CLINICAL STUDY

Graft-versus-host disease management

Mistrik M, Bojtarova E, Sopko L, Masakova L, Roziakova L, Martinka J, Batorova A

Department of Hematology and Transfusiology Medical Faculty, Commenius University, Slovak Medical University and University Hospital Bratislava, Slovakia. **mistrik@pe.unb.sk**

ABSTRACT

Graft-versus-host disease (GVHD) remains a major problem of allogeneic hematopoietic-stem cell transplantation (HSCT) and an obstacle for successful outcome. Clinically significant acute GVHD (grade II or higher) developed in 20 to 65 percent of the patients. Death due to this complication accounts for approximately 50 percent of the deaths that are not due to a relapse of the neoplasm. Up to 70 % of patients who survive beyond day 100 develop chronic GVHD and it is the leading cause of nonrelapse mortality more than 2 years after allogeneic HSCT. In addition, chronic GVHD is associated with decreased quality of life, impaired functional status, and ongoing need for immunosuppressive medications. The incidence of chronic GVHD is increasing because of expansion of the donor population beyond HLA-identical siblings, older recipient age, use of peripheral blood cells as the graft source, and infusion of donor lymphocytes for treatment of recurrent malignancy after HSCT. With the current rush in new findings related to GVHD, we see a significant advancement in its management. Given these various new options and challenges, it is important to identify the minimal requirements for diagnosis and treatment of GVHD, as access to the most sophisticated advances may vary depending on local circumstances *(Tab. 4, Fig. 1, Ref. 51)*. Text in PDF *www.elis.sk.*

KEY WORDS: allogeneic hematopoietic cell transplantation, acute graft-versus-host disease, chronic graft-versus-host disease, graft-versus-host disease, first line graft-versus-host disease treatment, steroid-refractory graft-versus-host disease.

Allogeneic hematopoietic stem cell transplantation (HSCT) has evolved into the treatment of choice for a wide variety of hematologic malignancies and non-malignant disorders. Increasing number of HSCT are being performed every year and their indications have expanded in the recent years especially in elderly patients due to the implementation of reduced intensity conditioning (RIC) and in patients without HLA-matched donors owing to the use of cord blood from public banks and haploidentical HSCT. Despite marked improvement in supportive care, immunosuppressive therapy and HLA typing, graft-versus-host disease (GVHD) remains a major cause of peritransplant morbidity and non-relapse mortality, and remains the major obstacle for successful allogeneic stem cell transplantation. Approximately half of the transplanted patients develop clinically significant forms which require therapy, and above 10 % of the patients may die of it. However, GVHD is connected with anti-tumor activity, called graft versus tumor (GVT) or leukemia (GVL) effect, thus suppressing GVHD completely may increase the relapse rate of original disease. GVHD presents with heterogeneous symptoms involving multiple organs including gastrointestinal system, skin, mucosa,

liver and lungs. In the past clinical features developing within 100 days from HSCT were called acute (aGVHD) and those occurring after 100 days were labelled chronic GVHD. This definition has been revised and new categories have been added to the classification such as late-onset aGVHD (acute GVHD occurring after 100 days) and overlap syndrome which includes features of both acute and chronic GVHD (1). And also new definitions of organ system involvement have been introduced (2). Nowadays, the categorization of GVHD is based on combinations of clinical symptoms and the time of onset:

- Classic acute GVHD – cases present within 100 days of HSCT and display features of acute GVHD. Diagnostic and distinctive features of chronic GVHD are absent.
- Persistent, recurrent, late onset acute GVHD – cases present more than 100 days post-HSCT with features of acute GVHD. Diagnostic and distinctive features of chronic GVHD are absent.
- Classic chronic GVHD – cases may present at any time post-HSCT. Diagnostic and distinctive features of chronic GVHD are present. There are no features of acute GVHD.
- Overlap syndrome – cases may present at any time post-HSCT with features of both chronic GVHD and acute GVHD. On occasion, this is informally referred to as „acute on chronic" GVHD.

The incidence of aGVHD varies with incidence of grade II–IV at 40 % in matched related donor (MRD) transplant to 50% in matched unrelated donor (MUD) transplant. Risk factors for aGVHD include degree of HLA disparity, donor and recipient

Department of Hematology and Transfusiology Medical Faculty, Commenius University, Slovak Medical University and University Hospital Bratislava, Slovakia

Address for correspondence: M. Mistrik, MD, Department of Hematology and Transfusiology, Medical Faculty, Commenius University, Slovak Medical University and University Hospital Bratislava – Nemocnica sv. CaM Petrzalka, Antolska 11, SK-851 07 Bratislava, Slovakia. Phone: + 421.2.63532166, Fax: +421.2.63532167

gender disparity (female donor to male recipient), intensity of the transplant conditioning regimen, patient age, source of graft, previous alloimmunization of the donor and the type of GVHD prophylaxis. However, risk factors differ by underlying disease, requiring distinct risk models for each condition. The incidence and severity of aGVHD also appears to increase with pre-transplant comorbidities. About 30–70 % of allogeneic HSCT recipients alive after 100 days develop cGVHD, and it is the leading cause of late death. One should expect increase in acute and chronic GVHD incidence in the future due to increasing use of mobilized peripheral blood graft, and unrelated and/ or mismatch HSCT (3). Despite the infusion of HLA class I and II disparate grafts, the incidence and severity of acute and chronic GVHD among unrelated umbilical cord blood recipients has thus far been lower than previously reported in recipients of matched unrelated donor marrow or partially-matched family member marrow allograft.

