Clinical Update on Graft-Versus-Host Disease in Children

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The last decade has yielded many significant advances in hematopoietic transplantation techniques, immunomodulatory prophylaxis, and diagnostic and treatment approaches to acute and chronic graft-versus-host disease (GVHD). Unfortunately, GVHD remains the cardinal complication in allogeneic hematopoietic stem cell transplantation, with significant associated rates of morbidity and mortality. In this review, we highlight the numerous strides that have been made in making hematopoietic transplantation more successful and provide an update on the clinical and histopathological features of both acute and chronic GVHD in the pediatric population. It is critical for dermatologists to be aware of the characteristic features of cutaneous acute and chronic GVHD and to remain up to date on the evolving spectrum of these conditions. We discuss 5 cases with clinico-pathologic correlation to illustrate the key concepts and principles underlying the diagnosis and management of both acute and chronic GVHD.
Semin Cutan Med Surg 29:92-105 © 2010 Published by Elsevier Inc.

Illustrative Case

Our pediatric dermatology inpatient consultation service was asked by the Pediatric Bone Marrow Transplant team to see a 5-month-old Native American male with severe combined immune deficiency (SCID) who was +111 days status-post a haplocompatible T-cell–depleted peripheral blood stem cell (PBSC) transplant. His mother was the donor. His course was complicated by poor engraftment and chronic rotavirus infection requiring 2 donor lymphocyte infusions (DLIs) on days +45 and +89 after transplant.

We had previously been asked to see this patient for a morbilliform eruption involving his face and extremities 30 days previously (day +76 after transplant) and biopsy at that time had shown spongiotic dermatitis, consistent with a viral exanthem. We then examined a new skin rash that had started on day +102 in the setting of liver abnormalities and persistent diarrhea with stool cultures positive for rotavirus.

The patient's examination revealed confluent erythema involving approximately 80% of his body surface area (BSA), with overlying 2- to 3- mm lichenoid papules accentuated on the dorsal hands and feet (Fig. 1A). Skin biopsy revealed vacuolization of basal keratinocytes with extension of apoptotic keratinocytes down acrosyringium with lymphocyte satellitosis, consistent with Lerner Grade II acute graft-versus-host disease (GVHD; Fig. 1B).

His eruption progressed quickly to involve more than 90% BSA with toxic epidermal necrolysis (TEN)-like dusky erythema, bullae and mucosal involvement (Fig. 1C, D). A frozen section biopsy of the roof of one of the bullae confirmed full-thickness epidermal necrosis. Given the clinical setting and results of his previous biopsy, the diagnosis of TEN-like acute GVHD (aGVHD) was given. The patient was treated with intravenous methylprednisolone, tacrolimus, daclizumab, high-dose intravenous immunoglobulin, and finally, as rescue therapy, mesenchymal stem cells (MSCs). After receiving his first dose of MSCs, he showed a marked response with decreased erythema within 24 to 48 hours, and his skin began to heal within 72 hours. He was slowly but successfully weaned off prednisolone and was discharged 2 months later on tacrolimus alone after a very complicated, morbid course.

During the subsequent 4 weeks, while his tacrolimus was being tapered, he developed a new rash involving 25% BSA

