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ORIGINAL ARTICLE

Complications of Allogeneic Hematopoietic Stem Cell Transplantation

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Infection, graft-versus-host disease (GVHD), and to a lesser extent sinusoidal obstructive syndrome (SOS) represent the major causes of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (AHST). During the last decade, progress in prevention and treatment of these complications led to improvement in the outcome of these patients. Despite the fact that nonmyeloablative regimens have been increasingly used in elderly patients and in patients with co-morbidities, the nonrelapse related mortality remains a challenge and long-term follow-up is required. The objective of this manuscript is to provide an updated concise review of the complications of AHST and of the available treatment interventions.

Keywords: Bone marrow transplant, Leukemias, Long-term survival and late effects

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (AHST) is considered a curative treatment option for many hematologic diseases (1). Acute and chronic leukemias, myelodysplastic and myeloproliferative syndromes account for approximately 70% of AHST cases in North America (2). The beneficial effect of this approach is not only due to the preparative chemo-radiotherapy regimen, but more importantly due to the immunologic effect induced by graft-versus-tumor (GVT) (3). Although the number of transplants from related donors has remained stable, unrelated donors have been increasingly used and currently contribute to almost 50% of AHST cases (4). Several complications including infection and GVHD have remained the major causes of mortality and morbidity. These complications are due to toxicities of the preparative regimen, the accompanying immunodeficiency, and the organ damage induced by GVHD. In the past two decades, there has been a steady increase in the use of nonmyeloablative or reduced-intensity conditioning (RIC) regimens, especially in elderly patients

and patients with co-morbidities. The treatment benefit of these regimens relies almost exclusively on the GVT effect. Several studies have shown improvement in non-relapse mortality rates due to decreased regimen-related toxicity (4).

Preparative regimens

The conventional preparative regimens have two major goals. The first goal is to provide adequate immunosuppression in order to prevent rejection of the transplanted graft and the second goal is to eradicate the underlying disease. The optimal myeloablative conditioning regimen remains unknown. High-dose oral busulfan combined with cyclophosphamide (BuCy) and cyclophosphamide in combination with total body irradiation (CyTBI) are well-established regimens and represent the two most commonly used conditioning regimens. Several studies have failed to show any difference in survival between these two regimens (5). However, more recently, intravenous (IV) busulfan conditioning was compared to total body irradiation (TBI) in patients with myeloid malignancies and was associated with superior survival with no increased risk of relapse or transplant-related mortality (6). Copelan et al. showed that a conditioning regimen using a combination of cyclophosphamide with IV busulfan was associated with better non-relapse mortality, relapse after one year post transplant, better leukemia-free survival and survival, compared to CyTBI in AML patients receiving a first AHST (7). Pharmacokinetic targeted IV busulfan combined with fludarabine was retrospectively compared to oral busulfan in combination with cyclophosphamide in patients undergoing AHST for AML, and was associated with reduced conditioning regimen related toxicity (8). Moderate dose (6.4 mg/kg total dose) IV busulfan was shown to be associated with lower toxicity and no change in OS compared to the full dose (12.8 mg/kg total dose) IV busulfan in fludarabine/busulfan/ATg RIC regimen (9).

Other regimens combining radiation in combination with etoposide or high dose cytarabine, with or without cyclophosphamide have also been used (10). Radiation noncontaining regimens were developed in an attempt

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to eliminate the toxicity of radiation. These regimens include busulfan and etoposide in patients with acute myeloid leukemia, cyclophosphamide, carmustine (BCNU), etoposide, and BCNU, etoposide, cytosine arabinoside and melphalan (BEAM) in lymphomas. Kahl et al. reported that the survival rate at 9.2 years was 88% in patients with aplastic anemia conditioned by cyclophosphamide combined with antithymocyte globulin (ATG) followed by allogeneic marrow transplants (11). Radio-labeled monoclonal antibodies (anti-CD20, anti-CD45, anti-CD66) use was also reported in small series, allowing the radiation to target the tumor cells and marrow, and minimizing radiation effects on other organs.

Common side effects of the preparative regimens include myelotoxicity, mucositis, nausea and vomiting, diarrhea, alopecia, rash, and peripheral neuropathy. Pulmonary and hepatic toxicities are also relatively common especially with busulfan. Cyclophosphamide is associated with an increased risk of renal toxicity (12). Recent studies have focused on the development of nonmyeloablative or RIC regimens. The main aim is to exploit the beneficial GVT effect and reduce regimen-related complications (13). Fludarabine in combination with busulfan, melphalan or low dose irradiation has been widely studied and has shown promising results (14, 15). Total lymphoid irradiation (TLI) with ATG also showed reduction in acute GVHD risk with retention of GVT effects (16). Comprehensive reviews of the role of nonmyeloablative therapy in AHSCT have been previously published (17, 18).