GVHD pathophysiology

Host antigen presenting cells (APCs) activation leads to donor T cells proliferation, differentiation and migration leading to destruction of target tissues (Fig. 1). Initial step in aGVHD is the activation of host APCs, which is mediated by the underlying disease process and the conditioning regimen through tissue damage. The damage to host tissues leads to production of chemokines and cytokines (tumor necrosis factor – TNF α, interleukin – IL-1, -2 and -6), and increased expression of major histocompatibility complex (MHC) antigens, adhesion molecules and costimulatory molecules in the tissue. The injury to the gastrointestinal tract from the conditioning regimen causes translocation of proinflammatory stimuli (such as bacterial lipopolysaccharide) that activate host APCs. In the second step the donor T cells are activated by

APCs and then differentiate and proliferate. The first interaction between APCs and T cells takes place in the lymphoid tissues associated with gastrointestinal (GI) tract (Peyer's patches). T cell activation is aided by costimulatory molecules on the surface of APCs. In HLA identical HSCT the GVHD is produced by CD4+ and CD8+ cells in response to minor histocompatibility antigen differences. Activation of the immune cells leads to transcription of genes leading to increased production of cytokines and their receptors. The effector phase in the aGVHD pathogenesis involves cytotoxic T cells. Chemokines direct T cell migration to the target organs where they cause damage. Expression of integrins and their respective ligands plays an important role in homing of donor T cells to Peyer's patches during aGVHD. The GI tract is especially susceptible to damage from TNFα and the GI tract play a major role in generation of the cytokine storm that is the characteristic of aGVHD (4). TNFα can be produced by both donor and host cells and produces myriad of effects including activation of APCs and alloantigen presentation, localization of immune effector cells to the target organs via increased chemokine production and causing direct tissue necrosis.

The pathophysiology of cGVHD is more complex. All the above mentioned mechanisms are relevant as well as other potential pathways. Thymic dysfunction caused by aGVHD has been implicated in the development of cGVHD. A newer role of B cells including immune regulation and immunostimulation via antigen presentation has been recognized in development of cGVHD (5). Antibodies to platelet-derived growth factor (PDGF) receptor, to extracellular matrix protein 1 and to Y chromosome mHA have been found in cGVHD patients, the levels of which are shown to be reduced with rituximab therapy (6). Multiple other auto and allo antibodies have been identified in patients with cGVHD, but the clear function of these antibodies in pathogenesis of cGVHD as
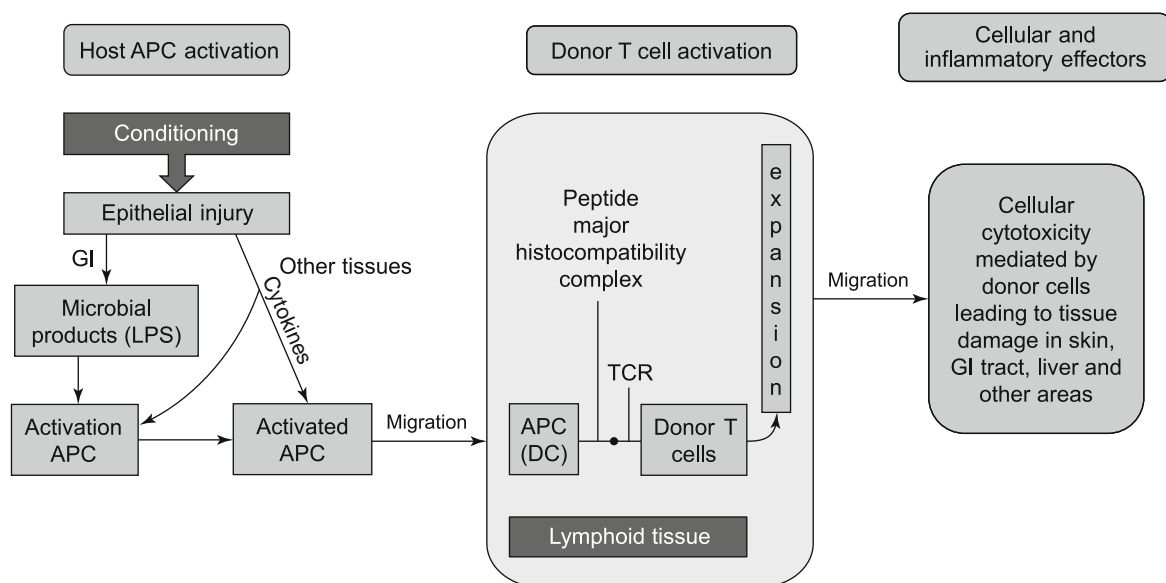

Fig. 1. A three-step GVHD pathogenesis. APC: antigen presenting cell; DC: dendritic cells; LPS: lipopolysaccharides; TCR = T cell receptor.

they have in other autoimmune diseases is unclear, and they possibly represent immune dysregulation which is a hallmark of GVHD.

