77 (55.0)	140 (73.2)
-Lichenoid hyperkeratosis	60 (51.7)	56 (48.2)	116 (60.7)
-Ulcers	3 (37.5)	5 (62.5)	8 (4.1)
-Atrophy	55 (47.8)	60 (52.1)	115 (60.2)
-Hyperkeratosis (sheet/plaque like)	71 (47.6)	78 (52.3)	149 (78.0)
-Mucocele	34 (57.6)	25 (42.3)	59 (30.8)

D. Oral symptoms at departure

A total of 33 patients reported mucosal sensitivity at departure (Table 10). There was no statistically significant association between mucosal sensitivity at departure and subsequent chronic GVHD development (p= 0.22).

Summarized in Table 10 is the frequency of oral GVHD and subsequent diagnosis of chronic GVHD among patients who had mucosal sensitivity. A total of 72.7% of patients with mucosal sensitivity reported mild mucosal sensitivity, 9.1 % reported moderate sensitivity and 3.0% reported severe sensitivity. Among all 538 patients, the odds of future chronic GVHD in patients with mucosal sensitivity were 0.6 times that of patients without mucosal sensitivity (OR=0.6 (0.3-1.3, p=.22).

Table 10: Oral GVHD and subsequent chronic GVHD among patients with mucosal sensitivity

Oral GVHD	Mucosal sensitivity	Subsequent diagnosis of chronic GVHD after departure No (n=22) N (%)	Yes (n=11) N (%)	Total (n= 33) N (%)
No	Mild	9 (27.2)	2 (6.06)	11 (33.3)
	Moderate	0	1 (3.03)	1 (3.03)
	Unspecified	1 (3.03)	1 (3.03)	2 (6.06)
Total		**10 (30.3)**	**4 (12.1)**	**14 (42.4)**
Yes	Mild	7 (21.2)	6 (18.1)	13 (39.3)
	Moderate	1 (3.03)	1 (3.03)	2 (6.06)
	Severe	1(3.03)	0	1 (3.03)
	Unspecified	3 (9.09)	0	3 (9.09)
Total		**12 (36.3)**	**7 (21.2)**	**19 (57.5)**

A total of 259 patients reported xerostomia at departure, and Table 11 summarizes the distribution of oral GVHD and development of subsequent chronic GVHD among these patients. Among all 538 patients, the odds of subsequent chronic GVHD among those with xerostomia were 0.8 times that of patients without xerostomia (OR=0.8 (0.6-1.2, p=.33).

Table 11: Oral GVHD and subsequent chronic GVHD among patients with xerostomia

	Subsequent diagnosis of chronic GVHD after departure		
Oral GVHD	No (n=152) N (%)	Yes (n=107) N (%)	Total (n=259) N (%)
No	96 (63.1)	56 (36.8)	152 (58.6)
Yes	56 (52.3)	51 (47.6)	107 (41.3)
OR (95% CI) = 0.8 (0.6, 1.2), p= 0.33, no association between xerostomia and future chronic GVHD development			

CHAPTER 5: DISCUSSION

Chronic GVHD remains one of the leading causes of morbidity and mortality post allogenic transplantation (Soares, et al., 2005). Early and timely diagnosis of chronic GVHD allows for optimum patient management and can improve the chances of disability-free survival (Soares, et al., 2005; Wagner, et al., 1998; Nakamura, et al., 1996; Flowers & Martin, 2014).

Early and timely diagnosis of chronic GVHD can be very challenging due to non-specific disease manifestations such as skin rashes, hepatitis and diarrhea that can mimic non-GVHD related conditions (Hiroki, Nakamura,, Shinohara, & Oka, 1994; Schubert M. M., et al., 1984; Jacobsohn, Montross, Anders, & Vogelsang, 2001). As a result of the difficulties in chronic GVHD diagnosis, studies have explored the value of oral exams and salivary gland alteration identified using lip biopsies to diagnose chronic GVHD (Soares, et al., 2005; Hiroki, Nakamura,, Shinohara, & Oka, 1994; Sale, et al., 1981). Oral examination and biopsies have been found to be of value in determining chronic GVHD status (Schubert M. M., et al., 1984).