Immunologic and infectious complications

Deficiencies in the cellular as well as humoral aspects of the immune system occur to some extent in all AHSCT patients for a variable period of time. However, in patients with GVHD receiving immunosuppressive therapy, these deficiencies are more profound and prolonged (19). Cellular immunodeficiency is a result of a lowered T-cell response to alloantigens and mitogens, decreased helper CD4+ cell function and reactivity to intradermal skin tests. Humoral immunodeficiency consists of a decrease in both IgG2 and IgG4. However, immunoglobulin levels may be within the reference range. The switch from primary (IgM) to secondary (IgG) production as well as antigen-specific responses is abnormal, leading to an impaired production of antibodies to pathogens. The recovery of the immune system consists of two phenomena, innate immunity or the numerical recovery of hematopoietic cells and adaptive immunity, the functional recovery of cellular interactions (19). Because of thymic involution in adult patients, T-cell function is dependent upon the peripheral expansion of the few donor T-cells that are present in the graft. Immune reconstitution is usually slow in adult patients reaching normal level 2 to 3 years after transplant. This is in contrast to immune reconstitution in children where cell function and T-cell receptor repertoire recover within the first 1 to 1½ years after transplant (20).

Infectious complications of AHSCT are summarized in Table 1. Viral infections are major causes of morbidity and

mortality in AHSCT recipients. Infections caused by the herpes virus group are common and are usually a consequence of reactivation of a latent virus. Infection with the herpes simplex virus may occur 1 to 2 weeks after transplantation in 80% of seropositive patients who are not receiving acyclovir prophylaxis, causing muco-cutaneous lesions of the oropharynx, esophagus, or genital tract (21–23). Other viruses, including varicella-zoster, respiratory syncytial virus, para-influenza virus type 3, human metapneumovirus, human herpes virus 6 and 8, and Epstein-Barr may also cause pulmonary infections.

Cytomegalovirus (CMV) is the most common viral pathogen associated with respiratory tract infections (24–26). CMV infection can be the result of a primary infection or a reactivation of the virus. It tends to occur 4 to 10 weeks after transplantation (22). Prophylaxis and empirical treatment lead to a significant decline in the rate of infections to less than 5% (27–29). Despite a reduction in CMV pneumonia in the early post-transplantation period, it continues to be a significant cause of morbidity and mortality in the later post-transplant period. Risk factors include older age, positive CMV serology, allogeneic graft and acute GVHD (30–34). Given the more liberal use of prophylactic therapy, CMV pneumonia can now occur later than 100 days after transplant (35, 36).

Prevention of CMV infections relies on the use of CMV-negative blood products in CMV-seronegative donor-patient pairs (37), intravenous immunoglobulin (38, 39), pre-storage of leukocyte-depleted blood products, leukocyte-filtered blood products, and prophylactic antiviral agents. Three randomized, placebo-controlled studies have shown that the prophylactic use of ganciclovir led to a significant reduction in the incidence and severity of CMV infections in CMV-seropositive AHSCT recipients (40–42).

Foscarnet sodium was used prophylactically in a small phase I-II study in CMV-seropositive patients (43). In this study, starting foscarnet 7 days before the transplant and continuing it for 75 days after transplantation was effective in preventing CMV infections in most patients.

CMV prophylaxis has been replaced with a pre-emptive strategy based upon active screening for CMV. Previously utilized antigenemia assays guiding institution of pre-emptive therapy, has been supplanted by real time polymerase chain reaction (PCR) tests for the quantification of CMV DNA. PCR tests have been shown to be more sensitive and particularly adept in patients with low cell counts or in which specimens need to be stored or shipped (44).

CMX001 is an orally bioavailable, lipid acyclic nucleoside phosphate that is absorbed and converted intracellularly to cidofovir diphosphate. In a multi-center trial, treatment with CMX001 at a dose of 100 mg twice weekly, in sero-positive CMV patients undergoing AHSCT, was shown to significantly lower the incidence of CMV events from 27% to approximately 10%. Myelosuppression and nephrotoxicity were not observed (45).

Over the years, given the improvement in prevention as well as treatment of bacterial infections, fungi have become

Table 1. Infectious Complications of Allogeneic Hematopoietic Stem Cell Transplantation

	Pre-engraftment: Early (<3 weeks)	Postengraftment: Immediate (<3 weeks–3 months)	Postengraftment: Late
Bacterial	Gram positive cocci Gram negative bacilli Anaerobes	Gram positive cocci Gram negative bacilli Anaerobes	Encapsulated
Fungal	Candida spp. Aspergillus spp.	Aspergillus spp. Pneumocystis carinii	Candida spp. Aspergillus spp. Pneumocystis carinii
Viral	HSV 1,2 RSV Influenza Adenovirus	Cytomegalovirus HHV 6 RSV Influenza Adenovirus	VZV EBV RSV Influenza Adenovirus

the foremost infectious cause of morbidity and mortality in AHSCT patients (46). Risk factors associated with increased susceptibility to late fungal infections in patients with chronic GVHD include impaired mucosal defense, chemotactic defects, functional asplenia, and qualitative and quantitative B- and T-cell abnormalities. Late-onset fungal infections with invasive candidiasis and aspergillosis can occur several months after transplantation, especially in patients with severe GVHD receiving immunosuppressive therapy.

