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Oral Long-Term Complications of Allogeneic Haematopoietic Stem Cell Transplantation

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A treatise submitted in partial fulfilment of the requirements for the degree of

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DECLARATION

This thesis describes the work carried out in the Bone Marrow Transplant Unit, Westmead Hospital and the Faculty of Dentistry, University of Sydney, Australia between March 2007 and October 2009. The research is entirely my own and has not been submitted in whole or in part for a degree at this or any other university. To the best of my knowledge it does not contain any material published or written by another person except where acknowledged in the text.

Dr Katrusha Hull

Date

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ABSTRACT

Allogeneic haematopoietic stem cell transplantation (HSCT) involves the infusion of haematopoietic progenitor and stem cells, obtained from donor marrow or other sources, which engraft and under optimal circumstances repopulate and reconstitute the host's immune system. Transplantation of allogeneic haematopoietic stem cells requires careful matching of donor and recipient. Failure to do so may lead to delayed or incomplete engraftment, complications such as graft-versus-host disease (GVHD) or graft failure.

Allogeneic HSCT is a treatment of increasing prevalence for a range of haematological malignancies, marrow failure states, immune deficiencies and some solid tumours. In Australia, major indications for allogeneic HSCT are acute myeloid leukaemia, non-Hodgkin's lymphoma and acute lymphoid leukaemia (Nivison-Smith, Bradstock et al. 2007). However, allogeneic HSCT is associated with serious and debilitating long-term complications which confer increased morbidity and mortality. These complications commonly involve the oral tissues and salivary glands potentially causing a detrimental impact on oral health and function. The most significant of these include the development of new or second malignant neoplasm's and oral chronic GVHD.

Of the 277 patients who received allogeneic HSCT from 2003 to 2008, 132 transplant recipients were still living and 88 participated in this study. Data analysis has shown a striking prevalence of oral manifestations of chronic GVHD with over 50% of subjects showing objective evidence of long-term complications from transplantation. Common presentations included salivary hypofunction, mucosal changes consistent with chronic GVHD, reduction in oral aperture and the presence of candidosis.

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LIST OF ABBREVIATIONS

aGVHD	Acute graft-versus-host disease
ALL	Acute lymphoblastic leukaemia
AML	Acute myelogenous leukaemia
Allo-HSCT	Allogeneic haematopoietic stem cell transplantation
APC	Antigen presenting cell
ABMRR	Australasian Bone Marrow Transplant Recipient Registry
BRONJ	Bisphosphonate-related osteonecrosis of the jaw
BMT	Bone marrow transplant
BMTU	Bone Marrow Transplant Unit
BSA	Body Surface Area
CO ₂	Carbon dioxide
CMC	Carboxymethylcellulose
cGVHD	Chronic graft-versus-host disease
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
EMBT	European Group for Blood and Bone Marrow Transplantation
GIT	Gastrointestinal tract
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukaemic effect
Gy	Gray
G-CSF	Granulocyte colony-stimulating factor

GM-CSF	Granulocyte macrophage colony-stimulating factor
HSCT	Haematopoietic stem cell transplantation
HLA	Human leukocyte antigen
IL	Interlukin
IFN- γ	Interferon gamma
LLLT	Low-level-laser therapy
MHC	Major histocompatibility complex
MUD	Matched unrelated donor
MMF	Mycophenolate mofetil
NIH	National Institutes of Health
NHL	Non-Hodgkin's lymphoma
PBSCT	Peripheral blood stem cell transplant
PUVA	8-methoxypsoralen ultraviolet light A
QOL	Quality of life
RIC	Reduced intensity conditioning
SCC	Squamous cell carcinoma
TBI	Total body irradiation
TNF α	Tumour necrosis factor alpha
UC	Umbilical cord
WHO	World Health Organisation

Chapter 1: LITERATURE REVIEW

Allogeneic Haematopoietic Stem Cell Transplantation

1.1 Definition

Stem cell transplantation involves eliminating the patient's (host) haematopoietic and immune system via chemotherapy and/or radiotherapy, termed conditioning therapy, and replacing it with stem cells derived from another individual (the donor) or with previously harvested stem cells from the patient themselves. Human stem-cell transplantation (HSCT) may be *syngeneic*, where stem cells are obtained from an identical twin, *allogeneic*, when donor stem cells are utilised or *autologous*, where the patient's own stem cells are used. Allogeneic HSCT involves the intravenous infusion of haematopoietic progenitor and stem cells, obtained from donor marrow or other sources, which then engraft and under optimal circumstances repopulate and reconstitute the host's immune system. Transplantation of allogeneic haematopoietic stem cells requires careful matching of donor and recipient. Failure to do so is associated with delayed or incomplete engraftment and with graft-versus-host disease (GVHD) (Hoffman, Benz et al. 2009).

1.2 Principles of Haematopoietic Allogeneic Stem Cell Transplantation

1.2.1 Selection of a donor

Successful allogeneic HSCT relies on molecular typing to facilitate precise human leukocyte antigen (HLA) matching between donor and host. If incompatible with the host, HLA antigens elicit both humoral and cell-mediated host responses which may progress to rejection and destruction of the graft.

The major histocompatibility complex (MHC) genes are a set of highly pleomorphic glycoproteins which are found on specific cell surfaces in mammals, they are intimately involved in antigen recognition and allow the immune system to distinguish self from non-self. MHC enable the presentation of peptides, derived from antigens, onto the cell surface and thus to the immune system. The human equivalent of MHC are the HLA which are located on the short arm of chromosome 6 and are divided into class I and class II antigens. The class I antigens (HLA-A, -B, -C) are expressed on all nucleated cells in the body. Class I HLA bind and present peptides derived from degraded intracellular proteins to CD8⁺ cells and thus allow the immune system to recognise self from non-self. The class II antigens (HLA-DR, -DP, -DQ) are located on antigen presenting cells and bind peptides derived from degraded extracellular proteins and thus help to regulate the immune response by allowing the recognition of foreign antigens.

The MHC genes are inherited as haplotypes, that is, they are inherited as a unit.

Molecular typing is performed to allow for precise HLA matching between prospective donors and the recipient however considerable genetic disparity may still exist via the

minor histocompatibility antigens (mHags). Minor histocompatibility antigens may reflect polymorphisms of normal cellular proteins that are not shared between donor and recipient (Cutler, Antin et al. 2006). Identification of these mHags by donor T-cells leads to the generation of an immune response which may result in GVHD and also the graft-versus leukaemic effect (GVL) (Copelan 2006). In contrast, recognition of the foreign donor cells by residual host T-cells may lead to graft rejection.

In the process of donor selection, donors are broadly categorised as HLA identical or non-identical donors (Table 1.2.1). For allogeneic HSCT a genotypically HLA-identical sibling is the ideal donor, namely, a sibling who has inherited the same paternal and maternal MHC genes. Donors which are a 2 haploid match are obtainable in 25% to 30% of patients who have living siblings (Hoffman, Benz et al. 2009). Specifically, the donor and recipient are matched for the amino acid sequence (allele) encoded by all HLA loci. It may also be possible to identify individuals within families (e.g. a parent, uncle, aunt etc) who share one haplotype. These donors have one identical haplotype and are phenotypically matched for the non-shared haplotype. Molecular techniques, typically polymerase chain reaction, allow the definition of the unique sequence of variants (alleles) which encode each HLA molecule.

Table 1.2.1: Donor types in Allogeneic HSCT (Hoffman Basic principles and practice, 5th ed)

Allogeneic HSCT	Donor types	Histocompatibility barrier	
		Minor	Major
	HLA genotypically/ phenotypically identical donor	+	-
	• Related	+	-/+
	• Unrelated	+++	-/+
	HLA non-identical donor		
	• Related	+	++
	• Unrelated	+++	++

Approximately 70% to 75% of patients, who could benefit from HSCT, lack a suitably matched related donor (Hoffman, Benz et al. 2009). This has led to the development of numerous donor registries. Currently there are an estimated ten million volunteer donors. For HSCT, HLA-A, HLA-B, and HLA-DR are routinely evaluated (Petersdorf, Anasetti et al. 1998). DNA-based methods have become established as the gold standard for HLA testing (Hoffman, Benz et al. 2009). Where possible, unrelated donors are completely phenotypically matched for critical HLA class I and II antigens. However, even in closely matched related donors mismatched mHags, encoded outside the MHC in the recipient, may be recognised as antigens by the donor T cells resulting in an immune response which may lead to graft rejection or GVHD. When HLA disparity cannot be avoided, selection of a donor with the least HLA mismatches may allow the avenue of transplantation for a wider group of patients. Clearly, the use of an as closely matched donor as possible increases the chances of successful engraftment and reduces the risk of GVHD (Flomenberg, Baxter-Lowe et al. 2004). Several studies

have shown that approximately 40% of recipients of HLA identical grafts develop acute GVHD however this increases to between 60%-80% in recipients of unrelated or one-antigen HLA mismatched grafts (Anasetti, Amos et al. 1989; Flomenberg, Baxter-Lowe et al. 2004).

Criteria independent of HLA are considered in donor selection when greater than one HLA-identical match is available for a given patient. For example, a younger donor age is significantly associated with an improved clinical outcome (Confer and Miller 2007). With each decade of life in a donor, the risk of acute and chronic GVHD increases by 10% (Hoffman, Benz et al. 2009). In contrast, donor ABO blood type has not been shown to influence the risk of GVHD or mortality (Hoffman, Benz et al. 2009).

Identified matched donors also undergo a screening medical history, physical examination and laboratory testing specifically for transmissible diseases: hepatitis B, C and A, human immunodeficiency virus, cytomegalovirus (CMV) and treponema pallidum.

1.2.2 Stem Cell Source

Mature haematopoietic cells are produced continuously by less-differentiated precursor cells which are ultimately, descendants from the haematopoietic stem cells. All stem cells have the capability to produce both mature differentiated cells and daughter cells. Daughter cells are self renewing and retain stem-cell properties and thus allow continual propagation of the haematopoietic cell cascade. Currently, for allogeneic HSCT, stem cells are harvested from three sites: the bone marrow, peripheral blood and umbilical cord blood. Classically, stem cells are collected from the donor bone marrow, most commonly from the posterior iliac crest and typically during a general anaesthetic or regional anaesthesia. Multiple aspirations are required with collection of approximately 5 mL of marrow from each puncture site. Bone marrow was the originally preferred source due to the ease and reliability of collecting adequate numbers of cells. In 1994, the first allogeneic transplant utilising peripheral blood-derived haematopoietic stem cells was performed in Australia (Nivison-Smith, Bradstock et al. 2005). Marrow stem cells continuously detached and enter the circulation making peripheral blood a convenient source of haematopoietic stem cells. The proportion of HSCT harvested from bone marrow have declined in recent years with peripheral blood being the currently preferred stem cell source in Australia and New Zealand, with 71% of allogeneic HSCT utilising this source in 2004 (Nivison-Smith, Bradstock et al. 2007).

Peripheral blood stem cells are collected using a cell-separator which is connected to the donor usually via peripheral cannulae. Mononuclear cells are collected by centrifugation whilst the erythrocytes are returned to the patient. The peripheral blood

contains significantly fewer stem cells relative to the bone marrow therefore pre-treatment of the marrow is frequently required. The number of peripheral blood stem cells is estimated by the cell surface molecule CD34+, which is used as a marker (Copelan 2006). The proportion of CD34+ cells in blood can be raised by the use of growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte colony-stimulating factor (G-CSF). Growth factors may be given to donors for 4-6 days (typically 10µg/kg/day) prior to collection to aid in stem cell mobilisation (Hoffbrand, Moss et al. 2006). G-CSF, causes the proliferation of neutrophils and the release of proteases. These proteases degrade the proteins which anchor the stem cells to the marrow stroma and, in combination with other factors, free the cells to enter the circulation (Levesque, Liu et al. 2004). Peripheral blood stem cell transplantation (PBSCT) is associated with significantly faster engraftment times relative to marrow in allogeneic HSCT (Nivison-Smith, Bradstock et al. 2007). However, due to a raised yield of T cells some studies have demonstrated an increased risk for the development of chronic GVHD in these recipients (Schaffer 2006).

The most recent advance in stem cell collection has been the introduction of umbilical cord blood (UCB) transplants, specifically for patients undergoing unrelated donor transplantation who lack an appropriate related or unrelated volunteer donor. Umbilical cord blood is collected from the placental vein after delivery of the infant and transection of the cord. Foetal blood contains a relatively large proportion of haematopoietic stem cells and UCB has been used successfully as a source of stem cells. The most significant hindrance in the utilisation of this source relates to the limited number of stem cells which can be collected from each sample. Umbilical cord blood transplantation has been linked to delayed haematopoietic recovery and therefore

a greater risk of posttransplant infections in combination with a higher incidence of graft failure (Schaffer 2006). Nevertheless a recent increase in the number of allogeneic HSCT using cord blood in Australia and New Zealand has been recorded with a rise from 12 such transplants in 2002 to 33 in 2004 (Nivison-Smith, Bradstock et al. 2007).

1.2.3 Processing

Manipulation of the harvested stem cell population, intended for haematopoietic stem cell rescue, aims to remove those components which are unwanted or may cause adverse effects, or to positively select a desired population such as the CD34+ cells (Hoffman, Benz et al. 2009). Routine minimal manipulation includes the removal of erythrocytes or plasma to overcome ABO blood group incompatibility between the recipient and donor. Elimination is achieved via centrifugation of the graft. Typically a maximal limit of incompatible erythrocytes can be tolerated by the recipient; exceeding this limit places the recipient at risk of hemolysis and transfusion reaction.

Another critical cell routinely manipulated in the haematopoietic stem cell graft is the T cell. Transplanted T cells have the potential to cause severe and at times lethal GVHD. Conversely T cells are also known to exert the potentially beneficial GVL effect. Many studies have attempted to identify the particular subpopulations involved in these two opposing processes. This would allow manipulation of allogeneic HSCT grafts to remove GVHD-producing T cells while sparing those subpopulations which initiate the desired GVL response (Hoffman, Benz et al. 2009). At this time there is no general consensus as to which populations should be targeted.

Several methods have been established to manipulate the graft T cell population prior to infusion. The technique currently favoured utilises specific monoclonal antibodies directed toward the antigens which identify unique T-lymphocyte subpopulations. The target population is then eliminated with superior efficiency using immunomagnetic separation. Various antigens have been identified including CD3 and CD2 for pan-T-cell depletions or CD4 and CD8 for the elimination of helper and suppressor T cells (Miller, Soignier et al. 2005).

Manipulation techniques mentioned to this point are *negative selection techniques* by nature as they eliminate identified populations, typically T cells or tumour cells, from the graft population. Conversely, identification of an antigen localised on the pluripotent cells essential for transplantation, namely the CD34 antigen, allowed development of manipulation techniques which cause enrichment of chosen cell populations. However, a concern raised about *positive selection techniques* is the fact that this process leads to the concomitant elimination of the non-target cell populations. These subpopulations may be of potential benefit to the host, for example, the T cell subpopulations involved in the GVL effect or those which facilitate engraftment (Hoffman, Benz et al. 2009). The future success of positive selection techniques relies on the identification of these subpopulations so that they may be recovered from the selected cell population and reintroduced to the final desirable graft cell population (Hoffman, Benz et al. 2009).

1.2.4 Conditioning

Prior to the infusion of the harvested haematopoietic stem cells a combination of anti-neoplastic or immunosuppressive agents are utilised (either chemical or physical agents), the aim being; to eradicate the host's immune and haematopoietic system so as to allow engraftment along with destruction of any residual malignancy. This preparative process, prescribed for the host, is known as *conditioning*. Conditioning regimens are classified as *myeloablative* or *non-myeloablative*. The object of myeloablative preparation is both to eradicate malignant cells and to induce immunosuppression to allow engraftment (Copelan 2006). A non-myeloablative regimen has been defined as a preparatory regime which should not eradicate host haematopoiesis and should allow haematopoietic recovery in less than 28 days without haematopoietic cell transplant (Champlin, Khouri et al. 2003). Non-myeloablative methods do not wholly eradicate host malignant cells and therefore rely on the immune-mediated effects of the donor cells to eliminate residual disease. Lastly, a *reduced intensity conditioning* (RIC) regime follows the principles of non-myeloablative techniques however HSCT is still required for marrow reconstitution and rescue.

Reduced intensity regimens are formulated to initiate less host tissue damage and inflammatory cytokine release, thus lowering the risk of transplant-related mortality (TRM) and, possibly, the incidence and intensity of GVHD (Wagner, Barker et al. 2002). Unlike myeloablative treatments, these regimens are primarily immunosuppressive and depend on the GVL effect to eradicate malignant cells. These features have allowed the application of HSCT to a wider group of patients who were previously excluded, most commonly due to comorbidities or age (Takahashi, Iseki et

al. 2004). Conversely, RIC regimes have a less potent antitumour effect and thus may not be ideal in all cases. Allogeneic HSCT, after the provision of RIC, is most effective in treating slower-growing malignancies such as chronic lymphocytic leukaemia. The most recently published European Group for Blood and Marrow Transplantation (EBMT) survey showed a continued rise in RIC HSCT from 3301 cases in 2005 to 3530 in 2006, with RIC used in 34% of all allogeneic HSCT (Gratwohl, Baldomero et al. 2007).

Conditioning protocols include various forms of chemotherapy in combination with, or in place of, total body irradiation (TBI). Specific agents used and their combinations vary widely amongst institutions and are influenced by the underlying disease process and transplant factors. As the name implies, TBI involves the delivery of radiation to the entire body however certain radiosensitive organs are commonly shielded, such as the lungs. TBI is myeloablative and immunosuppressive in nature and addresses sites not affected by chemotherapy (Copelan 2006). More recently, low dose TBI, in combination with immunosuppressive drugs, has been used as RIC regimens with lower toxicity.

The total dose of TBI used in HSCT typically ranges from 8-15 Gray (Gy) (Thomas, Buckner et al. 1979) which may be administered as a single dose or divided into smaller doses delivered over a course of days (fractionated) through the use of high energy photon beams, generally delivered by linear accelerators. TBI maximises malignant cell destruction with minimal damage to healthy cells by exploitation of the relatively

limited capacity of malignant cells to undergo repair. Although TBI does decrease the malignant cell load, the dosages commonly utilised are not intended as a standalone treatment but as a preparatory procedure to establish host immunosuppression to prevent graft rejection and to reduce the proportion of malignant cells.

Fractionated TBI (8–15 Gy in single or fractionated doses) combined with cyclophosphamide (commonly 60 mg/kg/day for 2 days) has been the standard myeloablative conditioning regimen since the 1980s (Thomas, Buckner et al. 1979). A common alternative utilises busulfan (typically 4 mg/kg/day for 4 days orally or 3.2 mg/kg/day intravenously) in place of TBI with cyclophosphamide (Hoffman, Benz et al. 2009). At least 36 hours are needed following the last chemotherapeutic dose for drug elimination prior to the infusion of the donor stem cells (Hoffbrand, Moss et al. 2006). Common conditioning related complications include mucositis and the consequent need for parenteral nutrition.

1.2.5 GVHD Prophylaxis

GVHD, a condition of T cell mediated alloreactivity, is a common outcome of allogeneic HSCT and may lead to significant morbidity and mortality. The most effective technique for the prevention of GVHD is ex-vivo depletion of donor T lymphocytes prior to infusion. Although this technique may reduce the incidence of aGVHD and hence cGVHD, it also places the patient at an increased risk of graft failure and relapse (Ho and Soiffer 2001).

Pharmacological immunosuppression is routinely prescribed following allogeneic HSCT with the aim to eliminate or blunt T lymphocyte recognition and proliferation which is responsible for the initiation of GVHD. The duration of immunosuppression varies between patients however several months (approximately 6 months) are generally required to allow the development of immune system tolerance and lymphohaematopoietic chimerism (Bolanos-Meade 2006). Agents which are frequently utilised, singularly or in combination, include methotrexate, corticosteroids, cyclosporine, and tacrolimus (Ruutu, Hermans et al. 1998).

1.2.6 Indications for allogeneic HSCT

Allogeneic HSCT has evolved as a curative therapy for a variety of haematological malignancies, marrow failure states, immune deficiencies and some solid tumours. This is reflected in a greater than 3-fold increase in the utilisation of this treatment modality in the past 15 years (Gratwahl, Baldomero et al. 2002). There are now an estimated 50,000 to 60,000 HSCT performed each year. The EBMT reported a 20% increase in allogeneic HSCT from 2004 to 2005 comprising 37% of all HSCT in 2005 (Gratwohl, Baldomero et al. 2007).

In Australia, the major indications for autologous HSCT in 2004 were non-Hodgkin's lymphoma (NHL) (37%) and multiple myeloma (38%). Whereas acute myelogenous leukaemia (AML), NHL, and acute lymphoblastic leukaemia (ALL) were the most common indications for allogeneic HSCT (Nivison-Smith, Bradstock et al. 2007). Multiple myeloma was also a significant indicator for allogeneic HSCT when a related donor was available. These indications are consistent with the reported indications for allogeneic HSCT in European centres (Table 1.2.6).

Table 1.2.6: Allograft indications by ABMRR and EMBT (Nivison-Smith, Bradstock et al. 2007)

Indications as a proportion of total allo-grafts		
Indication for HSCT	ABMTRR*	EBMT⁰
Acute myelogenous leukaemia	43%	32%
Acute lymphocytic leukaemia	19%	19%
Chronic myeloid leukaemia	8%	11%
Non-Hodgkin's lymphoma	9%	7%

*Australasian Bone Marrow Transplant Recipient Registry (ABMRR) ⁰ European Group for Blood and Marrow Transplantation (EBMT)

There has been a steady rise in HSCT in Australia and New Zealand with an increase of 7% per annum since 2001 (Nivison-Smith, Bradstock et al. 2007). Influencing factors include the greater proportion of older patients undergoing transplantation using reduced intensity conditioning regimens. The use of allogeneic HSCT for chronic myeloid leukaemia (CML) dropped sharply since 2001 and has been relatively low since that time (Nivison-Smith, Bradstock et al. 2007). This is a direct result of the implementation of imatinib mesylate therapy (Glivec™) for the majority of newly diagnosed patients (Goldman 2002).