Studies by Wagner et al. and Loughran et al. have examined the value of oral examinations and oral biopsies at day 100 post HCT in predicting chronic GVHD development (Wagner, et al., 1998; Loughran, et al., 1990). Loughran et al. examined the significance of "subclinical oral GVHD" which was traditionally defined as the presence of histological changes suggestive of GVHD without any clinical signs of the disease in the mouth (Demarosi, et al., 2007). Loughran's study found no value in screening for subclinical disease since treating patients positive for subclinical disease preemptively did not prevent disease progression as 70% still

went onto develop chronic GVHD (Loughran, et al., 1990). Oral biopsies have fallen out of favor, as they are invasive with risk for bleeding and infection and lack of predictive value in the absence of clinical findings. Oral examinations, on the other hand are fairly simple and with sufficient training providers can identify oral manifestation of GVHD which makes it a valuable tool to predict future chronic GVHD development. Wagner's study considered oral exam findings at departure to predict subsequent chronic GVHD development, but the methodology used in gathering oral GVHD data and criteria used for diagnosis of oral GVHD were unclear. Our study examined the association of oral mucosal GVHD between day+70-120 with the subsequent development of chronic GVHD. The findings in our study supports our contention that patients presenting with oral GVHD mucosal changes between days 70-120 and without any history of chronic GVHD activity in other organ systems, have an increased risk for subsequent development of chronic GVHD after controlling for known risk factors. Therefore, patients with oral GVHD at departure should be educated on the increased risk for subsequent chronic GVHD development.

At times, manifestation of oral mucosal GVHD are not recognized due to absence of distinctive clinical findings of oral GVHD (especially in the early stages of the disease around departure) (Jagasia, et al., 2015; Carpenter, et al., 2015; Woo, Lee, & Schubert, 1997). Therefore, to avoid a false positive diagnosis, it is necessary to rule out conditions that may have present similarly to GVHD, including candidiasis, malignancy and drug induced changes (Jagasia, et al., 2015; Carpenter, et al., 2015; Woo, Lee, & Schubert, 1997). Additional diagnostic tools such as culture and biopsy are extremely valuable in such cases (Woo, Lee, & Schubert, 1997). A diagnosis of oral mucosal GVHD is positive if the following characteristic features are present in any

intraoral site: lichenoid hyperkeratosis (which may appear reticular like the Wickham striae seen in lichen planus or as hyperkeratotic, patches, plaques, and sheets interspersed with erythema), loss of gingival stippling with erythema and atrophy (particularly in the anterior maxillary gingiva), atrophy or loss of papillation in the dorsal surface of the tongue surface, and the presence of pseudomembranous ulcerations (Schubert & Correa, 2008; Kuten-Shorrer, Woo, & Treister, 2014; Jagasia, et al., 2015; Carpenter, et al., 2015; Schubert M. M., et al., 1984). If oral changes are subtle, a combination of clinical findings, patient reported symptoms, and other systemic signs of chronic GVHD (if present) can aid in oral GVHD diagnosis. It is important to keep in mind that all patients in our study population were taking prophylactic immunosuppressants to prevent GVHD at departure examination and that many were also being treated with medications (including prednisone) due to history of acute GVHD. It has been previously shown that corticosteroid use around day 50 alters histological findings in GVHD (Wagner, et al., 1998). Therefore, it is possible for these immunosuppressants to alter clinical presentation of oral mucosa thereby masking the presentation of GVHD related changes.

Mucosal sensitivity is a common symptom in patients with oral GVHD (Schubert & Correa, 2008; Kuten-Shorrer, Woo, & Treister, 2014; Schubert M. M., et al., 1984). Consistent with the existing literature, a higher number of patients in the “oral GVHD” group reported mucosal sensitivity compared to patients in the “no oral GVHD” group. However, only a small proportion of patients with oral GVHD at departure reported mucosal sensitivity. Low symptom report may be due to either the presence of resolving acute GVHD (i.e. visible mucosal findings remain while symptoms have resolved) or early manifestation of chronic GVHD, in which oral mucosal damage has not yet reached the point where symptoms become apparent.