Regulatory T Cells (CD4+, CD25+) (Tregs) play important roles in the modulation of GVHD, and Treg deficiency is in cGVHD patients (7). Tregs suppress proliferation and function of TH1 cells which are the main effector of GVHD (8). Donor CD4+ T and B cells are essential for development of cGVHD. Early donor dendritic cell reconstitution is associated with decreased incidence of severe GVHD (9). From day 100 onwards after allogeneic HSCT the persistence of host dendritic cells correlates with onset of severe GVHD. Regulatory dendritic cells have a protective effect against cGVHD which is mediated by generation of alloreactive Tregs (10). The upregulation of natural killer cells (NK cells) is associated with reduced incidence of GVHD (11).

Acute GVHD clinical and histological manifestations

Acute GVHD classically presents in the early post-transplantation period. The initial signs and symptoms of aGVHD most commonly occur around the time of white blood cell engraftment. Although initial definitions of aGVHD required an onset of symptoms before 100 days post transplantation, the current National Institutes of Health (NIH) consensus criteria use clinical findings, rather than a set time period, to differentiate between acute and chronic GVHD. As such, patients presenting with typical findings of aGVHD prior to day 100 are considered to have "classic acute GVHD," whereas patients presenting with the same findings after day 100, typically upon reduction of immunosuppression, are categorized as having "late onset acute GVHD". Some clinicians also use the terms "early onset acute GVHD" or "hyperacute GVHD" to describe symptoms of aGVHD occurring within 14 days of transplant.

The immune system, skin, gastrointestinal tract, and liver are the principal target organs in patients with acute GVHD. In most patients, the first most common clinical manifestation of acute GVHD is skin involvement, a maculopapular rash, usually occurring at or near the time of the white blood cell engraftment. The rash initially involves the nape of the neck, ears, shoulders, the palms of the hands, and the soles of the feet. It can be described as a sunburn and may be pruritic or painful. From these initial areas of presentation, the rash may spread to involve the whole integument, eventually becoming confluent. In severe GVHD, the maculopapular rash forms bullous lesions with toxic epidermal necrolysis mimicking Stevens–Johnson syndrome. Histologic examination of the skin reveals changes in the dermal and epidermal layers. Characteristic findings include exocytosed lymphocytes, dyskeratotic epidermal keratinocytes, follicular involvement, satellite lymphocytes adjacent to or surrounding dyskeratotic epidermal keratinocytes, and dermal perivascular lymphocytic infiltration. The most consistent histologic feature is individual cell death (apoptosis) at the base of crypts. However, similar changes can result from cytotoxic therapy used in the preparative regimen for HCT, and bacterial or viral infections or reactivations.

Acute GVHD frequently involves both the upper and lower gastrointestinal tract. Gastrointestinal involvement usually presents with diarrhea and abdominal pain, but may also manifest as nausea, vomiting, and anorexia. Confirmation of the diagnosis is provided by pathologic evaluation of tissue obtained by upper endoscopy, colonoscopy. And most cases of acute GVHD of the gastrointestinal tract can be identified by rectal biopsy. However, a negative rectal biopsy does not rule out gastrointestinal GVHD. Further evaluation with upper endoscopy or colonoscopy should be performed for patients with clinical symptoms suggestive of gastrointestinal involvement in the setting of a negative rectal biopsy.

The diagnosis of gastrointestinal involvement requires pathologic evaluation of the tissue.

Involvement of the lower gastrointestinal tract with acute GVHD is often severe, and is characterized by diarrhea, with or without hematochezia, and abdominal cramping. Confirmation of the diagnosis is performed by pathologic evaluation of tissue obtained by rectal biopsy or colonoscopy. Patients with acute GVHD can develop severe diarrhea, occasionally exceeding 10 liters a day. The stool may initially be watery, but frequently becomes bloody. Maintenance of adequate fluid balance may be extremely difficult in such patients. The blood loss can result in significant transfusion requirements. It is not unusual for patients to require frequent transfusions of packed red blood cells per day to maintain a stable hematocrit. The diarrhea is secretory and characteristically continues despite fasting and occurs day and night. It can be accompanied by crampy abdominal pain that can also be difficult to manage. Severe ileus may develop in association with acute GVHD or result from increased opioid use required to control the physical discomfort.