Prevention of fungal infections is clearly preferred due to the difficulty in treating established infections. Multiple agents have been utilized to prevent invasive fungal infections in AHSCT recipients, including azoles as well as echinocandins. Prophylactic therapy with fluconazole, 400 mg/day, is associated with fewer positive fungal cultures, systemic fungal infections, and improved mortality (47, 48). In a randomized, double-blind trial, posaconazole prophylaxis was found to be superior in preventing invasive aspergillosis and reducing the rate of deaths related to fungal infections (49). Though relatively well tolerated, liver function tests should be monitored at baseline and throughout posaconazole therapy and treatment should be discontinued if serious hepatic abnormalities occur. Other rare serious adverse events that were reported in this study include hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and pulmonary embolus. Prolongation of the QT interval may be seen with posaconazole as well as with the other azole antifungal agents.

Invasive aspergillosis has been reported to occur in 3.6 to 28% of patients (50). Patients generally present with nonspecific symptoms including, fever, productive cough, and pleuritic chest pain (51, 52). Radiographic findings including the “halo sign” which corresponds to a nodule surrounded by ground-glass attenuation and the “hypodense sign” which reflects the presence of low density within nodules or areas of consolidation, are reported to have 30% sensitivity and 100% specificity for the diagnosis of invasive pulmonary aspergillosis (53).

Targeted screening for markers of fungal colonization, in attempt to prevent invasive infection, is being utilized frequently. Available tests include, Aspergillus PCR of serum, (1–3)- β -D-Glucan and/or the galactomannan antigen (GAL) assay (54). Initiation of therapy should be considered in patients at high risk with positive screening tests. The drug of

choice for invasive aspergillosis is voriconazole, which has been shown to lead to better responses and improved survival with fewer side effects in comparison to the old standard approach using amphotericin B deoxycholate (55).

After invasive aspergillosis, pathogens of mucormycosis are the second most common cause of mold infections, and can lead to life threatening rhino-orbital, pulmonary, cerebral, or disseminated infection (56). Treatment involves combination of surgical debridement and antifungal therapy, with intravenous liposomal amphotericin B remaining the drug of choice. Unfortunately, prognosis for recovery from mucormycosis remains poor (56, 57).

PCP (Pneumocystis Carinii Pneumonia) infection has decreased dramatically with the use of trimethoprim-sulfamethoxazole (TMP-SMZ) prophylaxis. For patients intolerant to TMP-SMZ, alternative treatments for prophylaxis include atovaquone, dapsone, or aerosolized pentamidine (58).

Sinusoidal obstructive syndrome

Sinusoidal obstructive syndrome (SOS), known in the past as veno-occlusive disease of the liver, is seen in about 10% of patients receiving myeloablative conditioning regimens (59). More recently, the use of intravenous busulfan-based regimens in conjunction with reduced intensity conditioning has resulted in a significant decline in the rate of SOS (60, 61). A definitive incidence is difficult to estimate due to differences in regimens as well as supportive care amongst transplant centers, though one large series showed an overall rate of about 5% (62).

With no established laboratory, imaging, or histologic test, SOS remains an elusive diagnosis. Most patients are diagnosed within 3 weeks of AHSCT. Clinical symptoms include jaundice, tender hepatomegaly, fluid retention and weight gain. In some cases, SOS may occur at the same time as acute GVHD, making clinical differentiation between the two conditions difficult. The pathogenesis of SOS remains unclear. One of the etiologies is thought to be the obstruction of the terminal hepatic and sublobular central venules by endothelial cell injury and thrombosis, leading to a shift of fluid containing sodium and albumin from the intravascular to the extravascular space. This fluid shift leads to a decrease in renal blood flow and activation of the renin-angiotensin system, resulting in sodium and fluid retention. An early decrease in

protein C and antithrombin III levels may play a role in the pathogenesis of SOS and is predictive of its development and severity (63).

Transjugular liver biopsies and manometric monitoring of hepatic blood flow can help in establishing the diagnosis of SOS (50, 51). Less invasive tests such as plasminogen activator-inhibitor-1, an inhibitor of the fibrinolytic system, can be useful, when elevated, in ruling out SOS with a high sensitivity (100% in one study) (64). Patients with mild disease, as evidenced by minimal elevation in the bilirubin level and mild weight gain, have an excellent prognosis. In contrast, patients who have a bilirubin level of 20 mg/dL or higher ($>342 \mu\text{mol/L}$) or a weight gain of 15% or more of original weight have 90% mortality before day 100 (65).

There has been no established therapy of SOS. Alteplase and heparin therapy have been historically associated with a modest response rate of approximately 30%, but severe bleeding rates of 25% were reported with these drugs (66).

The most promising agent, defibrotide, is an oligonucleotide derived from porcine tissue that has antithrombotic, thrombolytic, anti-ischemic and anti-inflammatory properties, and an affinity for small vessels. It was shown to have little systemic anticoagulant activity compared to alteplase and heparin. Initial studies showed that the use of defibrotide, in patients with severe SOS, induced complete response rates of 36–55% with 35–53% survival at day 100 (67, 68).

In the largest prospective evaluation of defibrotide for the treatment of severe SOS and multiorgan failure, data from an interim analysis, based on 269 patients, revealed that 32% achieved a CR and 50% survived to day 100. Toxicity proved to be manageable with 22% of patients experienced a total of 81 related AEs, primarily consisting of bleeding and hypotension. Defibrotide was given at a dose of 6.25 mg/kg IV q6h with treatment duration recommended for at least 21 days (69).