1.3 Early complications of allogeneic transplant

1.3.1 Mucositis

Mucositis, associated with HSCT, is characterised by widespread ulceration of the moveable, non-keratinised mucosae of the oral cavity most commonly involving the buccal mucosa and the ventrolateral tongue (Scully, Sonis et al. 2006). Oesophageal and gastrointestinal systems are also affected. It is a common acute toxicity associated with both intense chemotherapy reserved for haematological malignancies as well as head and neck radiation, both being integral components of HSCT. Oropharyngeal mucositis may result in severe discomfort and is the most common symptom and distressing complication of HSCT (Bellm, Epstein et al. 2000). Furthermore, concurrent therapy related immunosuppression, specifically neutropoenia, places patients at risk of bacteraemia, septicaemia and fungaemia (Ruescher, Sodeifi et al. 1998). Clinically, mucositis first presents as generalised erythema approximately 4-5 days following the initiation of chemotherapy or following a cumulative radiation dose of 10Gy to the head and neck region. At 7-10 days following chemotherapy frank ulceration occurs, associated with significant pain and impaired function. Mucositis commonly extends to involve the oesophageal and gastrointestinal mucosa. Opioid analgesics are often essential at this stage and total parenteral nutrition may be required.

At a cumulative dose of 30Gy ulceration develops in radiation associated mucositis. Unlike chemotherapy, radiation leads to ulceration over any radiation-exposed area. TBI is the most common application of radiation in the HSCT setting. Due to the low radiation dosages utilised in allogeneic HSCT, mucositis is more commonly associated

with intensive chemotherapy in this setting. Chemotherapy induced mucositis may persist for approximately 1 week and generally resolves within 3 weeks after the initiation of chemotherapy (Scully, Sonis et al. 2006). Mucositis is seen in 75-99% of patients receiving myeloablative conditioning regimes for HSCT particularly those which also involve TBI (Donnelly, Muus et al. 1992). Conditioning regimes which are at high risk for mucositis include high-dose melphalan, busulphan-cyclophosphamide and cyclophosphamide-TBI (Wardley, Jayson et al. 2000). Recent literature has shown reduced severity and prevalence of mucositis with RIC where severe mucositis developed in 32% of patients treated with myeloablative regimens and only 7% in patients who received RIC (Vela-Ojeda, Garcia-Ruiz et al. 2004).

Numerous interventions have been developed for the prevention or management of oral mucositis. A recent Cochrane review concluded benefit from ice chips and GM-CSF with only minimal benefit from amifostine and povidone (Clarkson 2003). Cryotherapy, with ice chips, causes mucosal cooling and constriction of blood vessels and is thought to reduce the exposure of the oral tissues to the causative chemotherapy agent (Scully, Sonis et al. 2006). This method has been shown to be useful for agents with a short half life. Ice chips, used 5 minutes prior to 5-FU bolus and continued 30 minutes after, were found to reduce mucositis by 50% (Loprinzi, Ghosh et al. 1997). Palifermin (Kepivance), a recombinant human keratinocytes growth factor, is the most recent drug designed to prevent mucositis. Benefits were seen when given for 3 days prior to conditioning (Scully, Sonis et al. 2006).

1.3.2 Infections

Transplant related infections are another common complication seen in the early and late phase post transplant. Two main mechanisms have been identified in patients at increased risk for infection. Firstly, the risk of infection is elevated when non-specific defences are compromised, as seen following intensive conditioning schedules where the integrity of surface barriers are hindered. Secondly, HSCT, by nature, causes deficiencies in both the humoral and cellular arms of the immune system rendering the patient at severe risk of infection. Recovery of granulocytes and natural killer cells is seen about 2 weeks following myeloablative conditioning whereas T and B cell function may be suppressed for prolonged periods. So, conditioning, immunosuppression and neutropenia, in isolation or in combination, place the patient at risk for early infection. RIC regimens are associated with a reduced rate of early infections (Copelan 2006).

Bacterial infections most commonly arise from the patient's own commensal flora. In the presence of neutropenia local infections can rapidly cause septicaemia. Typically, gram positive skin organisms such as *Staphylococcus* and *Streptococcus* colonise central venous lines, whereas Gram-negative gut bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) may lead to septicaemia (Hoffbrand, Moss et al. 2006). Oral mucositis is acknowledged to be the principal risk factor for bacteraemia due to *viridians streptococci* and coagulase-negative *staphylococci* (Epstein, Raber-Drulacher et al. 2009). Chronic infections associated with the dentition may also represent a critical source of systemic infection during myelosuppression. Periodontal infections have been

specifically highlighted as a significant cause of systemic infection in neutropenic patients (Raber-Drulacher, Epstein et al. 2002) however this is not universally accepted.

Viral reactivation of latent herpes viruses are a common and serious complications in immunosuppression associated with HSCT. Reactivation of previously acquired latent herpes simplex, varicella zoster, CMV and Epstein-Barr virus are commonly encountered in the immediate post-transplant phase. Reactivation of CMV is a particularly serious complication which has become significantly less common due to superior detection of subclinical infection, donor screening and advances in anti-viral therapies and prophylaxis. CMV reactivation places the patient at risk of a potentially fatal interstitial pneumonitis as well as hepatitis. CMV pneumonitis was once fatal to 15% of allogeneic HSCT recipients (Meyers, Flournoy et al. 1982).

Fungal infections are a significant cause of morbidity and mortality following HSCT. The two most common subtypes are yeasts, such as the *Candida species*, and moulds, of which *Aspergillus* is most common (Hoffbrand, Moss et al. 2006). Prophylaxis for bacterial, fungal and viral infections involves a combination of agents which vary between transplant units. For example, in our unit prophylaxis typically consists of Bactrim DS™ (sulfamethoxazole 800mg, trimethoprim 160 mg), penicillin V (250mg) and Valtrex™ (valaciclovir).

1.3.3 Acute graft-versus-host disease

1.3.3.1 Classification

Acute graft-versus-host disease (aGVHD) occurs following allogeneic HSCT and arises due to the reaction of donor immune cells against host tissues. Traditionally, clinical GVHD occurring prior to 100 days after HSCT was called aGVHD (Sullivan, Agura et al. 1991). The significant advances in HSCT practice have led to profound alterations in the natural history and clinical picture of both acute and chronic GVHD. For example, in patients receiving RIC aGVHD may present more than 3 months following transplantation due to the extended period prior to engraftment (Mielcarek, Martin et al. 2003). These changes have led to a recent National Institutes of Health (NIH) consensus document on the classification of GVHD which recognises the unique clinical and pathophysiological features of acute versus chronic GVHD, removing the temporal basis of classification (Toubai, Sun et al. 2008).

The current consensus classification system focuses on clinical manifestations, not the time to symptomatic onset, as determining whether the clinical picture is considered acute or chronic GVHD (Filipovich, Weisdorf et al. 2005). Specifically, the updated NIH classification system specifies that in the absence of clinical or histological features suggestive of cGVHD, the persistence, recurrence or new onset of manifestations characteristic of aGVHD should be categorised as such, regardless of time since transplantation (Schaffer 2006) (Table 1.3.3.1). This will be discussed in greater detail at a later stage.

Table 1.3.3.1: NIH classification system of GVHD (Filipovich, Weisdorf et al. 2005)

Category	Onset of symptoms after HSCT	Presence of aGVHD features	Presence of cGVHD features
Acute GVHD			
Classic	≤100 days	Yes	No
Persistent, recurrent or late-onset	> 100 days	Yes	No
Chronic GVHD			
Overlap syndrome	No time limit	Yes	Yes
Classic	No time limit	No	Yes

1.3.3.2 Clinical presentation and staging

Acute GVHD is characterised by cellular apoptosis and necrosis affecting the skin, liver and gastrointestinal tract. Clinically, this may present as a maculopapular rash with tenderness and pruritis. The palmar and plantar surfaces are often initially involved which may become confluent with blister formation (Figure 1.3.3.2). The onset of aGVHD usually correlates with donor engraftment. Gastrointestinal involvement manifests as diarrhoea, cramping, nausea and/or vomiting. Hyperbilirubinemia, evidenced by jaundice, is a hallmark of liver involvement (Jacobsohn and Vogelsang 2007).

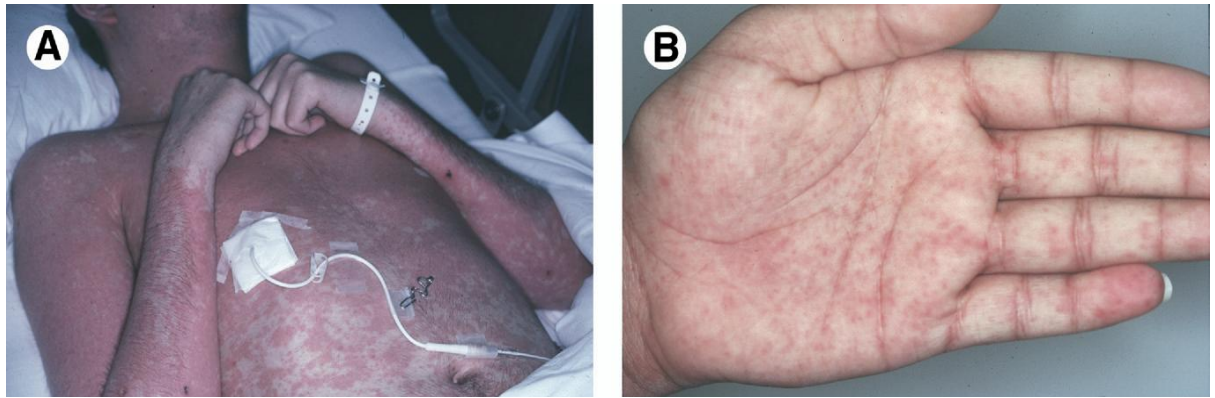


Figure 1.3.3.2: Acute GVHD. (A) Generalised eruption (B) Maculopapular rash on palm (Schaffer 2006)

The current staging system for aGVHD (Glucksberg 1994) subdivides patients into one of four grades (I-IV) based on the number and extent of organ involvement (Table 1.3.3.2).

Table 1.3.3.2: Staging system for aGVHD (Jacobsohn and Vogelsang 2007)

Stage	Skin (BSA)	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dl	< 500 ml/day or persistent nausea.
1	Maculopapular rash < 25%	BSA 2–3 mg/dl	500–999 ml/day
2	Maculopapular rash 25 – 50% BSA	3.1–6 mg/dl	1000–1500 ml/day
3	Maculopapular rash >50% BSA	6.1–15 mg/dl	Adult: >1500 ml/day
4	Generalized erythroderma plus bullous formation	>15 mg/dl	Severe abdominal pain with or without ileus
Grade			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	-

BSA- Body surface area

1.3.3.3 Pathophysiology

The pathophysiology of aGVHD was described by Ferrara and colleagues as a three-phase process (Ferrara, Levy et al. 1999). The initial step involves damage to the host tissues with inflammation, from the preparative chemotherapy and/or radiotherapy regimen. Donor T cells are activated by the host antigens expressed by the damaged tissues. In the second phase, the activation phase, both recipient and donor antigen-presenting cells (APCs), in combination with inflammatory cytokines, trigger the activation of donor-derived T cells, which then expand and differentiate into effector cells (Ferrara, Cooke et al. 2003). As mentioned previously, donor and recipient are usually matched at the HLA major histocompatibility loci, however, mismatches may arise at mHags loci. Minor histocompatibility antigens may play a role in allowing graft and host to recognise each other as foreign. These discrepancies may lead to activation of immunologically competent donor T cells which respond to the phenotypically disparate host antigens, derived from these polymorphic mHags, presented on antigen presenting cells (Toubai, Sun et al. 2008). T-cell activation pathways lead to transcription of genes for cytokines. In the effector phase, these activated T cells and cytokines mediate cytotoxicity against the target host cells.

1.3.3.4 Incidence and Risk Factors

The incidence of grade II-IV aGVHD is reported as 35%-50% (Jacobsohn and Vogelsang 2007). The chief risk factor for aGVHD is HLA disparity. With unrelated donor transplants, the greater the degree of HLA mismatch, the higher the likelihood of developing aGVHD and the worse the overall outcome (Beatty, Clift et al. 1985). Furthermore, the risk of GVHD has been associated with the conditioning regimen used. Regimens which cause extensive injury to epithelial and endothelial surfaces (Hill and Ferrara 2000) due to the resulting increase in permeability of epithelial barriers, leakage of microbial products such as lipopolysaccharide and the release of inflammatory cytokines (Imanguli, Alevizos et al. 2008) are especially implicated. This includes higher doses of irradiation and myeloablative regimens. Conversely, RIC was initially predicted to lead to less severe GVHD due to reduced mucosal toxicity and consequent initiation of proinflammatory cytokines. As yet, no conclusive evidence supports this hypothesis. This feature is thought to be a product of the increasing age of patients treated under RIC regimens (Epstein, Raber-Drulacher et al. 2009).

Donor stem cell source has also been identified as an important factor in the development of aGVHD with recipients of mismatched unrelated UCB transplant (4/6 or 5/6 HLA group match) having a similar risk of aGVHD as sibling HSCT (Jacobsohn, Hewlett et al. 2004). This has been attributed to the immunological naiveté of the UCB stem cells which offers some tolerance to greater degrees of mismatch. Other known risk factors for aGVHD include; unrelated donors, HLA mismatched donor, older age of donor, multiparous female donor and older age of the recipient (Jacobsohn and Vogelsang 2007).

1.3.3.5 Prophylaxis and Management

The currently favoured schedules for the prophylaxis of aGVHD are based on a combination of cyclosporine (alternatively tacrolimus) with a short course of methotrexate. This regimen has been shown to consistently result in a desirable inducement of GVHD and consequent beneficial graft-versus-tumour effect (Jacobsohn and Vogelsang 2007). Once GVHD occurs, most centers manage aGVHD by continuing prophylactic immunosuppression and adding methylprednisolone at 2 or 2.5mg/kg/day (Ruutu, Niederwieser et al. 1997). Approximately 40%-50% of patients will have a response to glucocorticosteroid therapy (Jacobsohn and Vogelsang 2007). Salvage therapy is implemented for steroid refractory cases. There is currently no standard approach for the management of steroid refractory aGVHD; some options include anti-thymocyte globulin, extracorporeal photopheresis and monoclonal antibodies, such as daclizumab and infliximab.

The prognosis of patients with aGVHD correlates with the initial clinical staging. Patients with Grade III aGVHD have a 30% probability of long term survival whereas those with Grade IV a GVHD show less than 5% survival (Cahn, Klein et al. 2005). Importantly, over half of the patients with aGVHD, regardless of the stage, will later go on to develop cGVHD (Jacobsohn and Vogelsang 2007).

1.3.4 Taste alteration

Alteration in taste (dysgeusia) or a reduction or loss in taste sensation (hypogeusia / ageusia) is commonly associated with both myeloablative and RIC preparative regimens. Furthermore, medications commonly utilised in GVHD prophylaxis, such as cyclosporine and tacrolimus, may also induce taste changes, commonly described as metallic, sweet, salty, sour and/or bitter (Epstein, Raber-Drulacher et al. 2009). Taste dysfunction may persist for days to months but typically fully recovers (Marinone, Rizzoni et al. 1991).

The precise aetiopathogenesis of taste dysfunction in HSCT is unknown however is thought to be influenced by direct toxicity of cytotoxic agents to taste buds, immune-mediated GVHD salivary damage and psychological changes, including conditioned food aversion (Epstein, Raber-Drulacher et al. 2009). Hyposalivation and xerostomia are common complications following HSCT with xerostomia being reported as the second most distressing symptom at discharge and at one year following HSCT (Larsen, Nordstom et al. 2007). Significant outcomes associated with taste derangement include weight loss, emesis and reduced quality of life.

1.4 Chronic complications of transplant

1.4.1 Graft-versus-leukaemic effect

In order for an allogeneic transplant to be maximally effective, malignant cells ideally must be subject to immunologic control. A critical therapeutic effect of HSCT in eliminating malignant cells is the graft-versus-leukaemic effect (GVL). The GVL effect is attributed to the immunological attack of the host tissue and, therefore, the leukaemia/malignancy by the allogeneic graft (Hoffman, Benz et al. 2009). This effect was first recognised over 50 years ago in experimental models by Barnes and Loutit (Barnes, Corp et al. 1956). The mechanisms behind the GVL effect are poorly understood. The GVL effect may be achieved through the presentation of molecules on the cell surfaces of the residual malignant cell population which can be recognised by the immune response. The antigens presented may be unique tumour antigens or, more likely, the same antigens which are responsible for inducing and sustaining GVHD against the hosts normal, non-malignant cells (Cutler, Antin et al. 2006). Of note, the literature demonstrates a clear relationship between the development of cGVHD and the anticancer effect of the GVL effect. Therefore it is clear that the GVL effect is a beneficial outcome associated with the development of cGVHD however, this cannot be separated from the substantial morbidity and mortality associated with cGVHD, if uncontrolled or severe.

1.4.2 Second malignancies

The development of new or second malignant neoplasms and lymphoproliferative disorders following HSCT have long been recognised as a rare but significant complication for long-term survivors (Demarosi, Lodi et al. 2005). Relative to the general population, HSCT survivors have a 13-fold higher risk for developing second malignancies, including leukaemias, lymphoproliferative disorders and solid tumours (Curtis, Rowlings et al. 1997). In the early post-transplant period, with a peak in the first 1 to 2 years, lymphoproliferative disorders and haematologic malignancies predominate as the most prevalent second malignancies. Common forms include leukaemia of the donor-derived cells and both Hodgkin's and non-Hodgkin's lymphoma.

In contrast to second leukaemias and lymphoproliferative disorders, solid tumours may develop many years following HSCT with their incidence continuing to rise over the years following transplantation. The most common solid malignancy is squamous cell carcinoma (SCC) however melanoma, glioblastoma and sarcoma have also been described (Epstein, Raber-Drulacher et al. 2009). The most significant risk factors identified in the literature include; the duration of immunosuppressive therapy for cGVHD, particularly the use of azathioprine (AZA), and the severity of cGVHD. Other factors consistently mentioned include the use of TBI, concurrent administration of cyclosporine/ AZA/ corticosteroids, young age at transplantation, and being male (Schubert and Correa 2008). In general, the risk of secondary malignancy is proportional to the survival time post HSCT (Schubert and Correa 2008). A recent international case control study found the median time from HSCT to solid tumour

diagnosis was 7.0 years (range 0.9-22.9 years) with a cumulative incidence of 1.1% at 20 years (Curtis, Metayer et al. 2005). The biologic mechanisms involved in the higher incidence of post-transplant SCC have not been fully elucidated, however, it is postulated that extended periods of tissue destruction and repair seen in cGVHD in combination with prolonged periods of immunosuppression may lead to propagation of oncogenic viruses and may render tumour development more likely (Curtis, Metayer et al. 2005).

Specific to the oral cavity, SCC and salivary gland malignancies (mucoepidermoid carcinoma) have been reported following HSCT (Curtis, Metayer et al. 2005; Demarosi, Lodi et al. 2005). Several authors have reported the development of solid malignancies at sites previously or concurrently affected with GVHD-related inflammatory processes (Abdelsayed, Sumner et al. 2002). Curtis et al reported that 72% of patients diagnosed with SCC following HSCT had cGVHD relative to 52% of patients in the control group. Critically, dysplastic pre-malignant lesions and frank malignancies may potentially be obscured by or may even resemble oral cGVHD and therefore can only be discriminated by biopsy (Schubert and Correa 2008). For this reason, long term follow-up of HSCT patients is essential so that suspicious lesions or frank malignancy can be detected at an early and potentially curable stage. The oral sites most frequently involved include the tongue, buccal mucosa, gingiva and lip in decreasing frequency (Abdelsayed, Sumner et al. 2002). Oral malignancies following HSCT have a tendency for aggressive behaviour and a tendency to recur. Median survival time following the diagnosis of invasive SCC of the oral cavity or the skin has been shown to be 1.7 and 4.1 years respectively (Abdelsayed, Sumner et al. 2002).

1.4.3 Bisphosphonate-related osteonecrosis of the jaw

Reduction in bone mineral density is a common long-term outcome following numerous treatment strategies for malignancy, including HSCT. Conditioning regimens, especially those comprising of irradiation, may initiate abnormalities in endocrine function. These effects may be exacerbated due to long-term corticosteroid therapy, the mainstay of cGVHD management. Of note, 15% of patients diagnosed with cGVHD will still be on immunosuppressive therapy as long as 7 years following transplantation (Stewart, Storer et al. 2004). Patients with a history of HSCT are therefore at risk of developing osteoporosis, fragility fractures and avascular osteonecrosis. Avascular osteonecrosis, death of bone, occurs as a result of impaired blood supply (Bamias, Kastiris et al. 2005) with the most commonly affected site being the femoral head. Risk factors which have been identified for avascular necrosis are the presence of acute and chronic GVHD, the dosage and duration of corticosteroid therapy, and young age with a reported 4 year cumulative incidence rate of 6.1% (Schulte and Beelen 2004).