From a diagnostic viewpoint, diarrhea, independent of the presence of acute GVHD, is a common occurrence following HSCT. During the first weeks, diarrhea may be due to the preparatory regimen or to the administration of nonabsorbable or systemic antibiotics. Later, superinfection and Clostridium difficile-associated diarrhea must be taken into consideration as possible causes. Radiologic findings are not diagnostic of GVHD, but if performed for other reasons may show luminal dilation with thickening of the small bowel wall (called a „ribbon sign") and air or fluid levels suggestive of ileus. On endoscopy, acute GVHD manifests as spotted erythema, aphthous lesions, and denudation of the mucosa. While acute GVHD of the intestine may be suggested by changes on endoscopy, pathologic evaluation of tissue is required for the diagnosis and visually normal mucosa does not eliminate the possibility of involvement.

A rectal biopsy is usually helpful in making the diagnosis of acute GVHD affecting the gastrointestinal tract. On histologic examination, crypt cell necrosis is observed with accumulation of degenerative material in the dead crypts. With severe disease, whole areas may be denuded with total loss of the epithelium, a finding similar to that observed in the skin. Colonoscopy or upper endoscopy is usually also performed. Infection of the gastrointestinal tract, principally with cytomegalovirus, may mimic the clinical and histologic features of acute GVHD. As a result, selective staining for such pathogens should be performed on the biopsy specimen.

Involvement of the upper gastrointestinal tract with acute GVHD often presents with anorexia, dyspepsia, food intoler-

ance, nausea, and vomiting. Patients may also display gingivitis and mucositis, although these findings are more common due to the effects of conditioning regimens. The diagnosis is verified by positive upper endoscopic biopsies of the esophagus and stomach. The differential diagnosis includes herpes simplex virus or candida esophagitis, gastritis, peptic ulcers, and gastrointestinal toxicity due to chemotherapy and/or radiation.

Acute GVHD affecting the upper gastrointestinal tract appears to be more responsive to immunosuppressive therapy than involvement of other areas of the gut. Patients with upper gastrointestinal disease who fail treatment progress to symptomatic lower gastrointestinal involvement, which suggests that this syndrome may be an earlier form of intestinal pathology. Initial studies suggest that patients with steroid refractory gastrointestinal tract involvement have expanded T cell clones within the gastrointestinal tract.

Liver involvement usually presents in patients with signs of cutaneous and/or gastrointestinal acute GVHD. Rarely, patients have moderate to severe hepatic GVHD without evidence of other organ involvement. Although liver involvement may be suggested by abnormalities in liver function tests in the setting of cutaneous or gastrointestinal GVHD, liver biopsy is required to document GVHD of the liver.

Hepatic involvement is manifested by abnormal liver function tests, with the earliest and most common finding being a rise in the serum levels of conjugated bilirubin and alkaline phosphatase. Serum cholesterol is usually elevated, while coagulopathy and hyperammonemia are very rare but may develop in severe cases. Patients may also demonstrate painful hepatomegaly, dark urine, pale stool, fluid retention, and pruritus. Fever, anorexia, and nausea are common nonspecific symptoms.

The abnormalities in liver function tests reflect the pathology associated with liver GVHD: damage to the bile canaliculi, leading to cholestasis. Temporary dilatation of the common bile duct has been described in this setting. However, a rise in the serum concentration of bilirubin or alkaline phosphatase is nonspecific. In this setting, the most common confounding disorders include:

- Hepatic sinusoidal obstructive syndrome (also known as hepatic veno-occlusive disease) is a relatively common toxicity associated with the use of high dose therapy.
- Hepatic infections (primarily viral hepatitis)
- Effects from the preparatory regimen
- Drug toxicity, including the drugs used for GVHD prophylaxis (cyclosporine and/or methotrexate)

Although the concurrent presence of the characteristic rash provides suggestive clinical evidence, biopsy is the most definitive method to diagnose GVHD of the liver. However, this may not be feasible because of the possibility of acute bleeding due to severe thrombocytopenia soon after HSCT. A transjugular hepatic biopsy may be preferred if an adequate amount of tissue can be obtained. The primary histologic finding is extensive bile duct damage (eg. atypical bile duct and degeneration, epithelial cell dropout, lymphocytic infiltration of small bile ducts), leading to occasionally severe cholestasis.

Acute GVHD diagnosis

The diagnosis of acute graft-versus-host disease should be considered in any patient who has undergone allogeneic hematopoietic cell transplantation. Acute GVHD can occur at any time point in the post-HSCT setting, but most commonly occurs within the first few months after transplantation or following a reduction of immunosuppression. The diagnosis of acute GVHD can be readily made on clinical grounds alone in the patient who presents with a classic rash, abdominal cramps with diarrhea, and a rising serum bilirubin concentration within the first 100 days following transplantation. In many cases, however, the diagnosis is less straightforward and competing causes for isolated abnormalities must be considered and excluded. Rash alone may be caused by antibiotics or a myriad of other drugs with which these patients are often treated, diarrhea may be infectious in nature, and hyperbilirubinemia may be related to biliary sludge or a side effect of multiple drugs. Histologic confirmation may be helpful to corroborate a clinical impression of possible acute GVHD. The skin and gastrointestinal tract are relatively easy to biopsy. As previously mentioned, percutaneous liver biopsy poses a significant risk of major bleeding since most patients are thrombocytopenic at the time of presentation with GVHD. Transjugular liver biopsy is a safer alternative if it can be adequately performed.