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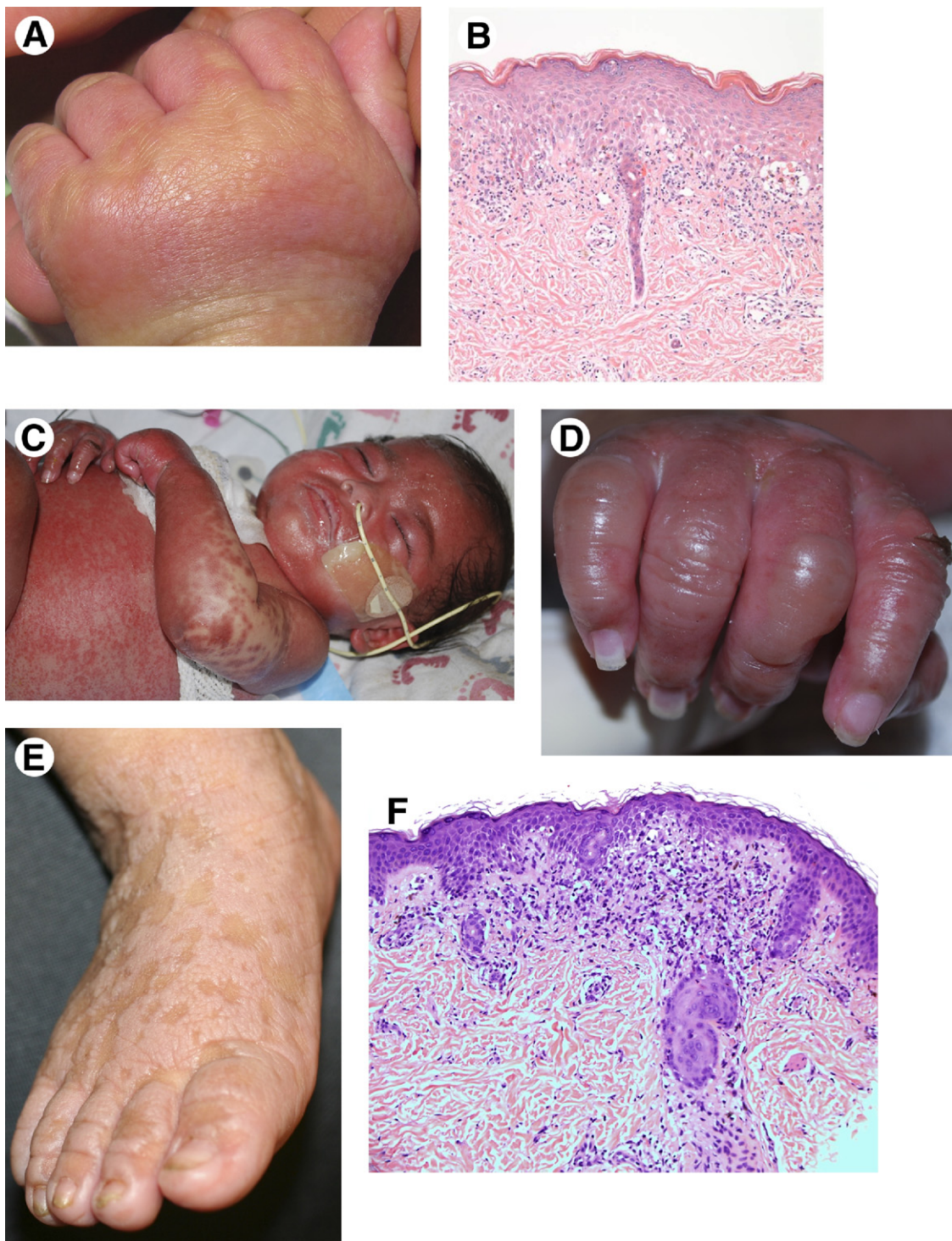
Figure 1 (A) Erythematous lichenoid papules distributed over the dorsal hand and fingers. (B) Histopathology showing vacuolization of basal keratinocytes with extension of apoptotic keratinocytes down acrosyringium and lymphocyte satellitosis (magnification ×20). (C) Widespread erythematous to dusky macules involving the face, trunk, arms, and legs (Used with permission.) (D) with bullae on acral sites. Histopathology of a blister roof showed full-thickness epidermal necrosis (not shown). (E) Lichenoid papules coalescing into plaques distributed over the dorsal foot. (F) Histopathology showing vacuolization and necrosis of basal keratinocytes with extension of apoptotic keratinocytes down acrosyringium (magnification ×20).

on his trunk and extremities, as well as vomiting and diarrhea. On examination day +218 after transplant, he demonstrated urticarial plaques on his trunk and proximal thighs and lichenoid papules on his proximal arms, dorsal hands, and feet (Fig. 1E). Biopsies of both morphologies confirmed

Lerner Grade II aGVHD (Fig. 1F). Immunosuppression was increased, including prednisolone and sirolimus, and his skin improved during the next 3 weeks.