More recently the attention has been focused on complications associated with the antiresorptive medications prescribed to ameliorate osteoporosis and the related complications, namely, bisphosphonate-related osteonecrosis of the jaws (BRONJ). Clinically, BRONJ is defined as an area of exposed bone in the maxillofacial area that does not heal within 8 weeks after identification by a health care provider, in a patient who was receiving or had been exposed to a bisphosphonate and had not had radiation therapy to the craniofacial region (Khodls, Burr et al. 2007). Bisphosphonates are synthetic analogues of the naturally occurring pyrophosphate which have been used in medicine since the 1970s. Currently, this group of drugs is widely utilised in the

treatment of osteoporosis, for the complications associated with metastatic bone disease and management of primary osteolytic pathology of bone (multiple myeloma, Paget's disease) (Hewitt and Farah 2007). In patients receiving oral bisphosphonate therapy for osteoporosis, the estimated incidence of BRONJ is very low, estimated between 1 in 10 000 and 1 in 1 000 000 patient-treatment years. In patients with malignancy, BRONJ has a significantly higher incidence estimated at 1-10 patients per 100 (Khodls, Burr et al. 2007). This is most likely associated with the higher dosages and more potent agents used for oncological indications combined with various comorbidities such as; concurrent corticosteroid therapy, a history of irradiation and general immunodeficiency.

Bisphosphonates bind and accumulate in bone from which they are slowly released over time as bone is turned over. Bisphosphonates are thought to primarily affect osteoclast function by reducing their resorptive capacity, hindering their recruitment and causing apoptosis (Hewitt and Farah 2007). The more potent, nitrogen-containing agents (pamidronate, zoledronate), have also been suggested to inhibit neoangiogenesis, capillary tube formation and vessel sprouting (Marx, Sawatari et al. 2005). In the HSCT setting bisphosphonate therapy is a critical component in the treatment of: cancer-related hypercalcaemia, primary osteolytic malignancies of bone and for the prevention of osteoporosis in patients on long-term corticosteroid therapy. Hypercalcaemia, if untreated, may lead to confusion, pain and ultimately renal failure and death. Clearly, by ameliorating these complications, bisphosphonate therapy has considerably enhanced the quality of life and life span in these patients (Marx, Sawatari et al. 2005).

BRONJ may arise spontaneously however more commonly arises following invasive dental treatment such as extractions, trauma from ill-fitting dental prostheses or overt local trauma (Zarychanski, Elphee et al. 2006). The most common oral sites involved are the mandibular molar region. Associated infection and pain is not always reported. Wide debate exists in the literature pertaining to the appropriate management of BRONJ and the dental management of patients on bisphosphonate therapy. Due to the only recent discovery of this bisphosphonate-associated adverse effect, long term data is unavailable and recommendations are generally based on expert opinion. Prior to the commencement of bisphosphonate therapy patients should undergo thorough dental evaluation and optimal dental health should be attained (Novince, Ward et al. 2009). Regular dental reviews are essential following the commencement of antiresorptive therapy so that early intervention is possible and invasive procedures, therefore avoided. In the event of the development of BRONJ, there is general consensus that the cessation of bisphosphonate therapy appears unwarranted due to the systemic incorporation and long-term bioavailability of this group of drugs (Bagger, Tanko et al. 2003). Management may include antimicrobial therapy when there is clinical evidence of infection, analgesics and gentle removal of loose bony sequestrae (Miglorati, Casiglia et al. 2005; Khosla, Burr et al. 2007).

1.4.4 Chronic graft-versus-host disease

1.4.4.1 Definition

HSCT may be the therapy of choice for a variety of diseases however it is associated with serious and debilitating complications, the most prevalent and significant being cGVHD. Unfortunately, despite significant advances in transplant techniques and post transplant immunosuppressive therapy, more than half of all allogeneic HSCT recipients develop GVHD, a major cause of morbidity and mortality (Schaffer 2006). Chronic GVHD remains the leading cause of non-malignancy related fatality, post allogeneic HSCT (Ferrara and Reddy 2006) accounting for approximately one-quarter of deaths in long term survivors of HSCT on the background of leukaemia and two-thirds of deaths in HSCT for aplastic anaemia (Ratanatharathorn, Ayash et al. 2001). GVHD is one of the principal impediments for wider applications of HSCT. GVHD was initially described almost half a century ago, in 1960, following the first bone marrow transplantations in humans (Mathe, Bernard et al. 1960). Since this time there have been many advances in our understanding of its pathogenesis and the clinical presentations.

Chronic GVHD is a multi-system long term complication of allogeneic HSCT which was traditionally classified as GVHD which persists or presents beyond the first 100 days post transplant. The use of allogeneic human stem cells allows transplantation enriched with haematopoietic progenitor cells however, there is concomitant transplantation of mature CD4⁺ and CD8⁺ T cells. Beneficial outcomes of T cell transplantation include; rapid promotion of haematopoietic engraftment, quicker

reconstitution of T-cell immunity and the development of the beneficial, potent GVL effect. Unfortunately, both of these donor T-cell subsets also cause GVHD, the broad attack against the host tissues by donor T cells (Shlomchik 2007). cGVHD is characterised by immune dysregulation, immunodeficiency, impaired organ function and decreased survival (Baird and Pavletic 2006).

1.4.4.2 Incidence

The incidence of cGVHD varies from 25% up to 80% in certain populations of allogeneic transplant recipients (Baird and Pavletic 2006). However, reliable incidence estimates are hindered by lack of standardised diagnostic guidelines. Of importance, cGVHD is increasing in prevalence as more patients survive transplantation and more transplantation procedures are performed using peripheral blood stem cell products (PBSCT), on older patients and with unrelated donors (Cutler, Antin et al. 2006). PBSCT is associated with a decreased relapse rate of haematological malignancies and an overall improvement in disease free status however it is also associated with a significant risk of extensive cGVHD. Prior diagnosis of aGVHD is the risk factor most consistently associated with subsequent cGVHD (Ferrara and Reddy 2006). The probability of developing cGVHD is approximately 49% in patients with a history of Grade I aGVHD and increases to 59%- 85% in patients with prior Grade II-IV aGVHD (Ratanatharathorn, Ayash et al. 2001). Other known risk factors include the use of female donors for male recipients, degree of HLA mismatch, TBI and the use of donor lymphocyte infusions (Pavletic S. Z, Smith L.M et al. 2005).

1.4.4.3 Pathophysiology

As discussed earlier, successful transplantation relies on molecular typing to facilitate precise HLA matching between donor and host. If incompatible with the host, HLA antigens elicit both humoral and cell-mediated host responses which may progress to destruction of the graft. Relative to our understanding of aGVHD, the underlying pathophysiology of cGVHD has yet to be completely deciphered, however, incompatible HLA loci in combination with alloreactive T-cells are thought to drive the process. Recent literature has identified a role for regulatory T-cells, B-cells and specific cytokines in the development and persistence of cGVHD (Imanguli, Alevizos et al. 2008; Toubai, Sun et al. 2008).

It has been established that for GVHD to eventuate the graft must contain immunologically competent cells, the recipient and donor must express significantly different tissue antigens to allow for the host tissue to be recognised as foreign and the recipient must be incapable of rejecting the graft (Barnes, Corp et al. 1956). The immunosuppression which ensues following HSCT, given for cGVHD prophylaxis, typically renders the recipient unable to reject the graft. Molecular typing is performed to allow for precise HLA matching between donor and recipient however considerable genetic disparity may still exist via the mHags. Critically, 1-10% of donor T cells recognise non-self HLAs on the host cells in transplantation leading to allogeneic recognition and graft rejection. Identification of these antigens by donor T-cells leads to the generation of an immune response which may result in GVHD. In contrast, recognition of the foreign donor cells by residual host T-cells may lead to graft rejection.

Clearly, a principal mechanism in the pathogenesis of cGVHD is the recognition, by donor T cells, of polymorphism in mHags between donor and recipient, generating an immune response with expansion of alloreactive T lymphocytes (Cutler, Antin et al. 2006). However it seems likely that T-cell mediated recognition of these antigens is not the only pathogenetic mechanism occurring in cGVHD. It is also believed that the dysregulated immune system cannot clearly distinguish autoantigens from alloantigens leading to a form of autoimmune reaction. Chronic GVHD shares several characteristics with systemic autoimmune conditions suggesting that there may be an element of humoral immunity involved in its pathogenesis.

Although alloreactive T cells are thought to be the effector cells in cGVHD the exact role of other specific T cell subsets, various cytokines and the involvement of B cells remains to be discovered (Epstein, Raber-Drulacher et al. 2009). Tumour necrosis factor- α (TNF- α) and interferon-gamma (IFN- γ) are also thought to be associated with the development and modulation of cGVHD (Baird and Pavletic 2006). Increased transcription of these cytokines has been found to independently predict the onset of extensive cGVHD (Ritchie D, Seconi J et al. 2005). The contribution of B-cell activity and autoantibody production in cGVHD still remains unclear. Increased B-cell lymphocyte subsets and increased antibody levels are frequently seen in cGVHD.

1.4.4.4 Diagnosis and classification

Historically cGVHD was defined as arising greater than 100 days post-HSCT either as a progression of aGVHD, following previous resolution of aGVHD or de novo (Sullivan, Weiden et al. 1981). In the past, any clinical manifestation present or arising after this time point was arbitrarily defined as cGVHD even if the clinical picture was indistinguishable from aGVHD. As mentioned earlier, significant advances in HSCT practice have led to profound alterations in the understanding of the natural history and clinical picture of both acute and chronic GVHD. The current consensus favours clinical manifestations, not the time to symptomatic onset, as the determinant of whether the clinical picture is considered acute or chronic GVHD (Filipovich, Weisdorf et al. 2005).

The recent NIH consensus classification of GVHD updated the diagnosis and classification of GVHD by recognising the unique clinical and pathophysiological features of acute versus chronic GVHD and removing the temporal basis of classification (Toubai, Sun et al. 2008). *Diagnostic* signs and symptoms are defined as those which establish the presence of cGVHD without the need for further investigations. Conversely, *distinctive* signs or symptoms refer to the manifestations which are not usually seen in aGVHD but are not considered sufficient to establish a definitive diagnosis of cGVHD. Rare or nonspecific features are classified as *other features* whereas *common* signs and symptoms are those which may be seen in both the acute and chronic spectrum. For the diagnosis of cGVHD the new diagnostic system requires at least one diagnostic manifestation or at least one distinctive manifestation with the supporting evidence via biopsy, laboratory testing or imaging (i.e. radiology). NIH diagnosis cGVHD shown in Table 1.4.4.4 (Filipovich, Weisdorf et al. 2005).

Table 1.4.4.4: Diagnosis of chronic GVHD (NIH diagnostic criteria 2005)

The diagnosis of chronic GVHD requires the following:

1. Distinction from acute GVHD.
2. Presence of at least 1 diagnostic clinical sign of chronic GVHD or presence of at least 1 distinctive manifestation confirmed by pertinent biopsy or other relevant tests.
3. Exclusion of other possible diagnoses.

Scoring of organ manifestations requires assessment of signs, symptoms, laboratory values, and other results.

A clinical scoring system (0-3) is provided for evaluation of the involvement of individual organs and sites. The proposed global assessment of severity (mild, moderate, or severe) is derived by combining organ and site-specific scores.

1.4.4.5 Global scoring

Clinical scoring of individual organ systems is divided into a 4 point scale (0-3) with 0 representing no involvement and 3 severe involvement. The scoring of organ systems allows for monitoring of disease extent and severity in individual organs over time.

Global scoring of cGVHD was originally divided into limited and extensive forms, dependant on the degree of overall clinical disease and the histology of involved tissues (Shulman, Sullivan et al. 1980). Recent revisions have been made to more accurately reflect the overall clinical effect of cGVHD on the patient's functional capacity and the degree of overall organ involvement (Table 1.4.4.5.1). Global scoring of cGVHD is divided into grades of mild, moderate and severe depending on number of organs affected and degree of individual involvement (Table 1.4.4.5.2).

Table 1.4.4.5.1: Organ scoring of cGVHD (FILIPOVICH, WEISDORF ET AL. 2005)

	1	2	3
Skin	<18% of BSA AND no sclerotic features	19 to 50% BSA OR superficial sclerosis	>50% BSA OR deep, “hidebound” sclerosis OR reduced mobility, ulceration
Mouth	Mild signs/symptoms not limiting oral intake	Moderate signs/symptoms with partial limitation of oral intake	Severe signs/symptoms with major limitation oral intake
Eyes	Mild dry eye symptoms (using eyedrops < 3x/d) OR asymptomatic but signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (using eyedrops > 3x/d or punctual plugs), no visual impairment	Severe dry eye symptoms significantly affecting ADL OR unable to work OR loss of vision
GIT	Symptoms without significant weight loss	Symptoms with 5 to 15% weight loss	Symptoms with >15% weight loss, requiring nutritional supplementation OR need for oesophageal dilation
Liver	Bilirubin, AP, AST, or ALT <2x upper normal limit	Bilirubin, AP, AST, or ALT 2-5x upper normal limit OR bilirubin >3 mg/dL	Bilirubin, AP, AST or ALT >5x upper normal limit
Lungs	Mild symptoms (SOB after 1 flight of steps); FEV1 60-79%	Moderate symptoms (SOB after walking on flat ground); FEV1 40-59%	Severe symptoms (SOB at rest or requiring O2); FEV1<39%
Joints/ Fascia	Mild tightness of arms or legs, mildly decreased range of motion	At least 1 of following: ○ Tightness and joint contractures ○ Erythema, decreased motion Mild-moderate limitation in daily activities	Contractures with significantly decreased range of motion and daily activities
Genital tract	Mild signs/symptoms, minimal pain on exam	Moderate signs/symptoms with discomfort on exam	Advanced signs like strictures, ulceration

Table 1.4.4.5.2: Global scoring of cGVHD modified from NIH consensus project

Score	Features
Mild	1-2 organs (excluding lungs) with maximum score of 1
Moderate	≥ 1 site with organ score of 2 OR ≥ 3 sites with score of 1 OR lung score of 1
Severe	Organ score of 3 OR lung score of 2

1.4.4.6 Clinical features

Clinically, cGVHD affects single or multiple target organs and produces a constellation of clinical manifestations (Filipovich, Weisdorf et al. 2005) with features resembling autoimmune and other immunologic disorders such as scleroderma, Sjögren syndrome and bronchiolitis obliterans. Symptoms generally arise within 3 years of allo-HSCT and are often preceded by aGVHD. The skin, conjunctiva, oral cavity, gastrointestinal tract and liver are variably affected in cGVHD. In addition to these more commonly involved systems, many other organ systems may be affected (Sullivan 1986).

1.4.5 Oral chronic graft-versus-host disease

The oral cavity is frequently involved in cGVHD and may, in some instances, be the primary or sole site of involvement (Woo, Lee et al. 1997). The frequency of oral cGVHD has been well documented with 72% to 83% of patients with cGVHD demonstrating oral involvement, making it one of the most common sites affected by cGVHD (Schubert and Correa 2008). In recent studies, the oral cavity was found to be involved in varying degrees relative to stem cell source with oral involvement in 70% of PBSCT recipients and 53% of BMT recipients who had cGVHD (Pavletic S. Z, Smith L.M et al. 2005). These manifestations closely resemble, both clinically and histologically, those of common autoimmune disorders including scleroderma and Sjögrens syndrome and immune-mediated oral lichen planus. Any oral site may be affected and the degree of involvement may be extensive. Oral mucosal lesions can be a source of significant pain, and when extensive, may limit nutritional intake and impede the maintenance of oral hygiene (Imanguli, Alevizos et al. 2008).

1.4.5.1 Mucosal lesions

Diagnostic features, highlighted by the NIH consensus project, for oral cGVHD include lichen planus-like changes which typically present as fixed white striations and hyperkeratotic plaques. Almost any oral site may be involved, however, the buccal mucosa, tongue and labial mucosa are most commonly affected (Treister, Woo et al. 2005) (Figure 1.4.5.1.1). Sclerotic changes in the perioral tissues, in patients with sclerodermatous cutaneous cGVHD, may result in a reduced oral aperture (da Fonseca, Schubert et al. 1998) hindering the provision of dental care and the maintenance of oral hygiene. Specifically, these classic mucosal changes mimic those seen in immune

mediated lichen planus whereas the fibrosis and restricted aperture resembles that seen with scleroderma (Imanguli, Alevizos et al. 2008).



Figure 1.4.5.1.1: Oral lichenoid cGVHD. (A) buccal mucosa (B) labial mucosa (Schaffer 2006)

Other oral lesions frequently associated with cGVHD but based on the NIH criteria, are not diagnostic of oral cGVHD include mucosal atrophy, development of pseudomembranes, ulceration and mucocelles (Woo, Lee et al. 1997; Filipovich, Weisdorf et al. 2005). Clinically, atrophic lesions may demonstrate a vasculitis-like or telangiectatic appearance. Chronic GVHD involving the maxillary anterior gingiva may be characterised by atrophy with loss of stippling (Schubert and Correa 2008).

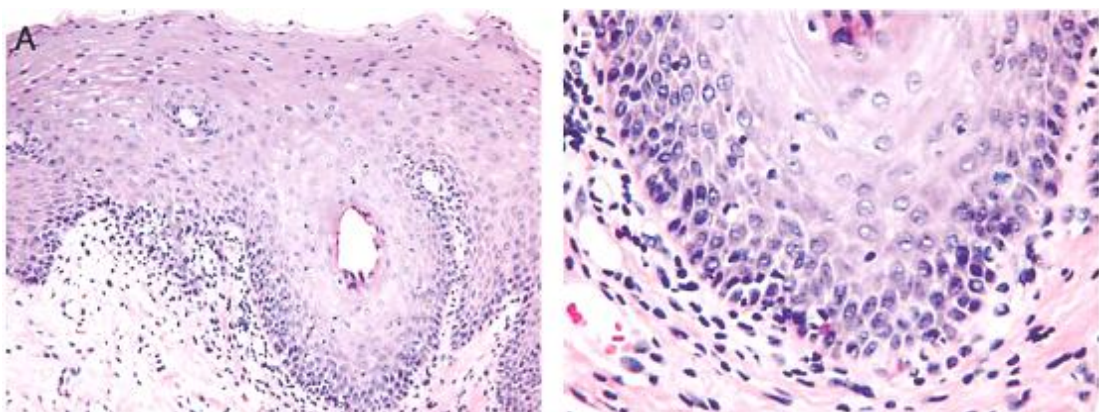


Figure 1.4.5.1.2: ORAL GVHD. A. Oral mucosa. Lymphocytic infiltrate along basal layer (magnified x160). **B.** High-powered: apoptotic changes along the rete ridge (Treister, Woo et al. 2005; Shulman, Kleiner et al. 2006)

The diagnosis of oral cGVHD is most commonly based on clinical findings however specific histopathological findings of both the oral mucosa and glandular tissue may confirm the clinical diagnosis. In 2006 the NIH Consensus Development Project Pathology Working Group (Shulman, Kleiner et al. 2006) provided an update on the interpretation of biopsy results in cGVHD and proposed histological criteria for GVHD. The minimal histological diagnostic criteria for oral mucosal cGVHD have essentially remained unchanged (Figure 1.4.5.1.2); features consist of localised or generalised epithelial changes which comprise of lichenoid interface inflammation, exocytosis, and apoptosis. The connective tissue is characterised by variable amounts of perivascular inflammation and lymphocytic infiltration with occasional subepithelial clefting (Woo, Lee et al. 1997). Histologically there may be little distinction between acute and chronic GVHD so mucosal biopsies must often be correlated with the clinical observations.

Immunohistochemical studies have established that the infiltrate in cGVHD is predominately lymphocytes and macrophages (Imanguli, Alevizos et al. 2008). The ratio of CD8 T cells to CD4 T cells has varied between studies, with some reporting a predominance of CD8 T cells (Soares, Faria et al. 2005) and others a predominance of CD4 T cells (Nakamura, Hiroki et al. 1996).

1.4.5.2 Xerostomia and salivary hypofunction

The effect of cGVHD on salivary gland secretions is well documented. Salivary gland dysfunction which arises in the acute stages following allo-HSCT are predominately attributed to conditioning regimen toxicity, especially in the case of TBI, and can persevere for many months. Late changes are most often ascribed to cGVHD and clinically resemble the features of Sjögren syndrome (Coracin, Pizzigatti Correa et al. 2006). Extensive involvement results in the total destruction of secretory units leading to permanent and profound salivary hypofunction (Schubert and Izutsu 1987). The most prominent risk factor which has been implicated in the aetiopathogenesis of cGVHD salivary gland disease is TBI (Hemidahl, Johnson et al. 1985). The reduction in salivary flow rate has been reported in the range of 55% to 90% in patients with cGVHD with the severity of hypofunction being proportional to the severity of systemic cGVHD (Schubert, Sullivan et al. 1984). Critically, patients report oral dryness as the second most distressing symptom at discharge and at 1 year after HSCT (Larsen, Nordstom et al. 2007). However, it has been reported that often the symptom of xerostomia does not correlate with the signs of salivary hypofunction; where clinical evidence may demonstrate a reduction in salivary flow in patients who do not complain of a dry mouth (Alborghetti M.R, Correa M.E et al. 2005).

Saliva plays a major role in maintaining oral health and oral function. A decrease in the quantity or quality of saliva can have a profound effect on the incidence of dental decay, oral candidosis, the retention of dentures and mucosal friability not to mention the adverse impact on speech, swallowing and mastication. Salivary dysfunction in HSCT patients is often not exclusively due to cGVHD. Confounding factors include

ionizing radiation, prolonged chemotherapy and the polytherapy often required for the management of these patients.