Given the ambiguity of diagnosis as well as costly and modest therapeutic response rates, prevention remains the most effective tactic in managing patients at risk for SOS. The risk factors for developing SOS include pretransplant elevation of serum aminotransferase levels (especially aspartate aminotransferase), intensive conditioning therapy (higher TBI and busulfan dose), graft from a mismatched or unrelated donor, and use of antimicrobial therapy with acyclovir, amphotericin B, or vancomycin (58). Sodium and fluid restriction and the judicious use of diuretics remain the main preventive approaches for this condition (70). Pentoxifylline, a xanthine derivative capable of downregulating tumor necrosis factor production, has been shown in randomized studies to be of no benefit in reducing the incidence of SOS (71–73). A randomized trial showed that use of low-dose heparin (100 U/kg/d) was capable of reducing the incidence of SOS without added risk of bleeding (74). Ursodiol administration reduced the incidence of SOS in a randomized, double-blind, and placebo-controlled trial in patients receiving a preparative regimen of cyclophosphamide and busulfan (75).

In a report of 58 patients who received defibrotide as prophylaxis without concurrent heparin or ursodiol, no patients

fulfilled the criteria for SOS or died of the condition within 100 days of AHSCT. In this study, patients received 5 mg/kg of intravenous defibrotide over 2 hr, twice-daily from day +1 to +21 (76).

Finally, minimizing liver insults by removing any hepatotoxic medication should be attempted at all time.

Graft-versus-host disease

Graft-versus-host disease (GVHD) remains one of the major causes of morbidity following AHSCT leading to prolonged use of immunosuppressive agents, organ dysfunction, increased risk of infection, and ultimately increased mortality (77).

Acute GVHD

The occurrence of acute GVHD has been defined historically to be limited to the first 100 days following AHSCT (78). However, a clear distinction can no longer apply due to the increased use of nonmyeloablative regimens and DLI both of which can potentially delay the appearance of acute GVHD. In 2005, a group at the NIH published criteria for the designation of acute and chronic GVHD as follows (79): Classic acute GVHD occurring within the first 100 days after AHSCT and persistent, recurrent or late-onset acute GVHD occurring more than 100 days after transplant both requiring the presence of acute features and absence of chronic features.

Early research provided insight into the immunologic factors essential to the development of GVHD and was summarized in Billingham's postulates in 1996 (78). The graft must contain immunocompetent lymphocytes, the recipient must be incapable of immunologically destroying the graft and the recipient must express antigens that are not present in the donor, leading to stimulation of the donor lymphocytes. Indeed, the greater the disparity in the minor and major histocompatibility antigens between the donor and the recipient, the greater the incidence and the severity of acute GVHD (80–82). Despite full HLA-matching between the patient and the donor, the incidence of acute GVHD still ranges between 26 and 32% in recipients of sibling donor grafts and between 42 and 52% in recipients of unrelated donor grafts (83). Risk factors for the development of acute GVHD other than the extent of HLA disparity include increased age of both the recipient and the donor, gender disparity (with multiparous female donors carrying particularly a higher risk), ineffective GVHD prophylaxis and the intensity of the transplant conditioning regimen (84–86). A risk model for clinical outcome of AHSCT including acute GVHD based on multiple single-nucleotide polymorphism was recently developed and reported (87). Also, a recent large retrospective study investigated risk factors that have a predictive value for acute GVHD (88). Results of this study indicated that regimens that do not use TBI are associated with lower risk for acute GVHD when bone marrow and not when peripheral blood was used as the stem cell source. Biomarker combinations that include regenerating islet-derived 3- α (Reg3 α), tumor necrosis factor receptor 1 (TNFR1), IL2 receptor α , IL8, hepatocyte growth factor, and elafin appear to have predictive value for

Table 2A. Staging of Acute Graft-versus-Host Disease

Clinical stage	Lower GI	Upper GI	Liver (Bilirubin mg/dL)	Skin
1	Diarrhea <500 mL/d	Nausea/Vomiting	2–3	Rash < 25% of BSA
2	Diarrhea 500–1000 mL/d		3–6	Rash 25–50% of BSA
3	Diarrhea 1000–1500 mL/d		6–15	Generalized erythroderma
4	>1500		> 15	Bullae/Desquamation

Weisdorf D (2006, November). Graft versus Host Disease and How to Report it. Retrieved April 17, 2014, from http://www.cibmtr.org/Meetings/Materials/CRPDMC/Documents/2006/november2006/Weisdorf2_RprtGVHD.pdf

etiology, response to treatment and treatment-related mortality in acute GVHD (89, 90).

Clinical manifestations of acute GVHD include specific derangements in the skin, the liver and the gastrointestinal tract. With the use of high-intensity conditioning, acute GVHD most commonly occurs 2 to 42 weeks after stem-cell infusion. A hyperacute form of GVHD can occur within the first 2 weeks of AHSCT, and is usually due to significant HLA mismatch or inadequate GVHD prophylaxis, and can be rapidly fatal (91).