Acute GVHD differential diagnosis

In most cases, acute graft-versus-host disease (GVHD) is a diagnosis of exclusion and other possible causes of clinical symptoms must be considered. The differential diagnosis depends upon the presenting signs and symptoms of acute GVHD. Most alternative diagnoses can be excluded with biopsy of the involved tissue.
- Skin involvement – The differential diagnosis of patients presenting with signs and symptoms of GVHD of the skin includes other causes of rash, including drug eruptions, viral exanthemas, engraftment syndrome, and radiation dermatitis.
- Gastrointestinal tract – The differential diagnosis of GVHD of the gastrointestinal tract includes other causes of nausea, vomiting, diarrhea, and weight loss. These include infectious causes (Clostridium difficile infection, CMV reactivation), drug effects, chemoradiation toxicity, inflammatory diarrhea, short bowel syndrome, peptic ulcer disease, neoplasms, and systemic disease (eg. diabetes mellitus).
- Liver involvement – The differential diagnosis of hepatic GVHD includes other causes of abnormal liver function tests (increased conjugated bilirubin and alkaline phosphatase). Common confounding disorders also include hepatic sinusoidal obstructive syndrome, hepatic infections (primarily viral hepatitis), effects of the preparatory regimen, and drug toxicity. At times a specific diagnosis is difficult without biopsy.

A continuum of clinical findings may be observed in patients with acute and chronic GVHD, as both disorders commonly affect similar organs, principally the skin, liver, and gastrointestinal tract. However, the target organs affected by, and the clinical and histologic features associated with, chronic GVHD may differ from

those observed with acute disease. As an example, autoimmune phenomena, such as autoantibody formation, are more common with chronic GVHD. Importantly, diagnostic or distinctive signs of chronic GVHD must be absent in order to make the diagnosis of acute GVHD. Patients with signs or symptoms seen in both entities are considered to have the overlap subtype of chronic GVHD.

Acute GVHD classification

Acute GVHD is staged and graded based on the degree of organ (skin, liver and gastrointestinal tract) involvement and clinical status of the patient. The degree of skin involvement is graded depending upon the range, degree and severity of the lesions, liver involvement upon the serum total bilirubin (μmol/l) level, and gastrointestinal involvement upon the severity of diarrhea (ml/day). The clinical features and staging and grading of aGVHD are described in Tables 1, 2 and 3, respectively (12; 13). The overall severity of aGVHD has major impact on HSCT outcomes, with transplant-related mortality ranging from 28 for stage 0 to 92 % for stage IV disease (14).

Treatment of aGVHD

Steroids and calcineurin inhibitor (CI) remain the gold standard for initial treatment of aGVHD (15). Mild skin aGVHD (grade I) can be treated with topical steroids alone. For more severe disease or any visceral involvement (grade II–IV) systemic steroids and CI are the mainstay of treatment. Complete or partial responses are achieved in 44 % patients with improvement in skin, liver and gut disease at 43, 35 and 53 %, respectively. The response to initial treatment correlates directly with post-transplant survival (16).

The treatment for grade II–IV aGVHD is started with methylprednisolone intravenously (especially in gut GVHD) at 2 mg/kg/

Tab. 3. Overall clinical grading of acute GVHD.

Grade	Skin	Liver	Gastrointestinal tract
I	Stage 1	None	None
II	Stage 2	Stage 1-2 or	Stage 1-2 (or both)
III	Stage 3 or	Stage 3 or	Stage 3
IV	Stage 4 or	Stage 4 or	Stage 4

day with CI, and continued at that dose 1 to 2 weeks depending on response. If the patient responds well, steroids dose is tapered down to 1.5 mg/kg/ day for 1 week, 1 mg/kg/day for 1 week, then tapering is continued at the rate of 10 mg/week, and slow down tapering at doses lower than 30 mg/ day. Initial steroid dose 1mg/kg/day is recommended for aGVHD of the upper GI tract which presents with symptoms of anorexia, nausea/vomiting and dyspepsia. Also in skin GVHD steroid treatment is being started often at a lower dose. Optimal tapering of steroids is over a period of 86–147 days after initial response to treatment, as treatment with steroids especially at higher doses can lead to significant side effects including immunosuppression, hyperglycemia and osteopenia. Many agents (etanercept, mycophenolate, denileukin or pentostatin) in addition to steroids and CI have been evaluated for initial treatment of aGVHD, but they have failed to show significant benefit. Other agents such as basiliximab, daclizumab, antithymocyte globulin (ATG), etanercept and infliximab have also been tested without convincing results (17). Therefore, the addition of agents to high-dose steroids in first-line treatment is only recommended in the setting of clinical trials.