Histopathologically, glandular tissue in cGVHD show similar features to those shown in Sjögren syndrome, except there are fewer lymphocytes which are not organised in foci (Lamey, Lundy et al. 2004). The diagnostic histological features seen in cGVHD involving the salivary glands include: intralobular, periductal lymphocytic infiltration with or without plasma cells and exocytosis of lymphocytes into the intralobular ducts and acini (Figure 1.4.5.3). Individual ductal epithelial cell apoptosis, and destruction of acinar tissues with periductal fibrosis is also often present (Soares, Faria et al. 2005; Shulman, Kleiner et al. 2006). The superficial mucoceles described in the oral cGVHD spectrum are most likely a result of GVHD induced inflammation and damage to the minor salivary gland ducts leading to duct obstruction and finally destruction (Schubert and Correa 2008). The lymphocytic infiltrate primarily contains T lymphocytes, with a slight predominance of CD8 T cells over CD4 T cells (Soares, Faria et al. 2005).

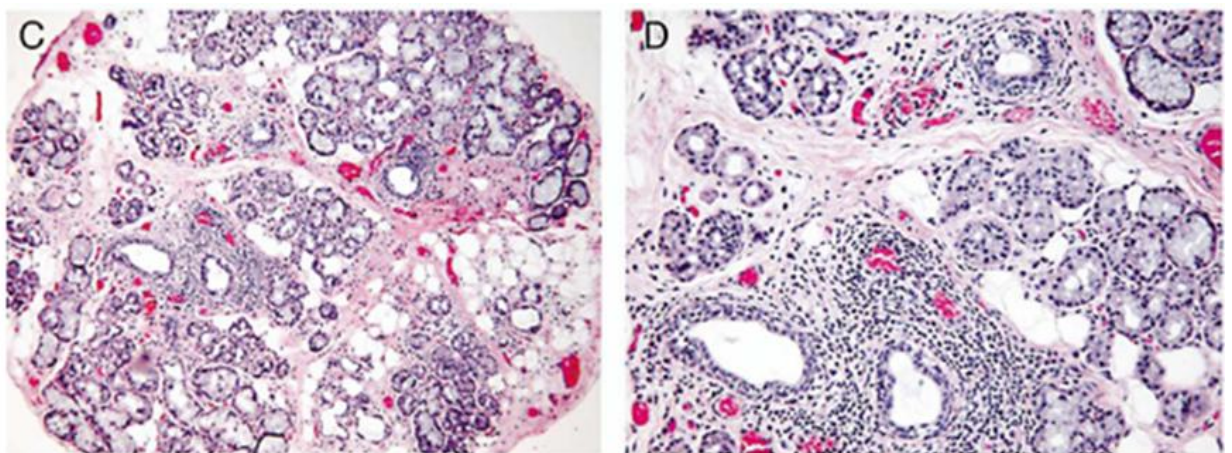


Figure 1.4.5.2: (C) Minor salivary gland. Early lobular change shows focal periductal lymphocytic infiltrates. (D) High-powered: marked lymphocytic infiltration, focal destruction of epithelium (Treister, Woo et al. 2005; Shulman, Kleiner et al. 2006)

1.4.5.3 Dysgeusia

Altered taste sensation (dysgeusia) is a common conditioning regimen-related phenomenon which typically resolves 1 to 2 months following HSCT (Comeau, Epstein et al. 2001). Dysgeusia and/or hypogeusia or ageusia can persist or arise in the later stages following HSCT and is often coupled with the onset of cGVHD. Patients may report a rapid reduction in their sense of taste during periods of cGVHD flare or onset (Epstein, Raber-Drulacher et al. 2009). The epithelial-derived taste receptor cell has been suggested as an immune based target in this setting (Marinone, Rizzoni et al. 1991). Importantly, calcineurin inhibitors commonly used in the management of GVHD (cyclosporine, tacrolimus) can also induce neurological changes which may result in dysgeusia (Schubert and Correa 2008). Patients commonly complain of taste changes which may be described as metallic, salty, sweet, sour or bitter. Furthermore, a reduction in the quantity of saliva seen where cGVHD involves the salivary glands, may further promote an altered taste perception due to the reduced capacity for saliva to act as a solvent.

1.4.6 Other commonly involved organs

1.4.6.1 Cutaneous chronic GVHD

Cutaneous changes seen in the spectrum of changes attributable to cGVHD can be of two main types, namely, lichenoid and sclerodermatous forms (Aractingi and Chosidow 1998).

Lichenoid cGVHD

Lichenoid lesions are traditionally considered the early presentation of cutaneous GVHD however lichenoid lesions can precede, develop simultaneously with, or follow sclerodermatous changes (Andrews, Robertson et al. 1997). Cutaneous lichenoid cGVHD typically involves the periorbital regions, hands, forearms and trunk (Schaffer 2006). Lesions appear as flat-topped, pink to violaceous, scaly papules. Rarely these changes extend to involve the glans penis and foreskin in males causing phimosis whereas in females, the vaginal mucosa may be affected, leading to stenosis (DeLord, Treleaven et al. 1999).

Sclerodermatous cGVHD: Morpheaform

In late stages, an often insidious progression to a sclerotic and even sclerodermatous stage may ensue. Early sclerodermatous lesions are also termed morpheaform lesions (Saurat 1981). Clinically these lesions present as localised patchy areas of firm, leathery and occasionally hyperpigmented plaques which typically involve the lower trunk (Schaffer 2006). With progression, lesions may coalesce and more closely resemble

scleroderma. Lichen sclerosis is the term given to the most superficial manifestation of sclerodermatous GVHD. These lesions appear as hypopigmented plaques with scaling and follicular plugging which typically favour the neck and trunk (Schaffer 2006).

Sclerodermatous cGVHD: Eosinophilic Fasciitis

A rare presentation of cGVHD is eosinophilic fasciitis which represents a deeper form of sclerodermatous cGVHD. The extremities are most commonly affected, with sparing of the hands and feet (Schaffer 2006). The condition is typified by pain and oedema followed by induration with bullae and ulcerations typically developing on the overlying surface (Schaffer, McNiff et al. 2005). The tissue surface may appear rippled and demonstrate a cellulite-like appearance.

Cutaneous cGVHD is often accompanied by dystrophic changes of the nails including vertical ridges, onycholysis and telangiectasia of the nail fold (Aractingi and Chosidow 1998). Skin sclerosis is thought to potentially arise due to excessive tissue repair resulting from immunologic injury by effector lymphocytes (Janin-Mercier, Devergie et al. 1984) which leads to replacement fibrosis and with time, joint contractures and debility (Sullivan, Weiden et al. 1981).

1.4.6.2 Ocular

Approximately 60% of patients with cGVHD develop ocular manifestations (Ratanatharathorn, Ayash et al. 2001). The most common presentations include keratoconjunctivitis sicca, sterile conjunctivitis and uveitis (Franklin, Kenyon et al. 1983). Patients may complain of dry, gritty or painful eyes which may be associated with photophobia and thick lacrimal secretions. It is recommended that a Schirmer's test be performed routinely on all patients in the early stages of cGVHD to facilitate early diagnosis of xerophthalmia. Extensive ocular involvement may lead to serious ocular complications such as corneal epithelial defects and ulceration (Ratanatharathorn, Ayash et al. 2001). Frequent use of topical lubricants and protective eyewear is essential in its management.

1.4.6.3 Gastrointestinal tract

Gastrointestinal involvement is typically not a prominent feature of cGVHD. As seen in aGVHD, dysphagia, nausea, diarrhoea and insidious weight loss may be presenting symptoms. Oesophageal webs, ring-like narrowing along with strictures formation, evident on a barium contrast radiograph or endoscopic visualisation, are diagnostic features of GIT cGVHD (Filipovich, Weisdorf et al. 2005).

1.4.6.4 Liver

Cholestasis most commonly predicts the clinical emergence of liver cGVHD (Ratanatharathorn, Ayash et al. 2001). Due to the existence of numerous alternate triggers for cholestasis, liver biopsy is essential for the confirmation of hepatic cGVHD

(Filipovich, Weisdorf et al. 2005). Liver failure, due to cGVHD alone, is uncommon in long-term survivors (Ratanatharathorn, Ayash et al. 2001).

1.4.6.5 Pulmonary

Bronchiolitis obliterans has been identified as a diagnostic feature of cGVHD involving the pulmonary system (Filipovich, Weisdorf et al. 2005). Clinical features range from a chronic cough to dyspnoea and progressive airflow obstruction. Patients with bronchiolitis obliterans have an exceptionally poor prognosis, and are often non-responsive to therapy with the end point of pulmonary cGVHD being pulmonary failure (Cooke, Krenger et al. 1998). Diagnosis is via pulmonary biopsy or clinical features such as poor pulmonary function or characteristic features on imaging.

Haematopoietic and Immune Systems

Markers of haematopoietic and immune system dysfunction are commonly associated with cGVHD however are not sufficient to support a diagnosis of cGVHD without diagnostic features in other organ systems. Cytopenias are common. Specifically, lymphopenia ($\leq 500/\mu\text{L}$), eosinophilia ($\geq 500/\mu\text{L}$), hypogammaglobulinemia or hypergammaglobulinemia (Filipovich, Weisdorf et al. 2005). A poor prognosis has been attributed to the development of thrombocytopenia at the time of cGVHD diagnosis (Filipovich, Weisdorf et al. 2005). Clinically, the resulting immunodeficiency places the patient at high risk of a wide range of opportunistic infections and often death (Ratanatharathorn, Ayash et al. 2001).

1.5 Management

The recently published NIH Working Group Report on Diagnosis and Staging defined that systemic therapy is indicated for the management of cGVHD when 3 or more organs are involved or, alternatively, when a single organ has a score of 2 or greater (Filipovich, Weisdorf et al. 2005). Other high-risk features such as thrombocytopenia and the underlying reason for transplantation (malignant versus non-malignant) may also be considered when assessing the need for systemic therapy. Early intervention may circumvent progression to severe cGVHD however the implementation of therapy alone increases the likelihood of significant infections and consequent TRM. Standard cGVHD therapy consists of cyclosporine with corticosteroids however only approximately 70% of patients respond to this empirical therapy. Multiple alternatives for salvage therapy are utilised however there is no standard approach, with an average response of 35% (Baird and Pavletic 2006). Critically, standard management strategies leave patients severely immunocompromised, frequently leading to fatal infections and other morbid complications (Pavletic S. Z, Smith L.M et al. 2005).

Therapeutic decisions for the management of oral cGVHD must be cognisant of the patient's global disease status and so often require planning with the treating physician. Pharmacological management of oral cGVHD may comprise of a single agent or may involve combinations of several agents which may be in topical or systemic form. Systemic therapy is often indicated in the treatment of severe oral cGVHD or where isolated oral lesions fail to respond to local measures. Primary treatment goals are to lessen pain, increase quality of life and circumvent further damage to the oral tissues. The effectiveness of various systemic therapies on oral cGVHD has not been

specifically studied with limited data in circulation on the therapeutic outcomes seen in oral cGVHD. There are no specific systemic agents tailored for the management of oral cGVHD. Often the selection of treatment will be determined by the availability of particular agents and formulations, patient acceptance and cost (Schubert and Correa 2008).

1.5.1 Systemic therapy in cGVHD

Systemic immunosuppressive therapy is implemented for extensive cGVHD involving numerous organs or sites (Imanguli, Alevizos et al. 2008). Initial treatment generally entails systemic corticosteroid therapy (e.g. prednisone 1.0mg/kg/day) with or without cyclosporine (Schubert and Correa 2008). Currently there is no standard second line therapy of cGVHD recalcitrant to steroid therapy. Limitations in the prescription of systemic therapy include an amplified risk for opportunistic infections and the prospect of a reduced graft-versus-leukaemic effect (Imanguli, Alevizos et al. 2008).

1.5.1.1 Corticosteroids

Corticosteroids, used therapeutically, are generally synthetic analogues of the glucocorticoid steroid hormones produced by the adrenal glands under the regulatory control of the hypothalamus and anterior pituitary. Synthetic steroid therapy is used as a replacement therapy for endocrine deficiency states and for many non-endocrine conditions; to suppress inflammation, allergy and, of specific relevance to GVHD, to suppress immune responses.

Naturally occurring cortisol and synthetic glucocorticoids are lipophilic and rapidly diffuse into target cells and bind to a cytoplasmic glucocorticoid receptor. This receptor-drug complex enters the nucleus and binds to specific regulatory elements on target DNA molecules which cause the initiation or inhibition of gene transcription (Goldman and Ausiello 2007). Synthetic glucocorticosteroids, rather than mineralocorticoids, are preferred in this setting due to their higher receptor affinity, slower inactivation and little or no salt retaining activity. Several preparations are utilised with different potencies, anti-inflammatory activity and half lives (Table 1.5.1.1). In the setting of GVHD, the major beneficial therapeutic effects of corticosteroid therapy are their immunosuppressive and anti-inflammatory properties.

TABLE 1.5.1.1: Properties of natural and synthetic steroids (Goldman and Ausiello 2007)

Steroid	Glucocorticoid effect	Equivalent dose (mg)	Mineralocorticoid effect	Plasma $\frac{1}{2}$ life (min)	Biological $\frac{1}{2}$life (hr)
Cortisone	0.8	25	2	30	8-12
Hydrocortisone	1	20	2	90	8-12
Prednisone	4	5	1	60	12-36
Prednisolone	4	5	1	200	12-36
Methylpred	5	4	0	180	12-36
Triamcinolone	5	4	0	300	12-36
Betamethasone	20-30	0.6	0	100-300	36-54
Dexamethasone	20-30	0.75	0	100-300	36-54
Aldosterone	0.1		400		
Fludrocortisone	10		400		

The synthetic corticosteroids utilised in the management of GVHD are immunosuppressive via their impact on the activation, production, circulation, function and survival of leukocytes. There is a decrease in the number of monocytes, lymphocytes, eosinophils and basophils. Of particular relevance to GVHD this effect is more pronounced on T cells than B cells, with the activation of apoptosis especially on immature or activated T cells (Goldman and Ausiello 2007). A rise in neutrophil numbers in the peripheral circulation is evident, however trafficking is impaired. Corticosteroids also are potent anti-inflammatory agents due to the inhibition of the arachidonic acid metabolites, with interference with the cyclo-oxygenase 2 enzyme and phospholipase A₂. Outcomes include the generalised reduction in pro-inflammatory cytokines and interleukins (IL-1, IL-2 IL-6, TNF- α) and the propagation of anti-

inflammatory cytokines such as IL-4, IL-10 and IL-13. However, long term therapy with corticosteroids, defined as systemic therapy for over 3 weeks or a dose of prednisolone >10mg/ day, may result in significant suppression of normal adrenal function and is therefore associated with numerous significant adverse effects.

Systemic corticosteroids predispose patients to opportunistic infections. This is further complicated by the anti-inflammatory properties which may mask many of the cardinal signs of infections, such as fever, inflammation and pain. Prolonged corticosteroid therapy is the most common cause of secondary hypoadrenalism, clinically presenting as Cushing's syndrome. Patients may develop a cushingoid facial appearance, which characteristically presents as a reddened (plethora) and swollen, moon face. This is exacerbated by weight gain, fluid retention and fat re-distribution from the extremities to the trunk and face. More significant metabolic complications include compromised glucose metabolism ranging from glucose intolerance to frank diabetes.

Disturbances in bone metabolism are possible, leading to osteoporosis, avascular necrosis and pathological fractures. Prophylactic therapy for prevention of osteoporosis is generally prescribed for all patients on long-term steroid therapy. Corticosteroid therapy may also cause depression and psychosis. Critically, cGVHD has a propensity to require prolonged treatment with only 5% of patients ceasing immunosuppressive therapy at 1 year after diagnosis (Schubert and Correa 2008) which clearly places these patients at risk of serious adverse treatment related outcomes. Steroid-resistant cGVHD is associated with significantly reduced treatment success rates.

1.5.1.2 Cyclosporine

Cyclosporine, a calcineurin inhibitor, functions by suppressing T cell proliferation by binding calcineurin and thereby preventing transcription of genes for IL-2, IL-2 receptor and IFN- γ (Imanguli, Pavletic et al. 2006). Major adverse effects include nephrotoxicity, neurotoxicity and immunosuppression leading to increased incidence of infections, hypertension and impaired glucose tolerance. Specific to the oral cavity, cyclosporine is associated with generalised gingival overgrowth.

1.5.1.3 Tacrolimus

Tacrolimus functions similarly to cyclosporine however it binds to a unique protein-FKBP-12 (Jacobsohn and Vogelsang 2002). This second line agent may be used in place of cyclosporine especially in patients with significant liver cGVHD due to this drugs concentration in this organ (Jacobsohn and Vogelsang 2002). Adverse effects are similar to those reported with cyclosporine, however a higher incidence of nephrotoxicity is seen. Gingival overgrowth is less frequent with tacrolimus use. Critically, the Food and Drug Administration have issued a “Black Box” warning attached to the use of these agents due to the increase risk of solid cancers (SCC and lymphoma) seen following prolonged use.

1.5.1.4 Sirolimus

Sirolimus, a macrolide compound, with a similar but not identical mode of action as tacrolimus, functions by inhibiting T cell co-stimulatory pathways (Kahan 2001).

Mounting evidence supports the use of this agent in steroid-refractory cases with a 63%

response rate observed in recent studies when combined with either tacrolimus or corticosteroids (Couriel, Saliba et al. 2005). The adverse event profile for infections and malignant neoplasms are similar to those reported with calcineurin inhibitors. Adverse reactions specific to sirolimus include renal impairment, thrombotic thrombocytopenic purpura (TTP), hyperlipidemia and cytopenias (Cutler, Antin et al. 2006).

1.5.1.5 Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an immunosuppressive agent commonly utilised for aGVHD prophylaxis. MMF is also the most commonly prescribed agent for the management of cGVHD in steroid-refractory patients. Studies have shown this agent to be well tolerated with a reliable response rate of approximately 75% when used as therapy for refractory cases. MMF is a reversible inhibitor of inosine monophosphate dehydrogenase and is cytostatic for both T and B lymphocytes (Imanguli, Pavletic et al. 2006). Gastrointestinal and haematological toxicity are the most frequent adverse effects and may limit the use of this agent (Imanguli, Pavletic et al. 2006).

1.5.1.6 Extracorporeal photopheresis

A method which is gaining popularity for the management of systemic cGVHD is extracorporeal photopheresis. The patient's mononuclear cells are isolated via apheresis and are then exposed to ultraviolet light A (UVA). The cells are subsequently re-infused into the patient. Although not fully elucidated, the suggested mechanisms of action include apoptosis of alloreactive T lymphocytes, normalisation of CD4/CD8 ratio and induction of regulatory T cell subsets (Fimiani, Di Renzo et al. 2004). Several small

studies have reported effectiveness in cGVHD particularly when the major site of involvement is the skin (Imanguli, Pavletic et al. 2006). Of particular interest, preliminary data has shown reasonable results in the management of oral GVHD (Imanguli, Pavletic et al. 2006) however the prolonged treatment time and the need for intravenous access are significant disadvantages.

1.5.1.7 Hydroxychloroquine

Hydroxychloroquine, an antimalarial agent, is widely used in many autoimmune and inflammatory disorders, most commonly lupus erythematosus and rheumatoid arthritis. Its mode of action is believed to be associated with altered antigen presentation, reduction in TNF- α , IL-2 and IL-6 activity, and lysosomal membrane stabilisation (Imanguli, Pavletic et al. 2006). The data supporting its applications in the management of cGVHD are few however the overall response rate in the oral cavity has been shown to be 38% (Gilman, Chan et al. 2000). The agent is generally well tolerated with nausea, vomiting and diarrhoea being the most significant side effects.

1.5.1.8 Thalidomide

Generally it was thought that thalidomide had limited efficacy in cGVHD, with its major applications being in inflammatory conditions such as Behcet's syndrome, ulcerative colitis and major aphthous ulceration. In recent years there has been a renewed interest in this immunomodulating agent in the treatment spectrum for cGVHD (Imanguli, Pavletic et al. 2006). The precise mode of action of thalidomide is unknown however observable outcomes include decrease in TNF- α activity, adhesion molecule

expression, inflammatory cell chemotaxis and CD4/CD8 ratio (Wood and Proctor 1990). Adverse effects may be considerable with sedation being the most common.

1.5.1.9 Methotrexate

Immunomodulatory and anti-inflammatory properties of this antimetabolite are commonly exploited in the management of rheumatoid arthritis. Methotrexate, a folic acid antagonist, competitively inhibits dihydrofolate reductase and halts regeneration of intermediates needed for the conversion of deoxyuridylic acid to thymidylic acid. As rapidly dividing cells require abundant supplies of deoxythymidylate for the synthesis of DNA, methotrexate selectively prevents the division of these cells.

Combined with cyclosporine, methotrexate is commonly used for the prophylaxis of aGVHD. Recently, several small studies have evaluated the efficacy of methotrexate in treatment of recalcitrant cGVHD. Data suggests that this agent deserves further investigation given its efficacy and favourable toxicity profile (Giaccone, Martin et al. 2005), significant adverse effects being hepatotoxicity and oral mucositis.

1.5.1.10 Other systemic therapies

Emerging systemic agents are increasingly being trialled in recalcitrant cGVHD specifically, monoclonal antibodies, including anti-CD20 (rituximab) with promising results seen. The recombinant anti-TNF- α antibodies infliximab and etanercept along with the IL-2 receptor antibody (daclizumab) have also been tested in patients.

1.5.2 Topical and local therapy

Topical preparations may be the sole therapy in oral cGVHD or may be part of a more complex management schedule. The advantages of topical or local therapies in oral cGVHD include the application of intensive treatments without necessarily increasing systemic immunosuppression and therefore maintaining the desirable GVL effects.