Acute GVHD is clinically graded and staged in severity from grades I to IV depending on the extent of skin, liver, upper GI tract and gut involvement. A commonly used staging and grading systems are shown in Tables 2A and 2B. The most common and the first organ to be affected by acute GVHD is the skin. Early cutaneous manifestations of acute GVHD could be palmar erythema and erythematous discoloration of the face and ears, followed by diffuse patchy erythema with follicular prominence, and a generalized morbilliform eruption. In severe cases, it can progress to a diffuse erythroderma with bullae formation and desquamation resembling Stevens-Johnson syndrome and toxic epidermal necrolysis (92). Histologically, vacuolar degeneration and lymphocytic infiltration involving the basal cell layer is seen in mild disease, and this picture changes to necrotic dyskeratotic cells with acantholysis and cell membrane separation in moderate disease. Epidermolysis can develop in severe cases (93). These findings are not pathognomonic of GVHD since chemo-radiotherapy and use of other drugs can induce similar changes. Upper GI symptoms including nausea and vomiting, and lower GI symptoms including abdominal pain and massive watery or bloody diarrhea, along with laboratory elevation of the bilirubin, alkaline phosphatase and aminotransferase levels characterize intestinal and hepatic involvement. Patients with upper GI tract involvement have a better response to immunosuppressive therapy compared to patients with involvement of other areas of the gut.

Table 2B. Grading of Acute Graft-versus-Host Disease

Overall Clinical Grade	GI	Upper GI	Liver Stage	Skin Stage
I	0	0	0	1–2
II	1	1	1	3
III	2–3		2–4	–
IV	4		–	4

Weisdorf D (2006, November). Graft versus Host Disease and How to Report it. Retrieved April 17, 2014, from http://www.cibmtr.org/Meetings/Materials/CRPDMC/Documents/2006/november2006/Weisdorf2_RprtGVHD.pdf

Failure of these patients to respond to treatment predicts progression to lower GI involvement (94, 95).

The diagnosis of acute GVHD can be made on clinical grounds only in patients presenting with a rash, diarrhea and elevation of bilirubin within the first several weeks of transplant. However, the diagnosis is frequently not straightforward. Mimickers of cutaneous GVHD include toxic erythema of chemotherapy, morbilliform drug eruptions or viral infections. The differential diagnosis of liver involvement with GVHD includes VOD, infectious hepatitis and drug-induced liver toxicity. Parasitic, viral and bacterial infections can present with similar gastrointestinal symptoms. The role of skin biopsies in diagnosing acute cutaneous GVHD is controversial as many studies showed the difficulty differentiating it from its mimickers (96–98).

In patients with isolated hepatic derangement, a liver biopsy is needed to confirm the diagnosis when it is deemed safe to perform and the histologic findings on liver biopsy include lymphocytic infiltration mostly of the portal triad with presence of hepatocellular necrosis sometimes similar to acute hepatitis. Rectal punch biopsies show epithelial cell necrosis, vacuolar degeneration, crypt dropout and in advanced cases, epithelial denudation. More recently, it has been proposed that a panel of plasma biomarkers including IL-2-receptor- α , TNF receptor-1, IL-8, and hepatocyte growth factor can confirm the diagnosis of acute GVHD at the onset of clinical symptoms and provide prognostic information independent of GVHD severity (99).

Prevention is an essential approach in the management of acute GVHD. The most commonly used pharmacologic regimen includes a combination of a calcineurin inhibitor (cyclosporine or tacrolimus) and prednisone with a short course of methotrexate. This regimen was initially established in 1986 (100). Several studies have compared the effectiveness of this combination with either agent alone and showed superiority in prevention of acute GVHD in the combination arm (101, 102). In a phase III study of patients receiving an HLA-identical sibling bone marrow graft, prophylactic therapy with tacrolimus in combination with methotrexate was shown to reduce the incidence of grade II to IV acute GVHD compared with cyclosporine and methotrexate therapy. In this study, there was no difference in the incidence of grade III or IV acute GVHD, chronic GVHD, relapse rates, or disease-free survival (DFS) or overall survival in patients with nonadvanced hematologic malignancy. However, there was a higher frequency of deaths caused by regimen related toxic effects in patients with advanced disease who received tacrolimus (103). Tacrolimus-MTX has been compared to

cyclosporine-MTX in another phase III study involving unrelated donors. The Tacrolimus arm demonstrated decreased incidence of grade II-IV acute GVHD but no overall survival advantage (104).

The optimal prophylactic regimen following reduced-intensity HSCT is yet to be established. A commonly used regimen in this setting includes a combination of a calcineurin inhibitor with mycophenolate mofetil (MMF) instead of methotrexate as some studies showed less mucositis and more rapid neutrophil engraftment with MMF compared to methotrexate (105). Some centers utilize T-cell depletion which has significantly reduced the incidence and severity of GVHD but has not gained wide acceptance because of the increased risk of graft rejection and relapse of leukemia and therefore no improvement in survival (106). The best evidence for *in vivo* antibody efficacy is for ATG in unrelated HSCT after myeloablative conditioning (107). In a randomized trial, patients were assigned to receive cyclosporine and methotrexate with or without anti-Jurkat rabbit ATG. ATG recipients had a significant reduction of grade II-IV acute GVHD. Another pharmacological approach for prevention of acute GVHD is through the use of sirolimus, an mTOR inhibitor as an addition to methotrexate and tacrolimus (108). However, due to increased risk of veno-occlusive disease, sirolimus should not be combined with myeloablative doses of busulfan or in the TBI-based myeloablative regimens if combined with methotrexate (109).