Treatment of steroid refractory aGVHD

If initial response to steroids is missing and aGVHD worsens in any organ during the first 3 days of steroid treatment or if there is no response during the first 5–14 days, a second agent is added and steroids are tapered 10 % or 10 mg every week from 2 mg/kg/day dose. The rate of the further tapering depends on the response. The 3 day criterion is especially used for lower GI aGVHD and secondary agents are introduced by fifth day. The decision to add second-line treatment should be made sooner for patients with more severe aGVHD and in patients who cannot tolerate steroid treatment. But none of the existing secondary agents provided convincing evidence for long-term benefits, and the outcome of steroid-refractory aGVHD remains very poor with mortality as high as 80 % (18).

The benefit of antithymocyte globulin (ATG) in steroid-refractory skin aGVHD is when used early. In a prospective randomized

Tab. 1. Symptoms of acute GVHD.

Skin	Maculopapular skin rash
Upper gastrointestinal tract	Nausea, anorexia, or both, and positive histological findings
Lower gastrointestinal tract	Watery diarrhea (≥ 500 ml) Severe abdominal pain Bloody diarrhea or ileus (after exclusion of infectious causes)
Liver	Cholestatic hyperbilirubinemia

Tab. 2. Organ staging of acute GVHD.

Stage	Skin	Liver (bilirubin; μmol/l)	Gastrointestinal (GI) (stool output in ml/ day)
0	No GVHD	<34	<500 ml (child <10 ml/kg/day) or persistent nausea
1	Maculopapular rash <25 % BSA	34–50	500–999 ml (child 10–19.9 ml/kg/day) or persistent nausea, vomiting or anorexia with positive upper GI biopsy
2	Maculopapular rash 25–50 % BSA	51–102	1000–1500 ml (child 20–30 ml/kg/day)
3	Maculopapular rash >50 % BSA or generalised erythroderma	103–255	1500-2000 ml (child >30 ml/kg/day)
4	Diffuse erythema with bullous formation or desquamation	>255	>2000 ml or severe abdominal pain, with or without ileus

BSA = body surface area

trial patients with steroid-refractory aGVHD were treated with 5 mg/kg/day methylprednisolone alone or in combination with rabbit ATG. There was no difference between the two arms in terms of response rates, survival or TRM (19). Alemtuzumab (Campath) is a very potent antibody with 50% responses even in grade III and IV aGVHD, but CMV reactivation and life-threatening infections occur. Thus caution should be taken not to use too high dose and it should be introduced earlier than later in the course.

Extracorporeal photopheresis (ECP) weekly until maximal disease response achieved CR rates of 82, 61 and 61 % for aGVHD of skin, liver and GI tract, respectively. Transplant-related mortality was only 14 % in patients treated with ECP (20). ECP shows benefit in the treatment of aGVHD, and is safe, without any increase in the rate of infections, secondary malignancies or mortality (21). Mycophenolate mofetil (MMF) treatment of steroid-refractory aGVHD was associated with responses, but this did not translate into long-term overall survival (22). Treatment of steroid-refractory grade III/IV aGVHD with sirolimus was associated with responses in 57 % patients (CR 24 %), but treatment was discontinued in 10 patients due to no response or toxicity (23). Pentostatin treatment achieved overall response rates greater than 50 % (24). Donor mesenchymal stem cells (MSCs) are helpful in the treatment of steroid-refractory aGVHD and their use in 55 patients was associated with CR in 30 patients and improvement in 9 additional patients (25).

Anti-interleukin 2 receptor antibody daclizumab was given as single second-line agent or added to cyclosporine and mycophenolate, and achieved significant amount of responses (26). Infliximab, a monoclonal antibody that binds to TNFα, has been shown to be associated with significant response although the proportion of patients with grade III–IV aGVHD was low and treatment was complicated by aspergillus infections (27). Etanercept, a soluble dimeric TNFα receptor 2 that competes for TNFα binding with cellular receptors, was shown to induce responses in patients with GVHD of the gastrointestinal tract (28). A study of pediatric patients of steroid-refractory aGVHD who were treated with a combination of daclizumab and infliximab showed response in 19 out of 22 patients (29). The published data suggests that treatment with TNFα inhibitors is associated with improved responses in steroid-refractory aGVHD, particularly the ones involving gastrointestinal tract.

Steroid-refractory gut aGVHD

When patient develops gut GVHD all medications should be changed to IV, particularly CI, for the concern of appropriate absorption, and TPN (total parenteral nutrition) is started. Methylprednisolone intravenously 2 mg/kg/day should be started, usually divided into twice a day doses. Prophylaxis for bacterial, fungal, Pneumocystis jirovecii and viral infections (acyclovir) should be initiated, or continued. If the patient does not respond to steroid for 3 days, he should start infliximab 5–10 mg/kg weekly × 4 doses. At the same time ECP should be initiated and budesonide should be given orally 3–6 mg daily to 3 times daily. When the patient is responding to the treatment, steroids are tapered first. Usually for these cases steroids are maintained 2 mg/kg/day for 2 weeks,

Tab. 4. Symptoms of chronic GVHD.