Topical therapy also permits the avoidance of the complications, toxicities and drug interactions often associated with systemic treatments (Schubert and Correa 2008).

These preparations are essentially identical to those utilised in the management of the oral manifestations of autoimmune and immune-mediated disorders such as oral lichen planus and vesiculobullous disorders. As mentioned with systemic therapies used in cGVHD, there is sparse literature on the efficacy of specific local and topical treatments and their usefulness relative to systemic treatments. Critical features needed in a topical or local therapy include substantivity, bioavailability when applied to oral mucosa, acceptable taste and a noninhibitory cost.

1.5.2.1 Corticosteroids

The most commonly used topical agents in the management of oral cGVHD are steroid preparations which may be formulated in a variety of media including rinses, gels and ointments (Schubert and Correa 2008). Typically, if there is extensive oral involvement or with areas difficult to access, such as the soft palate, a mouthrinse is considered the most appropriate vehicle. Where distinct lesions can be visualised and easily accessed, gels, ointments and creams may be effective. The various formulations and agents are listed in Table 1.5.2.1. Transient burning and oral candidosis are the most common

adverse effects of topical steroid therapy. Oral candidosis may lead to an acute flare in sensitivity and oral discomfort. Resolution is usually achieved with topical antifungal agents. When symptomatic oral GVHD impedes in activities of daily living and nutritional intake, topical analgesics, such as xylocaine viscous 2% (15ml swished 30seconds/every 3hrs) may also be helpful.

The available literature shows predictable results with dexamethasone solution (0.1mg/ml used 3-6x/daily). Wolff et al. showed a positive response in 11 out of 16 patients with histological evidence of oral cGVHD treated with dexamethasone mouthwash (0.1 mg/ml dexamethasone, 4x/daily in combination with topical antifungal prophylaxis), with 9 patients showing complete resolution (Wolff, Anders et al. 2004). The authors observed that treatment was successful even in cases of where oral lesions were resistant to systemic steroids. Critically 12 of the 16 patients were receiving topical therapy as part of a multi-modality treatment for systemic cGVHD. Topical budesonide rinse (3mg capsule dissolved in 5ml water, 2-3x/daily) showed an overall success rate of 58% in 12 patients with oral GVHD in a study by Elad et al, however, interpretation of these results is difficult due to the concurrent use of systemic therapies (Elad, Or et al. 2003). The proposed benefits of using budesonide, a highly potent steroid, was its high topical-to-systemic activity unlike other corticosteroids such as beclamethasone and prednisolone (Brogdan and McTravish 1992). This quality is attributed to the low bioavailability when absorbed through mucosal surfaces and therefore associated with minimal risk for systemic side effects.

TABLE 1.5.2.1: Topical and local treatment for oral GVHD (Schubert and Correa 2008)

Formulation	Specific agent	Concentration	Instructions for use
Rinses	Dexamethasone	0.1-0.4 mg/ml solution	5-10ml swish for 3-5min then spit out. Repeat 3-6 x/day
	Budesonide	0.3-0.6mg/ml solution	10ml swished for 15min then spit out. 2-4x/day
	Betamethasone	0.5ml tablet dissolved in 10ml	Held for 3min then spit out. Repeat 3-4x/day
	Prednisolone	3mg/ml	5ml swish for 4-6min then spit out. Repeat 3-6x/day
	Triamcinolone	1% aqueous solution	5ml swish for 4-6min then spit out. Repeat 3-6x/day
Sprays and inhalers	Beclamethasone		1-2 puffs without inhaling. Repeat 2-4x/day
	Fluticasone		As above
	Betamethasone		As above
	Triamcinalone		As above
Gels, creams and ointments	Clobetasol	0.05% cream, ointment, gel or solution	Apply to lesion 2x/day
	Halobetasol	0.05% cream, ointment	Apply to lesion 2x/day
	Fluocinonide	0.05% cream, ointment or gel	Apply to lesion 2x/day
	Betamethasone	0.05-0.1% cream or ointment	Apply to lesion 2x/day
	Triamcinalone	0.1-0.5% cream	Apply to lesion 2x/day

1.5.2.2 Other immunomodulatory agents

Cyclosporine rinse or gel has been described in the literature as a successful treatment in oral lichen planus along with other mucocutaneous disorders (Eisen, Ellis et al. 1990). A number of small studies have explored the use of topical cyclosporine in the management of oral cGVHD showing a response rate between 45%- 75% (Epstein and Reece 1994; Epstein and Truelove 1996). Epstein et al. reported a 45% clinical response in a group of 11 patients with oral cGVHD who were shown to be refractory to topical corticosteroid therapy where the clinical response was measured as a reduction in total area of ulceration and erythema (Epstein and Reece 1994). Side effects are generally mild and consist of transient burning however topical cyclosporine is prohibitively expensive for routine use.

Tacrolimus and pimecrolimus are newer calcineurin inhibitors. In its systemic forms, tacrolimus has been used successfully in the management of cGVHD for many years. Topical tacrolimus is widely used in the treatment of atopic dermatitis and cutaneous cGVHD. More recently, topical tacrolimus ointment (Table 1.5.2.3) has been investigated as a treatment alternative for oral cGVHD. A limited number of studies have been published with preliminary findings suggesting a therapeutic benefit with minimal adverse effects (Eckardt, Starke et al. 2004). However the Food and Drug Administration (FDA) have issued a “Black Box” warning assigned to the use of these newer agents due to the theoretical increased risk of malignancy, specifically SCC and lymphoma, in patients using these agents for cutaneous psoriasis. Thus, the applications of these agents are generally restricted.

Lastly, azathioprine, a purine analogue used extensively for the prevention of transplant rejection and inflammatory dermatological conditions, has been assessed as a topical agent for oral cGVHD. Azathioprine rinse and gel (Table 1.5.2.3) have been assessed in a small study where the patients recruited were known to be previously recalcitrant to both topical corticosteroids and cyclosporine. A 60% response rate over approximately 16 weeks of treatment with topical azathioprine with concurrent systemic therapy was reported. Potential adverse effects identified in systemic therapy with azathioprine includes dose related marrow aplasia along with a suspected risk for second malignancies in the transplant setting (Schubert and Correa 2008). Due to the limited data on its topical use it is unknown if topical azathioprine use is associated with a risk for oral malignancy.

1.5.2.3 Light-based therapies

Exposure to UVA following the oral administration of 8-methoxypsoralen (PUVA) has been described for the treatment of several cutaneous conditions. Essentially, 8-methoxypsoralen diffuses into the cell nucleus, causes cross-linkage of DNA once exposed to UVA, thus leading to cellular apoptosis. It has been suggested that rapidly proliferating cells, such as T lymphocytes and antigen presenting cells, may be particularly sensitive to the effects of PUVA (Yoo, Rook et al. 1996). PUVA is more commonly employed for the management of cutaneous GVHD however several case series have reported a beneficial response with local intraoral PUVA therapy for steroid-refractory oral cGVHD (Vogelsang, Wolff et al. 1996; Wolff, Anders et al. 2004). Vogelsang et al. suggested a potential benefit of intraoral PUVA therapy in a small number of patients (Vogelsang, Wolff et al. 1996). More recently Wolff et al.

reported promising results when seven patients with recalcitrant oral cGVHD were treated with intraoral PUVA therapy using a flexible glass fibre extension. Of note, all but one patient received concurrent systemic immunosuppression. Overall 6 of the 7 patients responded to intraoral PUVA therapy with 4 patients showing complete resolution of active oral cGVHD requiring a median of 36 treatments (range from 11-92) (Wolff, Anders et al. 2004). Patients who responded to therapy remained stable over the 2-3 year review period.

Common adverse effects associated with PUVA therapy include nausea associated with methoxypsoralen administration and phototoxicity. This is generally well managed with antiemetic pre-medication. Potential adverse effects include methoxypsoralen-associated hepatotoxicity, requiring regular monitoring of liver function. Potential cataract development has been reported with UVA exposure following photosensitiser administration necessitating appropriate eye protection following ingestion of methoxypsoralen. The use of topical psoralens has been suggested as a method of avoiding the side effects attributable to systemic administration (Al-Hashimi, Schifter et al. 2007). Of importance a long term side effect identified with cutaneous PUVA is the risk for cutaneous malignancies, particularly SCC and basal cell carcinoma, over an extended time frame of 10–20 years (Lindelof, Sigurgeirsson et al. 1991). At this stage there is insufficient data available to determine the actual risk for oral malignancy (Schubert and Correa 2008).

Of significance, there has been increasing interest in the applications of ultraviolet light B (UVB), instead of UVA, to treat skin conditions. The proposed benefits of UVB (Table 1.5.2.3) include its efficacy without the addition of a photosensitising agent

combined with the fact that an increased malignancy risk has not been reported with UVB (Schubert and Correa 2008). Wackernagel et al. compared the efficacy of PUVA and UVB therapy in patients with diffuse oral lichen planus. Out of the 15 patients treated with PUVA 67% (n=10) showed a complete response following therapy whereas only 31% (n=4) of the 13 patients in the UVB group showed complete resolution. There was no statistically significant difference between the duration of treatment or number of treatments (Wackernagel, Legat et al. 2007). There is limited data on the application of UVB therapy in oral cGVHD. Elad et al. reported two cases of steroid-refractory oral cGVHD which were treated with UVB radiation. Both patients responded early and effectively, displaying only minimal side effects at a relatively low cumulative dose (Elad, Garfunkel et al. 1999). More work is needed on both the use of PUVA and UVB in the treatment of oral cGVHD.

Lastly, the use of low-level laser therapy (LLLT) and CO₂ laser treatment has been described in a small number of case reports (Table 1.5.2.3). The positive features of LLLT, include its regenerative effects and its immunomodulatory potential demonstrated, in vivo, as inhibition of the proliferation of lymphocytes (Dyson, Agaiby et al. 2002). Chor et al. reported marked improvement in oral cGVHD lesions in a patient treated with LLLT (Chor, Mello de Azevedo et al. 2004). Elad et al. investigated the use of CO₂ laser for pain control in oral cGVHD in a recent pilot study. Using a low power emission (1W) treatment was provided without local analgesia; patients reporting an immediate reduction in pain following 82% of sessions (Elad, Or et al. 2003). CO₂ laser emits infrared radiation at a wavelength of 10.6 mm. allowing high water and soft-tissue absorption. The underlying mechanism for the analgesic effect seen with CO₂

laser has not been fully elucidated. Explanations proposed include activation of c-fibers leading to induction of a central somatosensory response (Tran, Inui et al. 2002).

Alternatively it has been postulated that there is a spinal inhibitory effect via peripheral nerve stimulation (Weng and Schouenborg 1996).

TABLE 1.5.2.3: Alternative non-steroidal local therapies (Schubert and Correa 2008)

Formulation	Specific agent	Concentration	Instructions for use
Rinse	Cyclosporine	100mg/ml	5ml swished for several min, spit out. 3 x/day
	Azathioprine	5-10mg/ml	5-10ml swished for 3-5min, spit out. Repeat 2-6 x/day
Gel or ointment	Cyclosporine	0.5mg/ml	Applied to lesions 2 x/day
	Azathioprine	5mg/ml in 3% methylcellulose base	1-2ml applied to lesion 3-4 x/day
	Tacrolimus	0.1% gel	Apply on gauze, hold on lesion 15-20min. 2 x/day
Phototherapy	PUVA	0.3mg/kg psoralen is given orally followed by 0.5-6.0 J/cm ² UVA radiation	UVA dose increased (by 0.5 J/cm ²) as tolerated. 3-4 x/week until resolution
	UVB	0.02mJ/cm ²	Escalating doses by 0.02mJ/cm ² every 4 th use
	Low-level laser therapy	632-660nm, 2-3J/cm ² per use	Treat 2-3 x/week until healed
	Carbon dioxide laser	Defocused 1W	Held 1cm from surface for 2-3 seconds. Surface kept moist

1.5.2.4 Others

Topical thalidomide in an ointment and rinse formulation, are currently undergoing investigation (Phase II trial NIH) for applications in oral cGVHD based on its anti-inflammatory effects, specifically, reduced TNF- α levels which have been proposed to decrease cGVHD-related stomatitis and oral pain (www.clinicaltrials.gov 2009).

1.5.3 Specific therapy for salivary dysfunction associated with oral cGVHD

Replacement of saliva in salivary hypofunction may range from simple measure such as frequent sips of water and water vapourisers to the prescription of sialogogues (Table 1.5.3). Temporary relief may be achieved through the use of oral moisturisers and saliva substitutes. Artificial saliva may have many different preparations with unique qualities and few studies have compared their effectiveness (Table 1.5.3). Visch et al. evaluated the commercially available mucin-based products and compared their efficacy to the carboxymethylcellulose (CMC) preparations. In general, it was found that the mucin-containing products were best tolerated and preferred by patients (Visch, Gravenmade et al. 1986). Most available products are CMC preparations which increase the viscosity but do not reproduce the physical properties of saliva. Critically, artificial saliva products do not replace the many salivary macromolecules critical to the varied functions of saliva (Agha-Hosseini, Mirzaii-Dizhag et al. 2007). Patient acceptance of these product is often hindered by taste, viscosity, lubrication properties, and poor retention in the mouth (Epstein and Stevenson-Moore 1992). Longer lasting results may be seen with the use of sialogogues such as pilocarpine hydrochloride and cevimeline,

which directly stimulate the salivary glands to increase output however, functional glandular tissue is required for successful outcomes of therapy.

Pilocarpine is a natural alkaloid (nitrogen containing compound) which is extracted from the South American shrub of the genus *Pilocarpus*. It is a hygroscopic, odourless, bitter powder which is soluble in water and alcohol. A direct acting muscarinic agonist, Pilocarpine functions to enhance the effects mediated by acetylcholine in the central and peripheral nervous system, a cholinergic effect, as well as producing a mild beta-agonist effect. By activating muscarinic receptors, pilocarpine hydrochloride, can increase the secretions by the exocrine glands including the salivary, lacrimal, sweat, gastric, pancreatic and intestinal glands along with the mucous cells of the respiratory tract. Pilocarpine is most commonly prescribed for the treatment of glaucoma as a locally acting miotic agent of the papillary muscles. Supplementary uses of pilocarpine are seen in the numerous conditions associated with salivary hypofunction, namely, Sjögrens syndrome and more recently salivary hypofunction in cGVHD (Table 1.5.3).

Along with the desired effect of causing an increase in salivation, pilocarpine hydrochloride may also cause some undesirable effects. Common adverse effects include urinary urgency, due to the relaxation of the sphincter of the bladder, along with an increase in perspiration and lacrimation. More significant adverse effects include an increase in airway resistance and secretions due to a rise in bronchial smooth muscle tone along with bradycardia and postural hypotension, due to the co-stimulation of the

muscarinic receptors of the heart. Pilocarpine should therefore be avoided in patients with significant pulmonary or gastrointestinal cGVHD.

Several studies have shown promising results of pilocarpine therapy in patients treated with head and neck radiotherapy and recently a number of small studies have looked at outcomes of therapy in patients with salivary cGVHD. Agha-Hosseini et al. reported a statistically significant difference in salivary flow rate 1 hour following administration of pilocarpine hydrochloride (5mg oral pilocarpine, Salagen™). There was no difference in salivary flow rate between the study group and control when further time had elapsed (7 days after completion) (Agha-Hosseini, Mirzaii-Dizhag et al. 2007). Nager et al. reported positive and enduring increases in both subjective and objective parameters of salivary flow following therapy with pilocarpine hydrochloride (30mg/day). Of interest, when treatment was ceased, there was a rapid reduction of salivary flow to baseline levels however when medication was reinstated, a rapid, profound increase was seen. The authors concluded that for the positive effects to be sustained continuous administration of pilocarpine is necessary (Nagler and Nagler 1999). Cevimeline (Evoxac™) is another parasympathomimetic agent which has been applied in the management of salivary hypofunction in cGVHD. Cevimeline is associated with a longer duration of action (Fox 2004) however is not available in Australia at this stage.

Table 1.5.3: General dental care and therapies for xerostomia (adapted from (Schubert and Correa 2008))

	Composition	Examples
Saliva substitutes	1. Aqueous ion solutions	Oralube® (Orion) spray - CMC base with electrolytes - pH 5-7 Aquae® (Hamilton) spray - pH 5.5 - ≤4m on PBS for palliative care patients Oralbalance® (Laclede Inc) gel - CMC base with enzymes lactoperoxidase, lysozyme, lactoferrin - pH 6.5
	2. Aqueous ion + carboxymethylcellulose	
	3. Mucin-containing solutions	
	4. Glycoprotein-containing	
	5. Enzyme containing gels	
Salivary stimulants	Non-pharmacological agents <ul style="list-style-type: none"> ○ Sucking on hard sweets (sugar free) ○ Bitter or acidic substances (discouraged in dentate patients, may cause erosion) 	Biotene Dry Mouth Gum Extra sugarfree gum
	Pharmacological <ul style="list-style-type: none"> ○ Pilocarpine hydrochloride: 5mg/5ml, 5ml QID po. Increase by 2.5mg/5ml if tolerated ○ Cevimeline: not available in Australia 	
Decay prevention	Fluoridated toothpaste <ul style="list-style-type: none"> ○ Avoid foaming agents e.g. sodium lauryl sulfate (SLS), may irritate mucosa ○ Strong flavourings (mint, cinnamon) may irritate ○ Avoid acidulated phosphate preparations as must etch tooth surface to be effective 	Biotene Dry Mouth Toothpaste (SLS free) NeutraFluor 5000 Plus (Colgate) 1.1% w/w Sodium Fluoride Gel-Kam (Colgate) gel with 0.4% stannous fluoride (may cause staining) PreviDent (Colgate) gel. 1.1% Sodium Fluoride (neutral pH) Savacol® mouthwash (Colgate) 0.2% with 12% alcohol Plaqacide® mouthwash (Oral B) 0.2% Curasept® mouthwash (Curaden) 0.05%, 0.12%, 0.2% Curasept® (Curaden) gel 0.2%
	Chlorhexidine mouthwash or gel <ul style="list-style-type: none"> ○ Cationic bisguanide ○ Broad spectrum antimicrobial with good adsorption onto mucous membranes ○ Effective against aciduric oral bacteria i.e. mutans streptococcus + lactobacilli (Hugo 1964) (Hugo and Longworth 1964) 	
General oral and dental care	Avoidance of <ul style="list-style-type: none"> ○ Known irritants such as spicy, acidic, hot food or carbonated beverages ○ Alcohol containing mouthwashes due to the associated desiccation of oral tissues ○ Xerogenic medications such as tricyclic antidepressants etc ○ High sugar/ acid diets which will potentiate rampant decay and erosion of enamel Patients are encouraged to maintain immaculate oral hygiene with regular dental reviews every 3-6months so early intervention and preventative measures may be implemented.	

1.6 Prognosis

Chronic GVHD is associated with a reduced quality of life (Sutherland, Fyles et al. 1997), impaired functional status (Duell, van Lint et al. 1997), the need for prolonged immunosuppressive therapy and reduced survival (Lee, Klein et al. 2002). This is the chief cause of late TRM following allogeneic HSCT with a 5-year survival rate in patients with cGVHD as low as 40% in certain populations (Baird and Pavletic 2006). Chronic GVHD accounts for roughly one-quarter of deaths in long term HSCT survivors for leukaemia and two-thirds of deaths in aplastic anaemia (Ratanatharathorn, Ayash et al. 2001). Even in long term survivors with cGVHD there is significant morbidity and reduction in quality of life. Despite these unfavourable outcomes, the presence of cGVHD is linked with fewer leukaemic relapses. Lee et al. reported a low relapse rate of 8-9% following the onset of cGVHD (Lee, Klein et al. 2002) which is thought to reflect the GVL effect. Clearly, the impact of cGVHD on survival depends on the balance between its adverse and desirable effects.

Numerous factors which are associated with a higher risk of TRM in cGVHD patients have been identified; these include the presence of cGVHD in multiple organs, a reduced clinical performance score, thrombocytopenia at the time of cGVHD diagnosis and the progressive development of cGVHD on the background of pre-existing aGVHD (Filipovich, Weisdorf et al. 2005). Lee et al. also reported that TRM increased with increasing global severity of cGVHD with a relative risk of 0.6 in mild cGVHD relative to 6.3 in severe cGVHD (Lee, Klein et al. 2002). Thus, it is clear that although some degree of cGVHD offers a protective element via the GVL effect, when severe, cGVHD is the primary cause for non-malignant mortality following HSCT.

Chapter 2: AIMS

The aims of this treatise are to:

1. Assess the extent and range of dental/oral long-term complications following allogeneic human stem cell transplantation
2. To explore factors associated with the dental/oral complications of allogeneic human stem cell transplantation.

Chapter 3: MATERIALS AND METHODS

3.1 Study design and inclusion criteria

All surviving patients, who had received an allogeneic HSCT (allo-HSCT) in the Westmead Hospital Bone Marrow Transplant Unit (BMTU) during the period of January 2003 to July 2008, and were at least 100 days post-transplant, were sent an invitation to participate in this research. Two hundred and sixty nine patients underwent allo-HSCT within the designated time period. Of those, one hundred and thirty nine patients were still living at the commencement of this study. These 139 patients made up the potential study population and were invited to participate.

The initial contact letter described the study aims and advised that they would be approached at their next clinical appointment with more information and invited to participate in this study. Participation was entirely voluntary. The sample included only those candidates who were at least 100 days (3 months) post allo-HSCT in an attempt to examine only the patients who may present with chronic, not acute complications, of transplantation.