There are many new preventive strategies that are currently under investigation. One study examined the use of the CCR5 antagonist Maraviroc (110). In this study, 35 high-risk AHSCT recipients had a cumulative incidence of grade 2–4 acute GVHD of 14.7% on day 100 and 23.6% on day 180. The cumulative incidence of grade 3–4 GVHD on day 180 was 5.9%, that was mainly attributed to a very low incidence of visceral GVHD. At 1 year, the rate of nonrelapse mortality was 11.7% and rates of relapse or infection were not increased.

Another novel preventive approach is the post-transplant administration of high-dose cyclophosphamide in an attempt to eradicate activated T cells (111). A study by the Johns Hopkins University investigators tested the use of cyclophosphamide as the only prophylaxis for GVHD after HLA-matched related and unrelated donors T cell-replete AHSCT (112). When cyclophosphamide is given early after transplantation, it depletes dividing alloreactive T cells rapidly. Aldehyde dehydrogenase, which is present at high levels in hematopoietic stem cells, convert 4-hydroxycyclophosphamide into a nonalkylating metabolite, sparing the stem cells from the antiproliferative effect of the drug. Cyclophosphamide was given on days 3 and 4 after AHSCT without the addition of an immunosuppressive agent. The results showed that, without the use of colony-stimulating factors, the median time to neutrophil engraftment was 23 days in the matched related donor group and 25 days in the unrelated donor group. The treatment-related mortality at 2 years was 13% in the matched related group and 21% in the matched unrelated group. There was a low

cumulative incidence of chronic GVHD of 10%. The incidence of grade II-IV acute GVHD was 42% and 46% in the related and unrelated groups respectively with grade III and IV occurring in 12% and 8% respectively. A major advantage to the use of cyclophosphamide is the selective elimination of host-reactive donor lymphocytes rapidly after transplantation, and the rapid immunologic recovery without the use of calcineurin inhibitors.

The treatment of acute isolated grade II cutaneous GVHD includes topical corticosteroids or tacrolimus (113). For acute grade III-IV GVHD, treatment includes high dose systemic steroids, cyclosporine, and antithymocyte globulin. Forty to 50% of patients respond to steroids and this response is predictive of prognosis and outcome as there is no well established second line therapy. Salvage therapy is under investigation and includes novel pharmacologic agents (e.g., HDAC inhibitors, proteasome inhibitors, antibodies targeting IL-21, or adhesion molecules) or upcoming cellular therapeutics such as Treg, NKT, MSC, or B-cell targeting (114). Patients with acute GVHD have a lower incidence of leukemia relapse owing to a presumed GVL effect (3).

Chronic GVHD

Chronic GVHD remains the most common late complication of AHSCT. It occurs in almost 50% of patients surviving more than 1 year posttransplant (115). The median time at presentation is 4 to 6 months after the transplant (116). Chronic GVHD is divided into two subtypes: classic GVHD (without features characteristic of acute GVHD) and overlap syndrome (with synchronous clinical features) (88). Risk factors include high degree of HLA mismatching, old age, gender disparity of donor and/or recipient, prior acute GVHD, cytomegalovirus seropositivity (donor and recipient) and the use of peripheral blood precursor cells (117). A recent phase 3, multicenter, randomized trial of transplantation of peripheral-blood stem cells versus bone marrow from unrelated donors showed no significant difference in survival. Peripheral-blood stem cell transplantation may reduce the risk of graft failure. On the other side, bone marrow transplantation may reduce the risk of chronic GVHD (118).

The pathogenesis is still poorly understood. Thymic atrophy, lymphocyte depletion and autoantibody formation have been described. Current evidence suggests that disruption of thymic function by conditioning regimens and acute GVHD leads to dysregulation of the negative selection process of potentially autoreactive T-lymphocytes. Dysregulated autoreactive T-cells will then activate autoreactive B-cells, which leads to autoantibody production and causes target organ damage. The continuous presence of T-helper 2 cells is required in chronic GVHD, and the role of interleukins, especially (IL)-12 and (IL)-18, has been demonstrated in murine models (119).

Chronic GVHD has similar clinical features to autoimmune diseases. The most common affected organ is the skin (lichen planus or scleroderma cutaneous manifestations). Other common target organs are the liver (bile duct damage with cholestasis), the gastrointestinal tract (erythema with

lichenoid lesions of the buccal and labial mucosa, ulceration and malabsorption), the lungs (bronchiolitis obliterans which may be associated with pulmonary failure and increased mortality) and the musculoskeletal system (fasciitis, stiffness and contractures) (120, 121). Diagnosis requires histologic confirmation which is usually by biopsy of the skin, mouth or liver.