Skin	lichen planus-like eruptions, or sclerotic features, dyspigmentation, new-onset alopecia, poikiloderma
Nails	dystrophy or loss
Eyes	sicca syndrome, dry eyes, cicatrical conjunctivitis
Mouth	ulcers, lichen-type features, xerostomia, restrictions of mouth opening from sclerosis
Muscles, fascia, joints	myositis, fasciitis, or joint stiffness from contractures
Gastrointestinal tract	weight loss, anorexia, esophageal web or strictures
Liver	jaundice, elevated liver function tests
Lungs	bronchiolitis obliterans, restrictive or obstructive defects on pulmonary function tests, pleural effusions
Heart	pericarditis
Female genitalia	ulcerations, vaginal sclerosis
Marrow	thrombocytopenia, anemia, neutropenia, eosinophilia, pancytopenia

then start tapering 10 % weekly. If diarrhea volume is more than 500 ml/24 h and watery the taper is hold off. When stool volume is less than 500 ml/day and contains some solid particles and is getting "pudding-like", PO intake is initiated, first with clear liquid, then full liquid, then step up the diet very carefully, adding one food item a day. Fat, protein, and dairy products may predispose to diarrhea, so these are food items added last. Treatment for steroid-refractory gut GVHD is a long, painful process, and has high mortality, but it may be possible to save some of these patients by treating them very carefully.

Chronic GVHD classification

Chronic GVHD is classified as mild, moderate or severe according to the NIH consensus criteria (30). The organs commonly affected by cGVHD include skin, eyes, mouth, liver, gastrointestinal tract, lungs and genitalia. The response to treatment in cGVHD is unpredictable. Mixed responses are seen in different organs in the same patient. The risk factors for the development of cGVHD are similar to aGVHD. The impact of cGVHD on survival must be considered in balance with the fact that cGVHD is associated with lower risk of relapse in leukemia (GVL effect). The correlation between GVHD severity and relapse is unclear (31). The main clinical features are mentioned in Table 4 (32).

Treatment of chronic GVHD

Mild form of cGVHD often respond to topical treatment with corticosteroids, while systemic therapy is usually needed for treatment of moderate-to-severe cGVHD. Prednisolone 1 mg/kg/day alone is usually the first-line treatment. The duration of treatment depends upon response and is often prolonged with median duration 2–3 years (33). Addition of cyclosporine (CSA) (a CI) to steroids lowers the rate of avascular hip necrosis, but not TRM, progression to secondary therapy or duration of immunosuppression. Other agents including MMF have failed to improve results of primary treatment of cGVHD when added to steroids, and are associated with increased mortality (34). Bortezomib in combination with prednisone have been associated with overall response rate of 80 % with very little toxicity (35).

Progression of cGVHD despite 1 mg/kg/day of corticosteroids for 2 weeks or lack of improvement in symptoms after 4–8 weeks of continuous therapy or inability to taper corticosteroids are considered as markers of refractory disease (36). There are no standard treatments for steroid-refractory cGVHD. In addition, continuation more than 20–30 mg/day of prednisolone for more than several months is associated with significant toxicities, thus many agents have been tested for steroid-sparing effect. Rituximab has shown response rate of 60 % to 70 % in steroid-refractory cGVHD, but responses were mainly partial and were limited to skin and musculoskeletal disease (37). A recent small prospective study evaluated combination of rituximab with alemtuzumab in 15 patients with steroid-refractory cGVHD (38). The overall response rate was 100 % with 5 patients achieving complete response.