3.2 Transplant Database Questionnaire

A standardised Transplant Database Questionnaire was completed for each patient who consented to participate. Information obtained from the medical file was divided into

1. Background information pertaining to diagnosis and details pertaining to the HSCT
2. Acute complications associated with HSCT
3. Chronic complications associated with HSCT

Demographics collected on each patient included; the original haematological diagnosis which led to HSCT, the number of years since HSCT and details of the transplant. This included the stem cell source, the nature of the donor (MUD or sibling donor), the conditioning therapy used, the use of TBI and the GVHD prophylaxis regimen. The second section of this Questionnaire focused on any early complications of HSCT, particularly mucositis and GVHD. The grade (severity) of mucositis was based on the World Health Organisation (WHO 1979) terminology criteria for common adverse events (Table 3.2). Details of aGVHD were gathered from the medical records, namely, the organs involved, the grade (Glucksberg 1994, Table 4) and management schedule.

Table 3.2: The World Health Organisation mucositis grading criteria

Grade	Clinical features
0	-
1	Soreness/erythema
2	Erythema, ulcers but able to eat solids
3	Ulcers but requires liquid diet
4	Oral alimentation not possible

Form 1: TRANSPLANT DATABASE QUESTIONNAIRE

Patient details:

Participant's name _____ Male / Female

Date of birth _____ MRN

Haematological transplantation:

Initial diagnosis

Date of diagnosis

Type of transplant Sibling Unrelated donor

Stem cell source Bone marrow Peripheral blood Umbilical

Conditioning Reduced intensity Myeloablative

TBI Yes No

Chemotherapy _____

Mucositis Yes No

Grade I II III IV

Acute GVHD Yes No

Time of onset Day + _____

Organs involved Skin Liver Gut

Disease severity I II III IV

Management Steroids Other

SYDNEY WEST
Area Health Service

Form 1: TRANSPLANT DATABASE QUESTIONNAIRE

Chronic GVHD: Yes No

Time of onset _____

Grade _____

Organs involved:

Dermatological	Yes	No	Clinical Dx	Histology
Ocular	Yes	No	Clinical Dx	Histology
Hepatic	Yes	No	Clinical Dx	Histology
Haematopoietic	Yes	No	Clinical Dx	Histology
Musculoskeletal	Yes	No	Clinical Dx	Histology
Gastrointestinal	Yes	No	Clinical Dx	Histology
Oral	Yes	No	Clinical Dx	Histology

3.3 Participant Questionnaire

Patients who agreed to participate in this study were seen in the Oral Medicine Clinic at the Westmead Centre for Oral Health, Westmead Hospital. On the day of examination an assessment was made using the Participant Questionnaire (Form 2) and the Oral Examination Form (Form 3). The Participant Questionnaire was completed by the researcher and detailed the responses to a series of questions about the experience of transplantation. Questions centred on

1. Assessment of subjects perceived oral complications associated with HSCT
2. Identification of potential confounding factors such as a previous diagnosis of Scleroderma or radiotherapy to the head and neck not associated with TBI
3. Questions pertaining to pre-transplant dental assessment and the current regularity of dental reviews
4. Each patient's assessment of the adequacy of information, pre-transplant, relating to potential oral complications.

The main purpose of the Participant Questionnaire was to obtain qualitative data on the severity and spectrum of subject's oral complications following HSCT. Specific questions explored alterations in the patient's oral aperture, taste, salivary function, decay rate and the overall impact these adverse effects had on their quality of life.

The questionnaire also examined transplant recipient's views regarding the adequacy of pre-transplant education about the long-term oral complications of HSCT, including their view on the optimal time for the delivery of education about oral health. This data was hoped to allow for future adjustment of educational methods to align with overall patient need.

Form 2: PARTICIPANT QUESTIONNAIRE

Patient details:

Participant's name

Medical record number

Limited medical history:

Current medications related to transplant _____

Other conditions:

Sjögrens syndrome	Yes	No
Systemic sclerosis	Yes	No
Oral lichen planus	Yes	No
Radiotherapy to the head and neck	Yes	No

Chronic GVHD Yes No

Subjective oral complications:

Xerostomia	None	Mild	Moderate	Severe
Reduction in oral aperture	None	Mild	Moderate	Severe
Alteration in taste	None	Mild	Moderate	Severe
Impact in quality of life	None	Mild	Moderate	Severe
Accelerated decay	None	Mild	Moderate	Severe

Form 2: PARTICIPANT QUESTIONNAIRE

Pre-transplant:

Did you have a dental assessment pre-transplant Yes No

How regularly were you seeing a dentist prior to transplant

Current oral management strategies:

Current regularity of dental reviews 3m 6m 12m >12m

If not, why are you not seeing a dentist regularly?

Cost Inconvenience Unaware of need Afraid

Where you aware of possible oral complications after transplant? Yes No

How was the information attained?

Transplant doctor Nurse Dentist Patient education day

BMT education book Other patient Friends/Family

Was this information useful?

Was the information sufficient?

Would you prefer the information relating to long-term oral complications at

Education day Pre-transplant Outpatient review If need arises

3.4 Oral Examination Questionnaire

Following the completion of the Participant Questionnaire each subject underwent a clinical examination which focused on the assessment of the oral and peri-oral tissues. This examination screened each participant for the spectrum of oral complications possible after allogeneic HSCT through assessment of;

1. Maximal unassisted oral aperture (interincisal distance)
2. Salivary hypofunction
3. The presence and location of mucosal pathology, including mucosal GVHD
4. Past diagnosis of an oral, peri-oral malignant or pre-malignant lesion

Maximal oral aperture was measured in millimetres from the incisal edge of the most vertically aligned maxillary central incisor to the labioincisial edge of the opposing mandibular incisor. The degree of incisal overlap was then added to the documented maximal opening to get the final recording. The measurements taken allowed each subject to be categorised relative to their degree of opening. Normal oral aperture was taken as any measurement higher than, and inclusive of, 50 millimetres (mm). The literature shows that the mean maximal unassisted opening falls in the range of 50mm to 60mm in normal, healthy individuals (Posselt 1962; Dworkin and LeResche 1992). Subjects were placed into one of five categories (normal, mild, moderate, severe) according to the severity of the reduction in unassisted maximal opening (Naylor, Douglass et al. 1984) (Table 3.4.1).

Table 3.4.1: Classification of the maximal unassisted oral aperture

Degree of reduction in oral aperture	Interincisal measurement (millimetres)
Normal	51 – 60
Mild	50 - 41
Moderate	40 - 31
Severe	< 30

Salivary hypofunction was established using a combination of visual inspection and the Saliva Check Buffer kit (GC Corporation). Resting, pooled salivary consistency was visually assessed by the examiner and subjects were placed in one of three groups. Salivary consistency was recorded as frothy/bubbly or sticky/frothy which represented saliva of increasing viscosity. Watery/clear saliva was indicative of saliva of normal viscosity.

The quantity of stimulated saliva was assessed using the Saliva Check Buffer kit. The subject was asked to chew on a piece of unflavoured wax to stimulate salivary flow. After 30 seconds had elapsed the subject was asked to expectorate any accumulated saliva. The subject then continued to chew for a further 5 minutes, expectorating all saliva, at regular intervals, into the measuring cup provided. The volume measured was the liquid component only, not any supervening frothy material, and this was read at the base of the meniscus. The final amount of saliva produced was measured from the millilitre markings on the measuring cup provided in the kit. Based on the normal stimulated salivary flow rate of 1ml/min – 1.6ml/min, subjects were grouped according to the total amount of stimulated saliva produced over this 5 minute period (Table 3.4.2).

Table 3.4.2: Quantity of stimulated saliva over a 5 minute period

Quantity (millilitres)	Category
< 3.5 ml	Very low
3.5 – 5.0	Low
> 5.0	Normal

The sample collected was then utilised to analyse the buffering capacity of each subject's stimulated saliva. Using a disposable pipette, saliva was drawn up and then dispensed onto the Buffer test strip (Figure 3.4.1). The Buffer test strip was made up of three identical test squares with one drop of saliva placed on each of the three test squares. Results were recorded after a two minute period. The colour of each of the three test squares ranged from green to blue and through to red. Colour readings were calibrated against the colour table provided. Saliva with a normal buffering capacity will turn each test square green, intermediate results will appear blue and poor buffering is represented via a red colour. Each of the three test squares are allocated a number from zero to 4 depending on the resulting colour (Table 3.4.3.1) creating a 12 point scale.



Figure 3.4.1: Instruments to test salivary buffering capacity

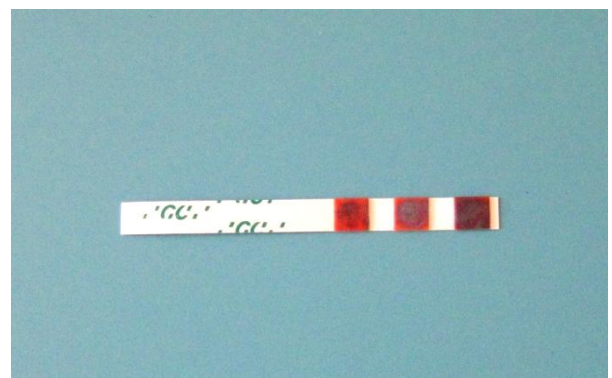


Figure 3.4.2: Buffer test strip after 2 minutes

Table 3.4.3: Conversion table of Buffer test strip

Colour of each square at 2minutes	Points
Green	4
Green / Blue	3
Blue	2
Blue / Red	1
Red	0

Conversion values for each square were summated, the final value placing each subject into one of three categories for salivary buffering capacity (Table 3.4.4).

Table 3.4.4: Buffering capacity of the collected stimulated saliva

Combined total	Buffering capacity of collected saliva
0 - 5	Very low
6 - 9	Low
10 - 12	Normal / High

Finally, peri-oral and intra-oral tissues were visually assessed using a dental mirror and gauze. Changes such as lichenoid lesions, sclerodermatous change and lesions suspicious for pre-malignancy/malignancy were described and recorded diagrammatically. Subjects were questioned regarding any past diagnosis of oral, peri-oral malignant or pre-malignant lesions. Statistical analysis was carried out using the Pearson Chi-Square test and Fisher's Exact Test where appropriate.

Ethics clearance was received by the NSW Health Human Research Ethics Committee – Sydney West Area Health Service, Westmead Campus.

Form 3: ORAL EXAMINATION

Patient details:

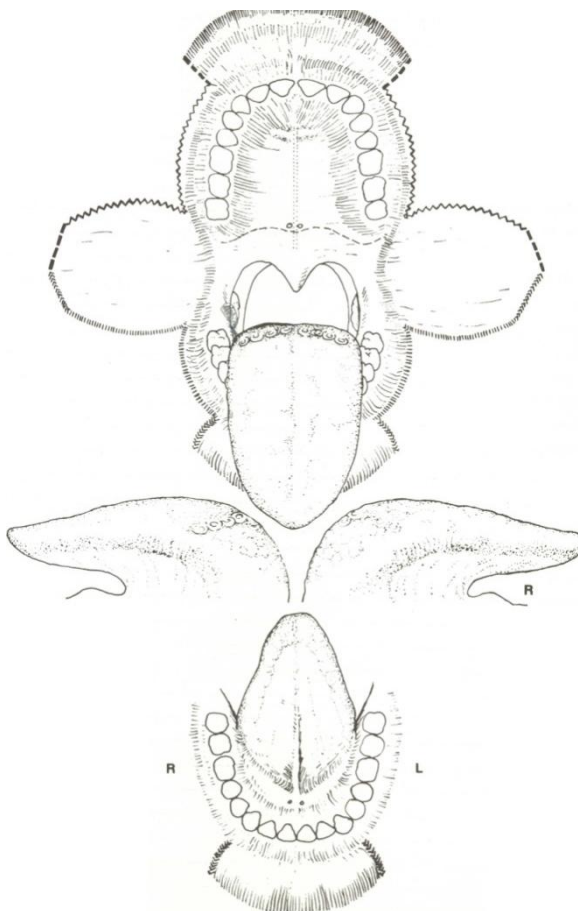
Participant's name

Medical record number

Oral examination:

Maximal oral aperture (mm)

Resting saliva consistency	Sticky, frothy	Frothy, bubbly	Watery
Stimulated saliva over 5minutes (ml)	<3.5	3.5 - 5	>5ml
Buffering capacity	0 - 5	6 - 9	10 - 12



Lichenoid lesions	Yes	No
Sclerodermatous change	Yes	No
Candidosis	Yes	No

Notes:

Previous diagnosis of oral malignancy

Yes No

Date of diagnosis:

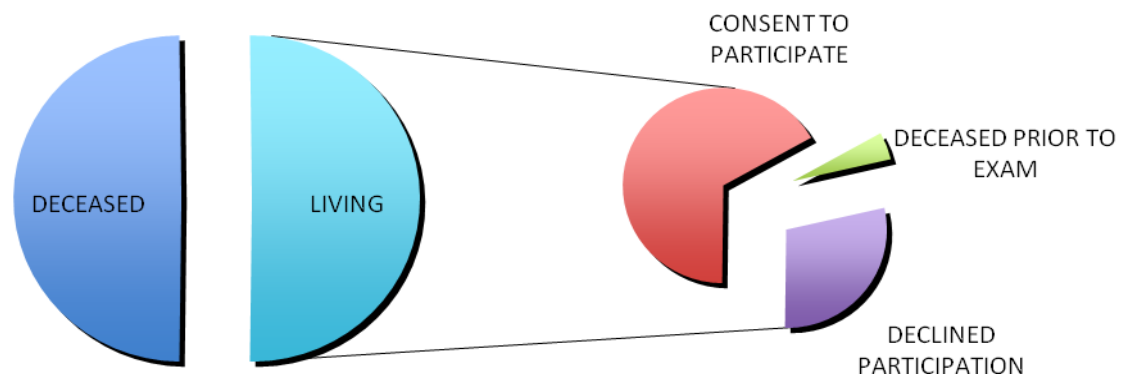
Site:

Chapter 4: RESULTS

4.1 Patient sample

Two hundred and sixty nine patients underwent allo-HSCT during the period of January 2003 to July 2008, and were at least 100 days post-transplant. Of those, one hundred and thirty nine patients were still living at the commencement of this study. These 139 patients made up the potential study population and were invited to participate. A further three subjects deceased prior to examination and thirty eight subjects declined participation primarily due to distance between their residence and the Westmead Hospital BMTU. The study population was therefore comprised of eighty eight subjects (Figure 4.1).

Figure 4.1: Study population characteristics



Subjects ranged in age from 19 to 65 years however the age at transplantation was most commonly between 50 and 65 years (39.8%), followed by 30 to 39 years (23.9%). Of the 88 subjects seen the majority were male (n=65). There was a range of underlying diseases which lead to the application of HSCT (Table 4.1) with over half of the subjects receiving HSCT on the background of AML (55.7%). Clearly, as a direct result

of the introduction of the targeted therapy, imatinib mesylate (Glivec™) in 2000 for the treatment of CML, there has been a significant decline in the number of allogeneic HSCT for this condition. The most recent ABMTRR and EBMT data is based on patient activity in 2004 and a small but considerable proportion of patients still received allogeneic transplantation for the treatment of CML. This practice is now not current clinical practice, reflected in this study population where no subjects had received allogeneic transplantation for CML.

Table 4.1: Initial diagnosis prior to HSCT

Diagnosis	Number of subjects	Percentage
Acute myeloid leukaemia	49	55.7
Acute lymphoblastic leukaemia	11	12.5
B-cell non-Hodgkin's lymphoma	13	14.8
Myeloproliferative disorder	9	10.2
Other	6	6.8
Total	88	100

Haematopoietic stem cells were harvested from the peripheral blood, bone marrow or umbilical cord tissue. The vast majority of subjects received PBSCT (79.5%), followed by BMT (15.9%), with only a small group receiving UC transplants (4.5%). The greater part of this population had sibling donors (71.6%) with a minority requiring a MUD (28.4%). More than half of all transplants were myeloablative (59.1%) with TBI-based conditioning regimens being the most frequently applied (36.4%) followed by the combination of Busulphan/Cyclophosphamide (23.9%). received a combination of cyclosporine and methotrexate as GVHD prophylaxis (79.8%).

4.2 Acute complications

4.2.1 Mucositis

Mucositis was frequently encountered in this population. Of the subjects where this data was available (n=69), 69.6% experienced some degree of mucositis. The severity was evenly distributed between those which were recorded as Grade 1-2 (30.4%) and those which were Grade 3-4 (32.1%). Mucositis was more common in participants with myeloablative transplants ($P < 0.001$) and a conditioning regimen involving TBI ($P = 0.006$) or busulphan combined with cyclophosphamide ($P = 0.004$). The source of stem cells (PBSCT, BMT or UC) did not statistically predict the development of mucositis.

4.2.2 Acute graft-versus-host disease

Acute GVHD was a regular complication following transplantation with 52 subjects (59%) being diagnosed with some degree of aGVHD. Of note, this information was not available for 23 subjects. The risk factors identified for the development of aGVHD included the use of myeloablative conditioning regimens (including TBI) where 68.6% of subjects who received myeloablative conditioning developed aGVHD, relative to 44.4% with RIC ($P = 0.024$). The use of a sibling or MUD was also significantly associated with the development of aGVHD ($P = 0.01$). Fifty percent of subjects who had sibling donors developed aGVHD relative to 80% with MUD transplants. A history of mucositis was also an independent variable, where 70.2% of subjects who had mucositis went on to develop aGVHD relative to 42.9% of subjects who did not have mucositis ($P = 0.032$). When recorded, the onset of aGVHD ranged from 14 to 126 days after HSCT with 86.5% of subjects with aGVHD being diagnosed between 30-100 days after transplantation.

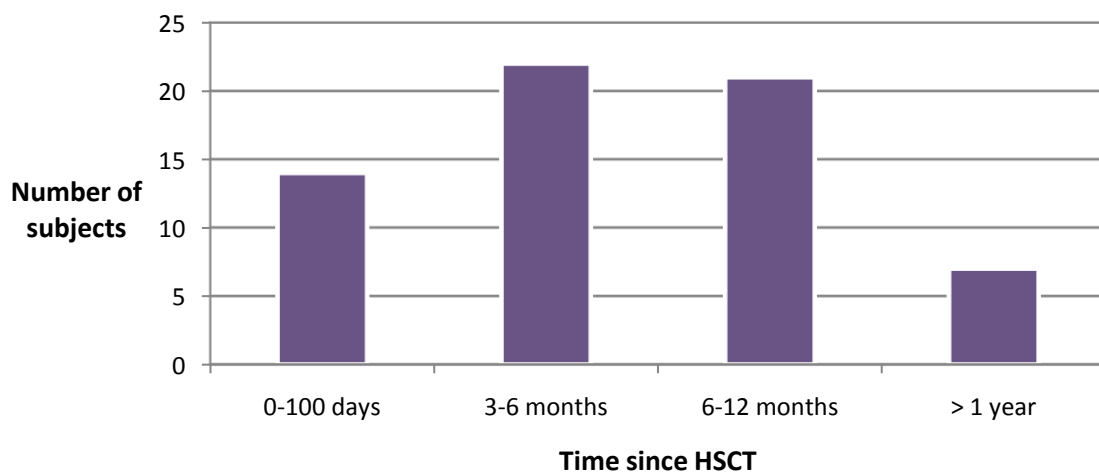
The majority of subjects had aGVHD of Grade 1-2 severity with only 6.2% of subjects experiencing Grade 3-4. TBI was the only features of transplantation that showed a significant association with the grade of aGVHD ($P=0.02$). Of note, age at transplantation, sex or stem cell source did not predict aGVHD or its severity. Clinically, the skin was most commonly affected in isolation (29%); multiple organ involvement was seen in 23% of subjects.

4.3 Chronic complications

4.3.1 Incidence and risk factors

Chronic GVHD arose in 64 of the 88 subjects (72.7%) after transplantation. Data pertaining to the grade of cGVHD was available for only 44 of these subjects with 70.5% of these subjects having limited disease as opposed to 29.5% who had extensive involvement. The onset of cGVHD was primarily clustered over the first 3-12 months after transplantation (Chart 4.3.1) however a few subjects did develop cGVHD as long as 30 months after transplantation. Subjects were more likely to develop cGVHD if they had a pre-existing diagnosis of aGVHD ($P=0.001$) with 86.3% of subjects with aGVHD going on to develop cGVHD in comparison to only 52.8% of subjects who did not have aGVHD. Of interest, no other factors were found to predict the onset of cGVHD including age at transplantation, sex, transplant type, donor type, myeloablative conditioning or a history of mucositis.

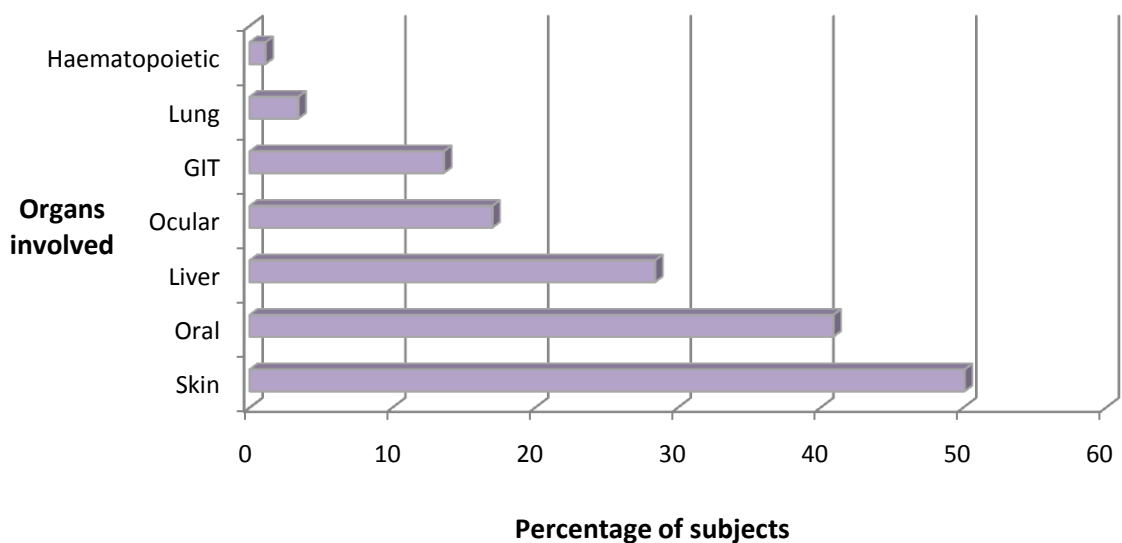
Chart 4.3.1: Onset of chronic GVHD



4.3.2 Clinical presentation

In those with chronic GVHD the most commonly involved organs (Chart 4.3.2.1) included the skin (70.3%), mouth (56.3%) and liver (39%). Diagnosis of both cutaneous and oral cGVHD was primarily based on clinical features (63.6% and 94.4% respectively) unlike gastrointestinal chronic GVHD, where histology was the basis of diagnosis in 75% of cases.