Xerostomia is another commonly reported symptom among patients post allogenic-HCT. GVHD may damage salivary gland structures resulting in a decrease in quantitative production of saliva and broad sialochemical changes in saliva that is produced. In this study, patient reported symptom of xerostomia was found to be higher amongst patients in the "no oral GVHD" group compared to the "oral GVHD" group. This finding could be explained by the lack of correlation between oral mucosal and salivary gland GVHD and a higher salivary flow rate among patients with oral mucosal GVHD (Imanguli, et al., 2010). Unlike the oral mucosa where chemoradiotherapy induced damage is transient, GVHD- related salivary gland damage can be permanent (Schubert, Sullivan, & Truelove, 1986). Therefore, it is possible that the xerostomia was related to effect of the conditioning regimen rather than GVHD, especially in protocols that include total body irradiation (Schubert, Sullivan, & Truelove, 1986; Schubert & Sullivan, 1989).

A. Strengths of the study

One of the primary strengths in our study is the large sample size of patients which strengthened the statistical power of our study results. Furthermore, in our center, oral examinations completed for all patients post-allogeneic HCT as part of standard assessment for chronic GVHD throughout the body. There are few cancer centers across the world that put such a strong emphasis on oral evaluation in the pre- and post-transplant periods. In addition, to oral examinations completed by highly trained medical professionals, SCCA maintains a specialty oral medicine clinic staffed by oral medicine specialists with extensive training and experience in the diagnosis of oral mucosal disease including GVHD. This enabled us to envision a study of

this kind and provided detailed records outlining oral signs and symptoms between day 70-120 post-HCT to address our fundamental research question

B. Limitations of the study

The study has several limitations. Given the retrospective nature of the study design, we had to rely on pre-existing data which posed challenges in the process of the study.

Oral examinations at departure were not collected specifically for the purpose of this research project using a standard protocol. Therefore, there were differences in the manner in which oral exams were conducted to determine oral GVHD status (though all providers used the NIH diagnostic criteria for oral GVHD). Differences existed in the description of clinical findings in the patient records provided by different OM providers at the time of departure oral exam. For several patients, a definitive GVHD diagnosis based on the clinical note was not possible. In these circumstances, oral GVHD diagnosis was subjected to our interpretation of previously gathered data. To decrease bias, we utilized an oral GVHD diagnostic criteria based on the most distinct mucosal manifestations of oral GVHD (Schubert & Correa, 2008; Kuten-Shorrer, Woo, & Treister, 2014; Schubert M. M., et al., 1984) and independently applied these criteria to the clinical note to determine oral GVHD diagnosis. Independent diagnosis was then reviewed as a group. In the case of disagreement, a discussion was held until consensus was reached. This ensured uniformity and consistency in oral GVHD diagnosis affecting overall study quality.

At our center, systematic oral examinations are not performed on a regular basis prior to departure. Therefore, it is possible that acute oral GVHD may have been underdiagnosed in the

absence of patient reported-symptoms. If acute oral GVHD had been present, but not formally diagnosed, subtle oral mucosal findings at departure examination may have represented residual or resolving acute oral GVHD, rather than emerging chronic GVHD. This would have altered the interpretation of our primary outcome by placing some individuals in the "Oral GVHD" group, who may not have had true oral GVHD at departure. We did not specifically account for this situation in our study; however, we did control for any history of acute systemic GVHD (and other known risk factors) prior to chronic GVHD diagnosis in the final analysis. The presence of oral GVHD at departure appeared to be associated with increased risk for chronic GVHD development even after controlling for history of acute systemic GVHD.

Following departure from SCCA, patients were monitored and treated for chronic GVHD by their local oncologists. We utilized reported data on chronic GVHD status for our study. This presents the potential for variation in clinical assessment and ultimate diagnosis of chronic GVHD between centers. Patients treated at SCCA come from across the world and reside in communities with widely variable medical resources. Differences in training and familiarity of providers could theoretically have impacted our primary outcome, though it reasonable to assume that oncology providers, regardless of location, possess adequate training and knowledge to successfully diagnose chronic GVHD. This consideration is further alleviated by regular communication between local providers and the providers in the SCCA transplant and long-term follow-up services and regularly scheduled post-transplant medical evaluations in which patients return to SCCA for clinical evaluation (with a focus on assessment for chronic GVHD).