Extracorporeal photopheresis (ECP) and PUVA have shown benefit in the treatment of steroid-refractory or steroid-dependent cGVHD (39). Approximately 50 % patients have reduction in symptoms (40). PUVA is using direct irradiation to the skin and it may be very effective in selected cases with skin cGVHD. The treatment with TKIs (imatinib) appears to be effective particularly in refractory sclerotic cGVHD. Mycophenolate mofetil has shown response rate of 45 % in salvage therapy for cGVHD, but randomized prospective trial of MMF vs placebo in addition to other treatment for cGVHD was terminated early due to no difference in response rate in control and study arms (41). Common side effects of MMF include cytopenias, infections and gastrointestinal toxicity which can mimic aGVHD. Since that study MMF has been less commonly used in the treatment of cGVHD. Sirolimus has been used in combination with tacrolimus and corticosteroids for the treatment of steroid-refractory cGVHD, and overall response rate was 63 % (42). Another retrospective study of patients with severe sclerodermatous cGVHD treated with sirolimus showed a response rate of 76 % (43). Toxicities included thrombotic microangiopathy and renal dysfunction. It is recommended to monitor patients for renal function, hyperlipidemia, myelosuppression particularly thrombocytopenia and thrombotic microangiopathy while on sirolimus. Pentostatin given every other week for a median of 12 doses reported an overall response rate of 55 %, despite that most patients were heavily pre-treated (44). Low-dose IL-2 administered daily for 8 weeks induces Treg expansion and achieves partial responses in 50 % of patients, probability and magnitude of response was proportional to the duration of treatment (45). Patients also had improvement in advanced fibrotic and sclerotic manifestations of cGVHD which were previously thought to be irreversible. Bortezomib in a retrospective study of 37 patients with multiple myeloma treated with reduced intensity allogeneic HSCT in 11 patients showed responses with 3 responses in patients with severe cGVHD. Eight patients with limited disease did not require any additional immunosuppressive therapy (46). Thalidomide therapy has shown complete or partial response in patients with refractory cGVHD. Treatment was associated with frequent discontinuation due to toxicity such as neutropenia and neurologic side effects (47). Low dose methotrexate achieves a marked benefit in cutaneous disease in combination with other immunosuppressants (48).

Special considerations in cGVHD treatment

Severe sclerotic skin cGVHD initial treatment includes a combination of steroid and cyclosporine that may be replaced with sirolimus. Rituximab and ECP should be introduced relatively early. Imatinib should be started at as low as 100 mg every other day, and the dose should be increased until the patient can tolerate it because higher dose may be more effective. Physiotherapy to keep the activity up is a very important part of the treatment. Patients usually have impaired sweating and should be careful to stay in a well air-conditioned room and keep taking a lot of water in summer to avoid heat shock. Blisters and skin infections are treated with oral (doxycycline) and local antibiotics (mupirocin). Patients are at high risk to develop skin cancers, so if they develop suspicious lesions, dermatology consultation must be pursued.

Oral cGVHD is treated with dexamethasone rinse (0.5 mg/5 ml) 2–4-times a day (instruct the patient to spit out after rinse, as it may be too much systemic steroid if they swallow it) followed by nystatin swish. Occasionally clobetasol gel application on the erosive lesions is beneficial. Also tacrolimus elixir or sirolimus syrup is used instead of pills to provide respective medications, and the patients are instructed to swish in the mouth before they swallow them.

Most of the patients develop dry eyes, thus artificial tears without preservative are necessary to keep eyes moist. Tear duct plugging has been done to keep eyes as moist as possible and often works well. For more symptomatic patients, cyclosporine and/or steroid eye drop may be used, but cyclosporine eye drop may irritate the eyes. Eye drops made of autologous serum have been tried and were very effective in some cases (49). Scleral contact lenses, a large size contact lens which rests on sclera and creates a tear-filled vault over the cornea, may help in refractory cases.

A very few effective bronchiolitis obliterans treatments are available (50). Steroids may work partially, but not for a long time, thus steroid should be tapered to a dose the patient can tolerate. Pulmonary rehabilitation is important, and providing support for these patients to change their lifestyle is necessary. For severe cases, lung transplantation may be the only option.

Many patients with cGVHD may be working or would like to be back to work, and it is necessary to support these patients to maintain or find jobs. One should be aware of the transformed self-images particularly female patients with skin GVHD and/or with steroid effect. These patients should be provided with appropriate support including mental aspects. And many patients cannot perform as much as they could before GVHD, thus providing help to accept the situation and set up a new goal is important.

Conclusions

GVHD is potentially lethal complication and continues to limit survival in patients undergoing HSCT. In the last decade a lot has been learned regarding the mechanisms involved in the pathophysiology of GVHD (51). Recent developments have led to remarkable improvements in the assessment and treatment of patients with GVHD. New technologies have become available to evaluate the extent of the disease. Novel pathways are being tar-

geted and new agents are being developed/tested for the treatment of GVHD. But a combination of cyclosporin with corticosteroids remains the cornerstone of initial GVHD management. Durable responses with steroids are seen in less than half of the patients treated for aGVHD and about 40–50 % of cGVHD depending upon severity of the disease. Due to the lack of randomized controlled trials for the treatment of steroid-refractory disease, there is no clear consensus on what comprises the best second- and third-line approach in the treatment of acute and chronic GVHD. Once GVHD is steroid refractory, a large group of potentially effective drugs and procedures have been introduced into clinical practice, which enable clinicians to significantly improve the outcome of patients but also pose new challenges for the prevention and management of their specific side effects. New options need to be considered with great attention paid to the type and stage/grade of GVHD, side effects profile, drug interactions and possible obstacles in administration of the treatment agents. With the current rush in new agents and new findings related to GVHD treatment, we will see a significant advancement in this field in next years. Given these various new options and challenges, it is important to identify the minimal requirements for diagnosis and treatment of GVHD, as access to the most sophisticated advances may vary depending on local circumstances.

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