Chart 4.3.2.1: Frequency of individual organ involvement in chronic GVHD



The clinical spectrum of oral cGVHD was inclusive of mucosal cGVHD, salivary hypofunction and reduced oral aperture. The risk factors identified for the development of oral GVHD included the presence of cutaneous cGVHD ($P < 0.001$). Subjects who had cutaneous cGVHD were much more likely to also have oral cGVHD (60%) where only 20.9% of subjects developed oral cGVHD without cutaneous involvement. Similar patterns were seen with hepatic and ocular GVHD (Chart 4.3.2.2). Oral cGVHD was also more likely to develop in subjects with a history of aGVHD ($P = 0.015$). However the development of oral cGVHD was not associated with a history of mucositis ($P = 0.675$).

4.3.3 Results for the subjective oral complications after transplantation

Subjects were investigated for both subjective and objective features of long-term oral complications after HSCT. Participants made their own assessment of any oral changes they ascribed to the transplant process. The specific subjective variables analysed were; a sensation of dry mouth (xerostomia), reduced oral opening, increased decay, reduced taste and the impact these factors had on each subject's quality of life (Chart 4.3.3.1).

4.3.3.1 Xerostomia

Forty four percent of subjects (n=39) reported oral dryness with 43.6% (n=17) of these subjects grading their degree of xerostomia as moderate to severe. The next most commonly reported complaint a reduction in taste (20.4%), closely followed by the subject's perception of accelerated decay rate (19.3%) (Chart 4.3.3.1). Interestingly, only 13 subject's (14.7%) thought these factors impacted on their quality of life.

4.3.3.2 Dysgeusia

Analysis of the data showed that the sensation of persistent reduction in taste was significantly associated with the time since transplantation, with progressively less subjects complaining of moderate to severe dysgeusia as this time increased (P=0.031). Dysgeusia was also related with transplant type, PBSCT showed a significantly higher proportion of subjects with a complaint of reduced taste (P=0.045). Of note, a history of TBI and myeloablative therapy was not associated with reduced taste. Both xerostomia and dysgeusia were significantly associated with the frequency of dental reviews after HSCT with the number of subjects with xerostomia increasing as dental reviews

became less frequent ($P=0.029$). Interestingly, the number of subjects who reported an accelerated decay rate post-HSCT was not associated with TBI, myeloablative therapy, time since HSCT or whether they had a dental examination prior to transplantation.

Chart 4.3.3.1: Percentage of subjects with subjective oral complications after HSCT

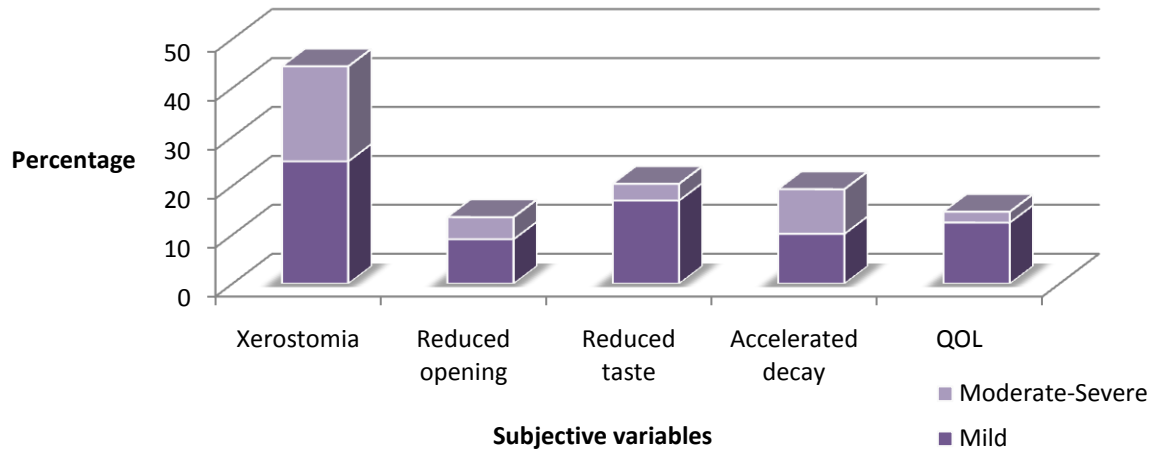
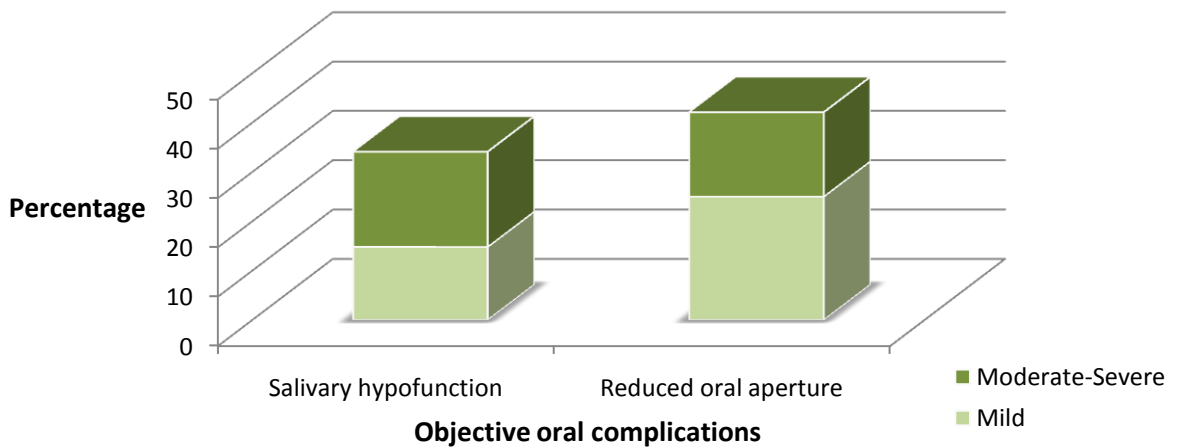


Chart 4.3.4: Percentage of subjects with objective oral complications after HSCT



4.3.4 Results for the objective complications after transplantation

The objective parameters which were investigated, representative of the clinical spectrum of oral cGVHD, included maximal oral opening (millimetres), stimulated saliva (over a 5 minutes period), presence of mucosal oral cGVHD and candidosis.

4.3.4.1 Analysis of salivary hypofunction

A reduction in stimulated saliva was the most common objective feature of oral cGVHD, with 34.1% of subjects demonstrating some degree of hypofunction. Severe salivary hypofunction, measured as less than 3.5ml of stimulated saliva over 5 minutes, was seen in 19.3% of subjects (Chart 4.3.4.1). A significant association was seen between the time since transplantation and the degree of salivary hypofunction (P=0.023). Only 44.4% of subjects at 6 months to 2 years after HSCT demonstrated normal (>5ml) stimulated saliva quantities compared to 80% (n=16) of subjects 2 to 3 years after transplantation (Table 4.3.4.1). A clear trend was also visible between the presence of aGVHD and the severity of salivary hypofunction (P=0.067). Subjects with a pre-existing history of aGVHD displayed greater salivary hypofunction in general (53.1% versus 22.2%) and a higher proportion of severe hypofunction (27.5% versus 8.3%). Candidosis was present on visual inspection in 11.4% of subjects.

Table 4.3.4.1: Stimulated saliva (ml) relative to time since HSCT

			Stimulated Saliva (5min)			Total
			>5ml	3.5-5ml	<3.5ml	
Time since	6mo-	Count	12	3	12	27
		% within this	44.4%	11.1%	44.4%	100.0%
	2-3yrs	Count	16	3	1	20
		% within this	80.0%	15.0%	5.0%	100.0%
	3-4yrs	Count	14	2	3	19
		% within this	73.7%	10.5%	15.8%	100.0%
	4-5yrs	Count	9	4	1	14
		% within this	64.3%	28.6%	7.1%	100.0%
	5-6yrs	Count	7	1	0	8
		% within this	87.5%	12.5%	.0%	100.0%
Total		Count	58	13	17	88
		% within Time	65.9%	14.8%	19.3%	100.0%

4.3.4.2 Analysis of mucosal oral chronic graft-versus-host disease

Mucosal oral cGVHD was seen in 20.5% of subjects, most commonly involving the buccal mucosa (88.9%) followed by the lateral tongue (44.4%) and gingiva (5.6%). Lesions generally presented bilaterally (77.8%). The pattern of presentation of oral mucosal cGVHD was generally reticular (66.7%) or plaque-like (38.9%) (Figure 4.3.4.2), ulcerative and atrophic areas were noted (27.8% and 5.6% respectively). More than one clinical pattern of mucosal oral cGVHD was seen in 38.9% of subjects at the time of examination. The likelihood of developing oral mucosal cGVHD was strongly associated with stem cell source ($P=0.051$), with all those with oral mucosal cGVHD having received PBSCT ($n=18$). Subjects who had received TBI displayed a clear trend for being free of oral mucosal cGVHD involvement ($P=0.051$), with 9.4% ($n=3$) of subjects who had received TBI developing lichenoid lesions relative to 26.8% ($n=15$) who had not received TBI. Of interest, when analysing the risk factors for the development of mucosal oral cGVHD there was no significant association with a history of aGVHD, mucositis, type of conditioning regimen, donor type and time since transplantation. This was in stark contrast to the findings for the overall risk of developing oral and cGVHD which were both strongly associated with aGVHD.

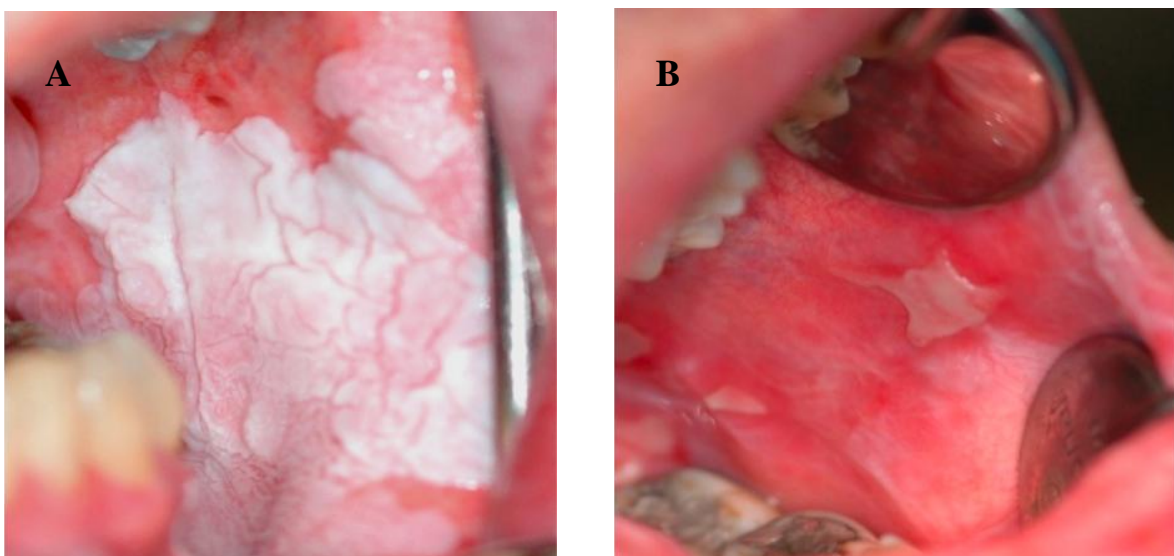


Figure 4.3.4.2: Presentation of oral mucosal cGVHD in two subjects. (A) Plaque-like mucosal lesion (B) Reticular cGVHD with ulceration

4.3.4.3 Analysis of maximal oral aperture

In 42.1% of subjects the maximum unassisted oral opening was reduced below the normal range reported for healthy adults. In 15 subjects (17.1%) oral aperture was less than 39mm (measured interincisally), placing them in a moderate to severe category. Of note, the presence of reduced oral opening was not found to be associated with; age at transplantation, history of aGVHD, TBI, stem cell source or conditioning regimen.

4.4 Pre-transplant education

The majority of subjects had a pre-transplant dental exam however a significant proportion (20.5%) did not. When asked about the frequency of their dental attendance prior to HSCT, most subjects stated they did not see a dentist regularly with over 1 year between visits. This pattern of attendance did not alter significantly after transplantation; the majority of subjects still recorded infrequent dental reviews (>1 year). Of the subjects who did not see a dentist regularly (>1 year between visits) 56.8% (n=25) were unaware of the need to seek regular dental reviews after HSCT while 18.2% were impeded by the cost involved. Over half of the subjects were aware that long-term oral complications could arise after HSCT (56.8%) however a significant number were unable to recall (15.9%) and 27.3% were not aware of these potential complications. The most useful education source pertaining to the oral outcomes of transplant was identified as the transplant physician (19.3%). Other valuable sources included the BMTU Education Day (15.9%) and the education book circulated by the BMTU in the pre-transplant phase. Only 18.2% of subjects reported that they did not receive enough information. Although most subjects' favoured pre-transplant delivery of information, a significant proportion preferred that this information be given at their first outpatient review (18.2%) or a combination of both (22.7%).

Chapter 5: DISCUSSION

Haematopoietic stem cell transplantation is now an established therapy for a range of haematological malignancies, conditions associated with marrow failure, immune deficiencies and some solid tumours however, effective therapy is associated with high mortality and morbidity. A range of oral complications may be seen following allogeneic HSCT which include mucosal cGVHD, salivary hypofunction, peri-oral fibrosis and second malignancies. Unfortunately the incidence of oral complications after allogeneic HSCT and the association with preparatory regimens and other factors has not been clearly elucidated. This study was designed to investigate the incidence and risk factors for the development of oral long-term complications following allogeneic HSCT.

The study population consisted of 88 patients who underwent allogeneic HSCT at a major Australian transplant unit (Westmead Hospital BMTU). The vast majority of these patients received PBSCT (79.5%) where AML was the most common underlying diagnosis preceding transplantation (55.7%) followed by NHL (14.8%) (Table 5.1). The population characteristics were consistent with the most recently published data from the ABMTRR and EBMTR.

Table 5.1: Proportion of allogeneic transplants for major indicators

Indicator	Indicator as a proportion of total allografts		
	<i>ABMTRR</i>	<i>EBMT</i>	<i>Study population</i>
AML	43%	32%	55.7%
ALL	19%	19%	12.5%
CML	8%	11%	Nil
NHL	9%	7%	14.8%

The long-term oral complications following allogeneic HSCT comprise of a significant clinical spectrum. Conditions identified as *diagnostic* presentations of oral cGVHD by the NIH consensus project (2005) include oral mucosal cGVHD, salivary hypofunction and sclerodermatous-like fibrosis of the peri-oral tissues. Other, associated lesions include mucoceles, candidosis and the development of pseudomembranes (Filipovich, Weisdorf et al. 2005). This study assessed the range and frequency of the dental/oral long-term complications in a cohort of patients who received allogeneic HSCT at the Westmead Hospital BMTU. Chronic GVHD arose in 72% of subjects after transplantation with 56% of subjects having some form of oral involvement. The development of oral cGVHD was significantly associated with a preceding history of aGVHD however the presence of oral cGVHD was not significantly influenced by a history of mucositis, the age at transplantation, donor type, transplant type or the conditioning regimen used.

Salivary hypofunction was the most common oral sign of cGVHD with 34% of subjects demonstrating some degree of involvement. Mucosal oral cGVHD was also relatively prevalent in this population with 20.5% of subjects showing clinical evidence of

lichenoid changes. Presumed peri-oral fibrosis, clinically presenting as reduced oral aperture, was present to a significant degree in 17% of subjects. Candidosis was not seen frequently in this subject group with only 11% of subjects demonstrating evidence of candidosis on visual inspection with angular cheilitis and denture-associated stomatitis being the most common forms. Due to the small number of subjects displaying features of candidosis at the time of examination, it was not possible to convincingly establish whether the presence of candidosis was a primary complication of cGVHD or secondary to features of transplantation such as current immunosuppression or salivary hypofunction.

Acute complications

Mucositis

Mucositis is a common acute toxicity related to both chemotherapy and on occasion the use of TBI associated with the conditioning regimen for allogeneic transplantation and can be the most distressing acute toxicity for patients (Bellm, Epstein et al. 2000). The literature suggests that 75-99% of patients receiving myeloablative conditioning regimens will develop some degree of mucositis (Donnelly, Muus et al. 1992). In this study population, when the information pertaining to the presence of mucositis was available (n=69), some 70% of subjects developed some degree of mucositis. A statistically significant correlation was seen between the risk for mucositis and its severity with the use of a myeloablative conditioning regimen ($P < 0.001$). In subjects who received myeloablative conditioning therapy (n=41) 87.8% developed mucositis relative to only 42.9% of subjects who received RIC. As expected, myeloablative conditioning regimens, including the combinations of busulphan-cyclophosphamide and cyclophosphamide-TBI, were identified as predicting a high risk for mucositis. These conditioning regimens were also significantly associated with a predisposition for grade 3-4 mucositis (WHO 1979) (53.8% and 47.4% respectively) in stark contrast to subjects receiving melphan based therapy, with no subject developing grade 3-4 mucositis. Subjects who received TBI almost always developed mucositis with 92.3% of subjects affected in contrast to 55.8% of subjects who did not receive TBI.

Acute graft-versus-host disease

The intensity of the preparative regimen has also been shown to correlate with the development of aGVHD with patients receiving myeloablative conditioning regimens at a higher risk of developing aGVHD. This effect is thought to be associated with the significant tissue damage caused by myeloablative preparative regimens which predispose the tissues to more inflammation from the alloreactive cells (Jacobsohn and Vogelsang 2007). The incidence of aGVHD has been reported as effecting between 35%-50% of allogeneic transplant patients (Jacobsohn and Vogelsang 2007). The most significant risk factors identified in the literature for the development of aGVHD are the degree of HLA mismatch, use of a MUD, myeloablative conditioning regimens and high dose irradiation (Beatty, Clift et al. 1985). This is attributed to the degree of epithelial damage associated with these therapies (Hill and Ferrara 2000). The data gathered on this set of subjects confirms these associations. Over half of subjects developed aGVHD (59%) with the use of myeloablative regimens, TBI and MUD being significantly associated with development of aGVHD. Conversely, some authors have postulated that RIC would be associated with a lower incidence and less severe aGVHD due to the reduction in treatment related mucosal toxicity (Epstein, Raber-Drulacher et al. 2009). This supposition was not supported in this study population. Although subjects who received RIC displayed a significantly lower incidence of aGVHD ($P=0.024$) there was surprisingly not a significant difference in the severity of aGVHD in subjects who received myeloablative or RIC regimens ($P=0.684$).

The literature highlights considerable changes in the clinical picture and natural history of aGVHD over the recent years which are attributed to the numerous advances in HSCT practice. Traditionally, clinical evidence of GVHD prior to 100 days post-

transplantation was presumed to be aGVHD however this is no longer definite. A clear distinction between acute and chronic GVHD, based on the time of occurrence alone, is no longer feasible and diagnosis should rely on the unique clinical and histological features to distinguish between these conditions. As such, the NIH classification system has recently been published with clear changes to the classification and diagnosis of both acute and chronic GVHD to acknowledge these developments (Filipovich, Weisdorf et al. 2005). RIC is one factor which has been suggested as changing the natural history of aGVHD, with patients presenting with features unique for aGVHD, such as maculopapular rash, profuse diarrhoea, or cholestatic hepatitis (Filipovich, Weisdorf et al. 2005), more than 100 days after transplantation (Mielcarek, Martin et al. 2003). In this patient population the majority of subjects who developed features of aGVHD did so within the first 100 days (86.5%) however 13.5% of subjects were diagnosed with aGVHD beyond 100 days post-transplantation.

Chronic complications

Chronic GVHD remains the leading cause of non-malignancy related fatality post allogeneic HSCT (Ferrara and Reddy 2006) accounting for approximately one quarter of deaths in long term survivors of HSCT for leukaemia (Ratanatharathorn, Ayash et al. 2001). A reliable estimate of the incidence of cGVHD is hindered by the lack of standardised diagnostic guidelines but the literature reports that between 25-80% of patients receiving allogeneic transplantation will develop cGVHD (Baird and Pavletic 2006). Known risk factors consistently associated with cGVHD include the use of PBSCT, increasing age of the patient, a matched unrelated donor, TBI and a preceding history of aGVHD (Cutler, Giri et al. 2001; Lee 2005; Baird and Pavletic 2006).