Lastly, an accuracy verification and quality assessment of the data collected were not performed using a randomized pool of patients. We identified errors in the interpretation of patient reported oral dryness status available in the oral medicine record during the data collection process versus the interpretation by an OM expert at a later point, however we do not expect any error that would have impacted the overall study results.

C. Future directions

This study has the potential to be a significant step in the timely diagnosis of chronic GVHD in patients post allogenic HCT. It will be valuable to conduct a prospective study to examine a similar relationship but also to investigate how providers treating these patients can clinically apply these findings going forward. A prospective study would also allow for robust calibration of providers through the use of training modules, sample cases, and photo atlases to ensure consistency in data collection. In addition, future studies in this area can assess the application of salivary biomarkers such as lactoperoxidase, lactotransferrin and salivary IL-ra1 as an adjunct tool along with oral examinations in determining chronic GVHD development and diagnosis (Bassim, et al., 2010; Presland, 2016). These tests are non-invasive and carry negligible risk for bleeding or infection.

Based on the study finding in conjunction with future studies evidencing the same, providers (particularly hematology-oncologists) involved in direct care for these patients can assess the clinical value and applicability of the study results. Providers across various cancer centers, can develop necessary guidelines/ protocols to perform oral examinations in a timely and accurate manner. Approximately 84% of clinicians in a study examining inter- and intra-observer

variability using NIH Oral chronic GVHD Activity Assessment instrument reported the need for formal training in diagnosing oral GVHD (Treister, et al., 2010). Clinicians can be trained using training modules, sample cases, and photo atlases.

Graduates in this field who provide clinical care to patients post allo- HCT should be trained and well equipped to diagnose and manage patients with oral GVHD. It might be noteworthy to conduct a multicenter study to evaluate the training and the skills oral medicine graduates have in recognizing and managing oral GVHD. It will help identify any setbacks in the current system, allow for improvements and help establish a standardized training methodology.

D. Conclusion

Early diagnosis of chronic GVHD and prompt treatment are key to reduce disease related morbidity and improve overall survival following HCT (Hiroki, Nakamura,, Shinohara, & Oka, 1994; Nakhleh, Miller, & Snover, 1989).

A clinical diagnosis of oral mucosal GVHD at the time of departure was found to be associated with increased risk of subsequent development of systemic chronic GVHD. This study highlights the potential value of examining patients 70-120 days' post HCT to determine the presence of oral GVHD-related changes. Patients with oral GVHD at departure should be educated on the increased risk for chronic GVHD and should be advised to report it to their oncologist.

With future studies supporting such an association, it would reaffirm the value of diagnosing oral GVHD accurately and in a timely manner to improve not only oral health and patient comfort but overall patient survival.

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CHAPTER 7: APPENDIX

Appendix A: Departure exam data

Patient information
UW# _____ **Did the patient have an oral exam around day 100?** o Yes o No o Inadequate information **Date of oral exam around departure**? _____ **Who provided the oral exam around departure?** o OM attending o OM hygienist o Medical team at SCCA o Outside provider
GVHD information
Diagnosis at oral medicine departure exam? o Clinical diagnosis of oral GVHD o Normal oral mucosa o Abnormal/ non-specific (oral changes but not GVHD) o Oral GVHD diagnosis unclear **Provide description of the clinical appearance of oral mucosal changes** o Ulcers o Lichenoid hyperkeratosis o Atrophy o Hyperkeratosis o Mucocele **What oral symptoms were reported by the patient?** o Mucosal sensitivity o Xerostomia **Patient reported grade of mucosal sensitivity** o Mild o Moderate o Severe o Unspecified **Did the patient have GVHD at any other site apart Yes from the mouth at departure?** o Yes o No **Other sites with acute GVHD during the time of departure from the center?** o Skin o GIT o Genitals/ vaginal o Liver o Lung o Muscles & joints o Eyes Subclinical skin

Appendix B: Demographic and GVHD data

Transplant diagnosis
Patient gender
Type and source of transplant
Conditioning regimen
GVHD prophylaxis agents
Immunosuppressive agents at departure
Dates and grades of acute GVHD
Dates and grades of NIH chronic GVHD diagnosis following departure
Dates of death, relapse and last contact with patient