There is no effective prophylaxis against chronic GVHD due to incomplete understanding of the pathophysiology of the disease (121). The risk may be reduced by selecting a younger, related male donor; using bone marrow rather than peripheral blood stem cells; and possibly limiting the infused doses of CD34⁺ and T-cell. Kottaridis et al studied a non-myeloablative preparative regimen, using CAMPATH-1H at a dose of 20 mg/day on Days -8 to -4, fludarabine 30 mg/m (2) on Days -7 to -3 and melphalan 140 mg/m (2) on Day -2, in 44 patients with hematologic malignancies. This approach was associated with durable engraftment, minimal toxicity and low incidence of GVHD. At a median follow-up of 9 months, only one patient was reported to have chronic GVHD (122). Hale et al. showed that the incidence of chronic GVHD was 3% in AML patients in first remission who were transplanted from HLA-identical siblings and where donor cells were incubated *in vitro* with Campath-1M. In this study, Campath-1G was infused *in vivo* before donor cell infusion, and no post-AHSCT immunosuppression was given (123). Barge et al, showed that the incidence of chronic GVHD was 19% in 73 patients who received myeloablative regimen and a transplant from HLA-identical siblings and received donor cells treated *in vitro* with Campath-1M (124). High-dose post-transplantation cyclophosphamide was also suggested to be an effective single-agent prophylaxis of acute and chronic GVHD in patients with advanced hematologic malignancies after BuCy conditioning and HLA-matched BMT (125). Data suggest that an anti-CD6 antibody plus complement for *in vitro* T-cell depletion resulted in an incidence of chronic GVHD of less than 15% in HLA-identical sibling transplants (126).

Patients with limited disease may not need systemic therapy. However, for patients with extensive involvement, immunosuppressive therapy consisting of alternating prednisone and cyclosporine has been shown to increase the overall survival and has been the standard first line therapy. Doses are usually tapered over time and the median duration of therapy is usually 2–3 years. Steroid-refractory patients should be entered in clinical trials. Extracorporeal photopheresis, mTOR inhibitors including sirolimus and tacrolimus, rituximab, pentostatin, and imatinib are suggested as second line treatment options in refractory chronic GVHD. MME, methotrexate, and pulse corticosteroids are considered for third line therapy (127). Mortality in chronic GVHD is largely due to infection, especially in the setting of immunosuppression. Therefore, all patients should receive antimicrobial prophylaxis against PCP and encapsulated organisms.

Poor prognostic factors include extensive skin involvement (>50%), thrombocytopenia (<100,000/uL), and progressive onset chronic GVHD without resolution of acute

GVHD. Other risk factors for poor outcome include old age, poor performance status, and hyperbilirubinemia (128).

GRAFT FAILURE

Graft failure is a life threatening complication that occurs occasionally following AHSCT. It can be early, as evidenced by lack of initial hematopoietic recovery, or late, in association with recurrence of the disease or reappearance of host cells after initial donor cell engraftment. The incidence of graft failure following AHSCT is below 5% (103). Risk factors include donor-recipient HLA or ABO mismatch, unrelated donor alloantibodies, less intense conditioning regimen, T-cell depletion, insufficient number of transplanted stem cells, and viral infections such as CMV infection (129, 130).

The pathophysiologic characteristics of failure of sustained and complete engraftment are not completely understood. There is some evidence to suggest that graft failure may result from a graft-versus-marrow effect or from an abnormal microenvironment (131). It is important to differentiate between graft failure and severe marrow suppression secondary to infections (CMV), administration of certain drugs (Ganciclovir), and chronic GVHD causing thrombocytopenia.

Management of graft failure includes augmentation by hematopoietic growth factors, administration of additional hematopoietic stem cells, and second transplantation with pre-infusion conditioning (129). Bone marrow or peripheral blood stem cells from the original donor or another donor may serve as the additional AHSCT. In a retrospective study looking at 82 patients with acute leukemia, aplastic anemia, or chronic myeloid leukemia who underwent a second transplant, the engraftment rate was 73% with a day-100 transplant-related mortality of 50% (132). Although the efficacy of a second transplant has been shown in some patients, the best source of AHSCT (bone marrow versus peripheral blood) remains unclear (133).

RELAPSE RISK

Relapse rates after AHSCT vary by disease type, disease status at transplantation, and by the presence of GVHD. For patients with AML/MDS, the relapse rate ranges from 20 to 90% (134, 135). The detection of minimal residual disease (MRD) from the peripheral blood and bone marrow has been shown to be a predictor of outcome in ALL, AML, and CML (136). When relapse occurs after AHSCT, the prognosis is usually poor (137, 138). The standard initial intervention, once disease recurrence has been identified, is reduction or discontinuation of immunosuppressive therapy. This approach has induced remission rates ranging from 84% in chronic phase CML, to 10% in AML, and 0% in ALL (139). In patients with acute and chronic leukemia, the duration of remission after the first transplant appears to be the most important prognostic factor (140, 141). More favorable outcomes are also seen in patients who achieve complete remission before a second AHSCT (140, 141). Two prospective studies have evaluated the strategy of reduction of disease burden with

chemotherapy followed by granulocyte colony stimulating factor-primed DLI in patients with advanced myeloid malignancies (142, 143). Patients in remission lasting greater than 6 months had a greater likelihood of response. The efficacy of DLI was retrospectively studied in patients with AML in first relapse after AHSCT (144). Benefit of DLI was seen in a minority of patients, including those with a lower tumor burden at relapse (<35% BM blasts), female sex, favorable cytogenetics, and remission before DLI. Low dose azacitidine, prior to DLI or second AHSCT, has been used to decrease the tumor burden especially in AML patients with MRD following AHSCT (145).