Chronic graft-versus-host disease

In the cohort of subjects studied cGVHD arose in 64 of 88 subjects (72.7%) with the time of onset primarily within the first 3-12 months after HSCT. Acute GVHD was a significant risk factor for the development of cGVHD in this population with 69.8% of subjects who developed cGVHD having a preceding diagnosis of aGVHD in contrast to the 30.2% of subjects with cGVHD who did not have aGVHD ($P=0.001$). The probability of developing cGVHD has also been linked to the grade of aGVHD. Patients who develop grade 2-4 aGVHD have been shown to be more likely to develop cGVHD (Ratanatharathorn, Ayash et al. 2001). This was not replicated in this study population however, since information pertaining to the severity of aGVHD was only recorded for 73% of subjects it is unclear if we have captured an accurate assessment of the proportion of subjects who developed grade 1-2 versus grade 3-4 aGVHD. No other risk factors predicted the onset of cGVHD including PBSCT, increasing age, MUD and TBI. It is possible that these associations were not evident due to the small subject pool.

Oral long-term complications of transplantation

The oral cavity is frequently involved in the spectrum of cGVHD, the literature suggesting that between 72% to 83% of patients diagnosed with cGVHD demonstrate some degree of oral involvement (Schubert and Correa 2008). The incidence of oral cGVHD was somewhat reduced in this study population with only 56.3% of subjects diagnosed with cGVHD demonstrating oral features of cGVHD. However the oral cavity was still one of the most commonly affected sites in cGVHD with 56.3% of subjects with cGVHD showing oral involvement, second only to the skin (70.3%).

This discrepancy in the prevalence of oral involvement in subjects diagnosed with cGVHD may be partially attributable to the way in which this information was attained.

In each patient the history of cGVHD is unique including; time of onset, organs involved and the duration of active disease. In order to capture a true incidence of cGVHD in this study population the medical records of each subject was assessed by the researcher for any documentation, at all outpatient reviews since transplantation, of oral cGVHD. It was thought that this method of assessing disease incidence would more accurately capture all subjects who developed oral cGVHD at some point following transplantation. So, the overall number of subjects affected by oral cGVHD was not based on the findings of the Participant Examination Form. This examination allowed only an isolated appraisal of each subject's complex and prolonged post-transplantation disease history and therefore was thought to not accurately represent disease incidence. Throughout the post-transplant period several transplant physicians and haematology registrars were involved in each subject's review and the documentation of clinical findings. Clearly, examination techniques vary between all clinicians and due to the lack of availability of a standardised approach for the

assessment of the oral cavity it is possible that documentation of oral involvement may have been either over, or more probably, under-diagnosed.

Risk factors identified for the development of oral cGVHD

The risk factors specific for the development of oral cGVHD remain largely unknown however, recent studies have suggested an association with transplant type; a higher degree of oral involvement (70%) seen in PBSCT relative to BMT (53%) (Pavletic S. Z, Smith L.M et al. 2005). This trend was replicated in this group of subjects where those treated with PBSCT showed the highest degree of oral cGVHD (44.3%) relative to BMT (28.6%) and UC transplantation (25%). However, due to the very small numbers of patients receiving umbilical cord transplants during the study period (n=4) it is unlikely that a true representation of this group is possible. Other associations seen in this group of subjects included a past history of aGVHD (P=0.015) and the presence of cutaneous, hepatic or ocular cGVHD. No other statistically significant risk factors were identified including mucositis, TBI, myeloablative conditioning or MUD.

Mucosal oral cGVHD

Oral cGVHD closely resembles, both clinically and histologically, other autoimmune disorders including scleroderma and Sjögren syndrome and the immune-mediated condition lichen planus. Oral mucosal lesions of cGVHD present, most commonly, as lichenoid lesions which typically arise as fixed, fine white striations and hyperkeratotic plaques. Although almost any oral site may be involved, the sites most commonly reported include the buccal mucosa, tongue and labial mucosa (Treister, Woo et al. 2005). In the sample of patients studied, mucosal oral cGVHD lesions were seen in 20.5% of subjects at the time of examination. The sites involved were consistent with

those reported in the literature with the vast majority of subjects showing involvement of the buccal mucosa (88.9%) followed by the lateral tongue (44.4%) and gingiva (5.6%). Mucosal lesions generally existed bilaterally with reticular lesions (66.7%) and plaque-like areas (38.9%) representing the most common pattern of presentation.

Statistically significant risk factors identified for the development of oral mucosal cGVHD were limited in number. A clear association was seen between the prevalence of oral mucosal cGVHD and the type of transplant, with all subjects who developed mucosal cGVHD having received PBSCT (n=18). This is in keeping with the known association of an increased risk of developing cGVHD in patients receiving PBSCT (Stem Cell Trialists' Collaborative Group 2005). However it is unlikely that the distribution of oral mucosal cGVHD relative to transplant type is justly represented in this sample due to the significantly inferior number of BMT and UC transplants in this group relative to PBSCT. Furthermore, it is difficult to know that if by clinically examining every subject once only allows a true representative assessment of this group. In this study, when analysing the risk factors for oral mucosal cGVHD alone, there was no significant association with aGVHD, mucositis, conditioning regimen, MUD and time since transplantation. The lack of association with aGVHD was in stark contrast to the strong association identified for developing chronic GVHD and oral cGVHD in general.

Salivary hypofunction

Acute post allogeneic HSCT salivary gland dysfunction is predominantly attributed to conditioning regimen toxicity, especially in the case of TBI, and can persist for many months. Late changes are most often ascribed to cGVHD and clinically resemble the features of Sjögren syndrome (Coracin, Pizzigatti Correa et al. 2006). Extensive involvement results in the total destruction of secretory units leading to permanent and profound salivary hypofunction (Schubert and Izutsu 1987). The most prominent risk factor in the aetiopathogenesis of salivary cGVHD, which has been implicated extensively in the literature, is TBI (Hemidahl, Johnson et al. 1985). Other confounding factors when assessing the cause of salivary hypofunction in these subjects include radiotherapy to the head and neck region and the polytherapy often required in the management of these patients following transplantation.

The reduction in salivary flow has been reported in 55 to 90% of patients diagnosed with cGVHD with the severity of hypofunction being proportional to the severity of systemic cGVHD (Schubert, Sullivan et al. 1984). In this group of subjects reduced stimulated salivary flow, measured over 5 minutes, was the most consistent, objectively measured, adverse effect with 34% demonstrating some degree of hypofunction. Severe hypofunction, measured as <3.5ml over 5 minutes, was seen in 19% of subjects. The prevalence of salivary hypofunction in this study population is clearly lower than that previously reported however Schubert et al utilised visual assessment only when documenting salivary hypofunction. Clearly, the use of different tools to diagnose salivary hypofunction in this study would explain the divergence in results. A significant inverse association was seen between the time elapsed since transplantation and the prevalence of salivary hypofunction ($P=0.023$) with only 44.4% of subjects 6

months to 2 years after HSCT having normal (>5ml) stimulated saliva quantities compared to 80% of subjects 2-3 years post transplant. This trend may not only reflect salivary cGVHD but may be prejudiced by the proximity of the time since TBI delivery and the intense conditioning regimens delivered during the early phases of transplantation. Salivary tissue is extremely radiosensitive however some degree of recovery is possible with time, which may explain the strong improvement in salivary function as the time since transplantation increases. An interesting correlation was also visible between the previous diagnosis of aGVHD and the severity of salivary hypofunction ($P=0.067$) with subjects who had a history of aGVHD having a higher proportion of severe hypofunction (27.5% versus 8%). Salivary hypofunction, in this study population, was not significantly associated with a history of TBI ($P=0.513$).

The literature shows oral dryness as the second most distressing symptom recorded by patients at discharge and at 1 year following HSCT (Larsen, Nordstom et al. 2007). Xerostomia, the subjective complaint of oral dryness, is by definition irrespective of the clinical signs of salivary hypofunction and is commonly reported in patients with clinically normal salivary function. In this study, xerostomia was reported in 44% of subjects ($n=39$) with 43.6% ($n=17$) of these subjects grading their degree of xerostomia as moderate to severe. Interestingly, in this group of subjects, a sensation of oral dryness was not statistically associated with a history of TBI and that the risk factors identified for clinically evident salivary hypofunction were not transferable to subjects who self reported a sensation of oral dryness (xerostomia). Specifically, the time since transplantation did not predict the prevalence of xerostomia ($P=0.461$) and a history of aGVHD did not preclude the complaint of oral dryness ($P=0.740$). Interestingly, only 13 subjects thought these factors impacted on their quality of life.

Previous authors have reported that often the symptom of xerostomia does not correlate with the objective clinical signs of salivary hypofunction; where clinical features may demonstrate a reduction in salivary flow in patients who do not complain of a dry mouth (Alborghetti M.R, Correa M.E et al. 2005). When the subjects who clinically demonstrated reduced salivary flow (stimulated saliva <5ml over 5 minutes) were compared to the subjects who self-reported a sensation of oral dryness (xerostomia) a measurement of agreement value of 0.288 (ideally 1.0) was obtained, demonstrating poor overlap between these subjects. For example, only 10 subjects of the 17 who were categorised as having severe salivary hypofunction on examination (<3.5ml) also self-reported a sensation of moderate to severe xerostomia (Table 5.2). Conversely, during clinical examination, 6 subjects who self-reported a sensation of severe xerostomia actually demonstrated normal salivary function during the clinical examination (>5ml). This is in agreement with the findings of other authors who suggested that the symptom of xerostomia does not necessarily correlate with clinical evidence of salivary hypofunction. Furthermore, this would suggest that in everyday clinical practice, basing our diagnosis of cGVHD salivary involvement on a patient's self-reported sensation of xerostomia is not an accurate indication of this feature.

Table 5.2: Analysis of agreement of the subjective and objective diagnosis of oral dryness

		Stimulated Saliva (5min)			Total
		>5ml	3.5-5ml	<3.5ml	
Xerostomia	None	39	8	2	49
	Mild	13	4	5	22
	Mod-Severe	6	1	10	17
Total		58	13	17	88

Dysgeusia

Altered taste sensation (dysgeusia) is a common conditioning regimen-related phenomenon which typically resolves 1 to 2 months following HSCT (Comeau, Epstein et al. 2001). Late dysgeusia and/or hypogeusia (reduced taste) or ageusia (absence of taste) can persist or arise in the later stages following HSCT and is often coupled with the onset of cGVHD. Also, a reduction in the quantity of saliva seen in cGVHD involving the salivary glands, may further promote an altered taste perception due to the reduced capacity for saliva to act as a solvent. In this study population, the complaint of altered or reduced taste more accurately predicted the subjects with clinically reduced salivary function ($P < 0.001$). All subjects who recorded severe taste disturbances ($n=3$) clinically demonstrated severe salivary hypofunction. Conversely, 72.9% ($n=51$) of subjects which did not record any disturbance in their sensation of taste clinically demonstrated normal salivary function. Subjects who reported only a mild disturbance in taste did not accurately reflect true mild salivary hypofunction. Approximately half of these subjects demonstrated normal salivary flow and the remainder had more severe hypofunction than predicted (Table 5.3). This information would suggest that in our clinical practice, questions pertaining to changes in taste may be a more accurate predictor of true salivary hypofunction compared to a patient's subjective reporting of oral dryness.

Table 5.3: Correlation of the complaint of altered taste with salivary function

			Stimulated Saliva (5min)			Total
			>5ml	3.5-5ml	<3.5ml	
Taste	None	Count	51	12	7	70
		% within Taste	72.9%	17.1%	10.0%	100.0%
	Mild	Count	7	1	7	15
		% within Taste	46.7%	6.7%	46.7%	100.0%
	Mod-Severe	Count	0	0	3	3
		% within Taste	.0%	.0%	100.0%	100.0%
Total	Count		58	13	17	88
	% within Taste		65.9%	14.8%	19.3%	100.0%

Analysis of the data showed that the subjective sensation of a persistent reduction in taste was also significantly inversely associated with the time since transplantation, with progressively less subjects complaining of moderate to severe dysgeusia as the time since transplantation increased (P=0.031). Of note, a history of TBI and myeloablative therapy was not associated with a reduced taste.

Alterations in maximal oral aperture

Sclerodermatous changes in the perioral tissues, in patients with cutaneous cGVHD, may result in a reduced oral aperture (da Fonseca, Schubert et al. 1998) potentially hindering the provision of dental care and the maintenance of oral hygiene. This perioral fibrosis and restricted oral range of motion resembles, both clinically and histologically, that seen in scleroderma (Imanguli, Alevizos et al. 2008). These clinical measurements allowed each subject to be categorised relative to their degree of oral opening. Normal oral aperture was taken as any measurement higher than, and inclusive of 50 millimetres (mm). The literature shows that the mean maximal unassisted opening falls in the range of 50mm to 60mm in normal, healthy adults (Posselt 1962; Dworkin and LeResche 1992). The maximal oral aperture (measured interincisally) was reduced in 42% of subjects with 17% (n=15) of subjects demonstrating moderate to severe reductions in opening (interincisal distance ranging from 39mm to 23mm). Of note, one of the subjects in this group, who measured a maximum of opening of 39mm, had a pre-existing diagnosis of scleroderma prior to transplantation.

Interestingly, when each subject was questioned about their perception of a reduced oral opening since transplantation, only 4 subjects (4.5%) complained of moderate to severe reduction. This was in stark difference to the number of subjects (n=15) who demonstrated clinical evidence of oral opening less than the normal range. Furthermore when these two groups of subjects were compared, 8 subjects of the 15 who clinically demonstrated reduced oral aperture self-reported their opening as normal. However, several clear confounding factors hinder the usability of this data. Numerous alternate causes for a reduced maximal oral opening are possible, some of the most noteworthy being myalgia of the muscles of mastication, arthralgia of the temporomandibular joint

and a pre-existing diagnosis of autoimmune scleroderma. Subjects were questioned on confounding factors such as true scleroderma however a complete temporomandibular examination was beyond the scope of this study and so cannot be excluded as a potential confounder in this group of subjects.

Lastly, this study was retrospective in design; subjects were not examined prior to transplantation. Ideally, if subjects were examined prior to the onset of the transplantation process obviously a more accurate assessment of changes in oral opening would have been possible. Without a baseline recording pre-transplant it is impossible to accurately predict which subjects showed a true reduction in oral aperture as a consequence of sclerodermatous cGVHD involving the peri-oral tissues.

Second malignancies

The development of new second malignant neoplasms and lymphoproliferative disorders following HSCT has long been recognised as an uncommon but significant complication for long-term survivors of HSCT (Demarosi, Lodi et al. 2005), patients having a 13-fold higher risk for developing second malignancies, including leukaemias, lymphoproliferative disorders and solid tumours (Curtis, Rowlings et al. 1997). In the early post-transplant period, with a peak in the first 2 years, lymphoproliferative disorders and haematologic malignancies predominate as the most common second malignancies. More commonly involving the oral cavity, solid tumours may develop many years following HSCT with their incidence continuing to rise over the years following transplantation, the most common malignancy involving the oral cavity being SCC (Epstein, Raber-Drulacher et al. 2009). The median time from HSCT to solid tumour diagnosis has been shown to be 7 years (0.9 to 22 years) with a cumulative incidence of 1.1% at 20 years (Curtis, Metayer et al. 2005). Within the oral cavity the tongue, buccal mucosa, gingiva and lip are most frequently involved (Abdelsayed, Sumner et al. 2002). The literature also shows the development of solid malignancies at sites previously or concurrently affected with GVHD (Abdelsayed, Sumner et al. 2002).

In this subject pool no solid malignancies of the oral cavity were diagnosed. Subjects in this study ranged from 6 months to 6 years post allogeneic HSCT. The majority (53.4%) in the period of 6 months to 3 years post transplantation with only 9.1% being 5 to 6 years post transplantation. So, it is clear that although all subjects were beyond the immediate post transplant phase, the majority of subjects had not progressed sufficiently in regards to duration post transplantation to accurately assess the incidence of certain long term complications such as the development of solid malignancies.

Pre-transplant education

The last section of this project examined transplant recipients' views concerning the adequacy of pre-transplant education in reference to the long-term oral complications of HSCT, the optimal time for the delivery of information about oral health and the overall compliance with pre-transplant dental assessment. This data was hoped to allow for future adjustment of educational methods to align with overall patient need.

Patients planned for allogeneic HSCT are encouraged to seek a dental assessment prior to the commencement of the transplantation process. This assessment is either completed by the patient's own general dentist or an examination is arranged with an Oral Medicine Specialist within Westmead Hospital BMTU. The majority of subjects in this study did have a pre-transplant dental assessment however a significant proportion (20.5%) did not. When asked about the frequency of their dental attendance prior to transplantation most subjects stated that they did not see a dentist regularly with over 1 year between visits. This pattern of attendance did not alter significantly after transplantation; the majority of subjects still recorded infrequent dental reviews (>1 year). Of the subjects which did not see a dentist regularly (>1 year between visits) 49% were unaware of the need to seek regular dental reviews while 9.8% did not seek regular dental care due to the cost involved.

Regular dental assessment following allogeneic transplantation is an essential component of outpatient care. As evident by this study, the oral cavity is a frequently involved site in the spectrum of cGVHD with 56.3% of subjects diagnosed with cGVHD demonstrating some degree of oral involvement making the oral cavity the second most common site affected by cGVHD. The oral manifestations of cGVHD are

varied and numerous and can include lichenoid lesions, salivary hypofunction, peri-oral fibrosis and second malignancies involving the oral cavity. The role of the Oral Medicine Specialist and general dentist is therefore crucial for the assessment and monitoring of these outcomes and for the management of the complex dental needs of these patients. In this subject group over half of the subjects were aware that long-term oral complications could occur after transplantation (57%) however a significant proportion were unable to recall (16%) or were not aware of these complications (27%). The most useful source of information pertaining to the oral complications of transplant was identified as the transplant physician (19.3%). Other valuable sources included the BMTU Education Day (16%) and the education book circulated by the BMTU. Although most participants favoured pre-transplant delivery of this information, a significant proportion preferred that this information be given at their first outpatient review appointment (18%) or both pre and post transplantation (22.7%). This finding may suggest that the second delivery of information at outpatient review, specific to the long-term oral complications post transplantation, may be well received.

Limitations of this study

This project was a retrospective analysis of the long-term oral complications faced by a series of subjects who received allogeneic HSCT at the Westmead Hospital BMTU.

The retrospective nature of this study made it difficult to truly assess the extent of some oral complications seen after transplantation; such as reduced oral aperture, salivary hypofunction and decay rate. Without a pre-treatment, baseline assessment it is difficult to uniformly state that the complications that were recorded were purely as a result of the transplantation procedure. Although subjects were questioned about the most frequent confounding factors like a prior diagnosis of Sjögren syndrome, oral lichen planus, scleroderma or previous head and neck radiotherapy, there is no clear method to exclude the subjects who may have displayed these oral changes prior to transplantation due to other causes.

Due to the considerable number of deaths within the initial cohort of patients who received HSCT within the designated time period and the substantial number of subjects who declined participation, there remains the possibility of selection bias. The characteristics of the non-participants may have been dissimilar to the final study population. This factor may have influenced the reported incidence of oral cGVHD and associated risk factors in this group.

Lastly, in this study the diagnosis of the majority of oral complications in the spectrum of oral cGVHD was completed using clinical tools only. Without histological analysis, subjects clinically displaying features of lichenoid cGVHD or reduced salivary capacity cannot be, without doubt, diagnosed with cGVHD. In the ideal setting these clinical diagnoses would be confirmed with histopathological analysis.

Implications of research and future directions

The aim of this study was to assess the extent and range of the long-term oral complications which can occur following allogeneic transplantation. From this data set, it can be seen that this clinical spectrum includes lichenoid-like mucosal lesions, salivary hypofunction, peri-oral fibrosis and altered taste sensation. For a minority of patients these oral manifestations impact on their quality of life post-transplantation. This study has identified a select number of risk factors which seem to be significantly associated with the development of cGVHD and specifically oral cGVHD. The most prominent risk factor identified for the development of oral involvement within the cGVHD spectrum included a past history of aGVHD ($P=0.015$). Subjects were also more likely to develop oral cGVHD with a history of cutaneous, hepatic or ocular cGVHD. Significantly, no other risk factors were identified; mucositis, TBI, myeloablative conditioning or MUD did not significantly predict the risk for oral cGVHD. With so few predictive factors known, this data highlights the unique difficulties faced when diagnosing and managing these patients.

The second aspect to this study was to assess the adequacy of education material for the long-term oral complications of allogeneic HSCT. The vast majority of subjects were aware of the potential oral complications that could arise however a strong proportion of subjects agreed that delivery of this information may be more useful in the outpatient setting or both pre and post transplantation. Ideally this data may be used to aid in tailoring future education material to further address patient needs.

Several new research directions may follow on from this preliminary work. Analysis of the histology of the clinical lesions and complications diagnosed in these subjects, via

incisional biopsy, would allow for more accurate diagnosis and a further understanding of the pathophysiology and natural history of these complications. Through this research it was clear that many subjects were not able to find successful and easily accessible management strategies for the oral complications experienced after transplantation. Further research could focus on developing patient education pamphlets which would help patients understand their oral changes and offer simple and accessible management strategies.

In conclusion, it is evident that oral long-term complications are a common occurrence following allogeneic haematopoietic stem cell transplantation with over half of the patients (56.3%) who developed cGVHD having some form of oral involvement. The most common clinical presentations were of salivary hypofunction and mucosal oral cGVHD. Although the majority of subjects did report receiving adequate information regarding these potential oral complications, a significant proportion of this study population showed interest in attaining further education and more detailed management strategies in the outpatient setting. This study has clearly shown that oral complications after transplantation are common and require regular review and appropriate management as part of the routine outpatient assessment of all haematopoietic stem cells transplant patients.

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