Other studies suggested that the use of prophylactic/maintenance azacitidine, following AHSCT, may help prevent relapse in high risk AML patients by augmenting GvL effect without increasing GVHD. Hypomethylating agents were also found to be beneficial as salvage treatment for AML patients who relapsed following AHSCT (146). Treatment with either azacitidine or decitabine was shown to induce a CR in 75% of patients with relapsed AML or with loss of donor chimerism (LDC) after AHSCT for both AML and high-risk MDS patients (147). A study using Low-dose azacitidine as a maintenance or salvage treatment after AHSCT for patients with AML/MDS showed a one year overall survival rate of 90% (145).

A second AHSCT should be considered in selected patients whose duration of remission is more than 6 months after the first HSCT. The choice of second HSCT versus DLI should be individualized on the basis of donor availability and achievement of remission before DLI or second HSCT.

LATE COMPLICATIONS

Several delayed complications after AHSCT can occur and require long-term follow-up (Table 3). The use of TBI and chemotherapy as part of the conditioning regimen prior to AHSCT can induce secondary malignancies. This is attributed to the mutagenic risk of irradiation and chemotherapy, the genetic predisposition of the patient to develop cancer, prolonged immunosuppression, and an age-related risk for elderly patients.

A large report, including more than 1000 patients transplanted before December of 1985, showed that the incidence of secondary malignancies is around 3.5% at 10 years and 12.8% at 15 years, which is a 3.8-fold higher than that of an age-matched control population (148). A recent update on the study demonstrated that the incidence of secondary malignancies continues to increase with longer follow-up time compared to the control population (149). Another large analysis, which included 28,874 patients showed that solid tumors are twice more frequent than expected in the general population (150). This risk reached threefold among patients followed for 15 years or more. In a study including acute and chronic myeloid leukemia patients receiving high-dose busulfan and cyclophosphamide conditioning, the cumulative incidence of solid cancers at 10 years was 1.2% for acute myeloid leukemia, which was 1.4 times higher than the ex-

Table 3. Late Complications

Secondary malignancies	Increased incidence for tumors of the oral cavity, esophagus, lung, breast, soft tissue, and brain.
Reproductive	Infertility in men and women.
Endocrine	Clinical hypothyroidism, osteoporosis Cataracts
Pulmonary	Obstructive pulmonary defects. Bronchiolitis obliterans.
Liver	Hepatitis B and hepatitis C infections leading to fulminant hepatitis or cirrhosis.
Hematologic	Iron overload.
Neurologic	Leukoencephalopathy. Cerebellar ataxia and seizures. Motor spinal cord syndromes.

pected rate (132). Significantly elevated risks were observed for tumors of the oral cavity, esophagus, lung, soft tissue, and brain (151). A joint EBMT/Seattle study revealed that the cumulative incidence of breast cancer is around 5% at 20 years and the median time to breast cancer diagnosis is around 12.5 years (152). Cumulative incidence was higher among survivors who received TBI than those who did not receive TBI. Lifelong surveillance is important as secondary malignancy rates increase over time after AHSCT and is greater among younger patients.

Most men and women treated with TBI become infertile, and although a few women were reported to have been able to become pregnant, recovery of spermatogenesis in men is unlikely. However if high dose cyclophosphamide is used alone, many women younger than 25 years experienced recovery of ovarian function and most men experienced recovery of spermatogenesis (153).

Clinical hypothyroidism is another late complication that can occur as early as 1 year and as late as 15 years after AHSCT. The use of glucocorticoids to treat GVHD is associated with an increased incidence of cataracts. In a large series evaluating a cohort of 1064 patients, factors independently associated with an increased risk of cataracts were older age, highest dose rate of TBI, AHSCT, and steroid administration (154). Obstructive pulmonary defects can happen in 10% of patients with chronic GVHD approximately one year after transplantation. Bronchiolitis obliterans has been reported to occur in 2% to 14% of AHSCT patients and has a mortality rate of 50% (155). Late liver complications have been described to include hepatitis B and hepatitis C infections, which may be asymptomatic or progress to fulminant hepatitis or cirrhosis (156). Iron overload is diagnosed in up to 88% of long-term survivors of AHSCT (157). Neurologic toxic effects that may arise include leukoencephalopathy, cerebellar ataxia, seizures, and motor spinal cord syndromes.

CONCLUSION

AHSCT provides prolonged remission in many patients with hematologic diseases and cure in some

patients. Complications such as acute and chronic GVHD, infections and to a lesser extent SOS, remain major obstacles for the success of AHSCT. Progress in prevention approaches as well as advances in the conventional management of the underlying malignancies, expansion of the donor pool and the use of nonmyeloablative regimens have improved the outcome of these patients. Lifelong observation and screening is of utmost importance because of the late serious complications that can develop in this patient population.

DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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