We begin with this complex case to illustrate some of the key concepts of both aGVHD and chronic GVHD (cGVHD).

Importantly, the clinical and histopathologic spectrum is broad, and overlap is observed between these entities. There can be discordance between the clinical morphology and the histopathologic findings, underscoring the need for an updated classification system and a better understanding of the spectrum of GVHD. We will return to this case throughout the following discussion as we dissect and attempt to clarify in a practical and useful manner the current body of knowledge surrounding aGVHD and cGVHD.

Introduction

GVHD remains one of the most exciting, challenging, and important diagnoses that physicians make. Its clinical manifestations and histologic features can closely mimic those of numerous other conditions in the posttransplantation period, especially viral exanthems and drug eruptions.[1] In some cases, the diagnoses of engraftment syndrome or eruption of lymphocyte recovery also need to be considered (discussed below). As the number of patients being transplanted grows each year, dermatologists, including pediatric dermatologists, are being asked to evaluate patients and consider this diagnosis at an increasing rate.

In the following article, we review advances in hematopoietic stem cell transplantation (HSCT) and discuss the current understanding of aGVHD and cGVHD pathophysiology and treatment, particularly as they pertain to children. We also provide clinical cases with pathologic correlation to illustrate some of the primary morphologic and histologic presentations observed in these complex patients.

Hematopoietic Stem Cell Transplantation

Stem cell transplantation has changed dramatically during the past 42 years since successful transplantation was originally described in humans.[2] There are an estimated 50,000 transplantation procedures undertaken annually worldwide, and this number continues to grow.[3] In North America, 20% to 25% of all transplantation procedures are performed in children.[3] The list of indications has rapidly expanded to include many life-threatening malignant and nonmalignant diseases, such as primary hematologic and metabolic disorders, immunodeficiency syndromes, and genodermatoses. In the pediatric setting, roughly two-thirds of transplants are performed for malignant conditions, whereas one-third are performed for nonmalignant diseases. The technology used in the procedure has dramatically changed since the late 1960s, and like many areas of medicine, continues to evolve constantly. We review here the various aspects of hematopoietic transplantation that have changed: donor type, graft source, degree of human leukocyte antigen (HLA) match, conditioning intensity, use of DLIs, and GVHD prophylaxis.

Graft Source

There are 3 potential donors for hematopoietic transplantation: autologous, syngeneic, and allogeneic. In autologous

scenarios, the donor and the recipient (host) are the same individual. In syngeneic cases, the donor and recipient share identical genotypes, as is the case for identical twins. Finally, in allogeneic settings, the donor and recipient have related but sufficiently dissimilar genotypes that they interact antigenically. In the pediatric setting, allogeneic transplantation is most common and is used for the majority of indications. Autologous transplant is reserved for some lymphomas and solid tumors.

In addition, there are now 3 potential sources of stem cells used for transplantation: bone marrow (BM), PBSCs, and umbilical cord blood (UCB). PBSC transplants are used increasingly in adults because they provide easier mobilization and faster hematopoietic recovery, with greater $CD34^+$-cell and T-cell counts. Research has shown that pediatric patients receiving allogeneic PBSC transplants have poorer outcomes than adult patients, including greater rates of treatment-related mortality, treatment failure, and cGVHD; thus, PBSC is used less in pediatric patients compared with adults.[4] UCB is increasingly used in both children and adults, and has made allogeneic HSCT available to many patients who do not have an HLA-identical sibling or unrelated donor. Although hematopoietic recovery is slower with UCB compared with BM or PBSCs, the benefits of using UCB as a source include (1) increased availability and faster access to cells (cryopreserved); (2) absence of risk to the donor; (3) expansion of the donor pool because of the tolerance of 1 or 2 in 6 HLA mismatches; (4) lower risk of latent virus transmission; and (5) lower severity and frequency of aGVHD (possibly because of the presence of a naive immune system).[5,6] Prospective randomized clinical trials are needed that compare outcomes of allele-matched BM and allele-mismatched UCB transplants in children.

Degree of HLA Matching

When available, an HLA-matched sibling is always the donor of choice for children who need HSCT. Unfortunately, only 30% of pediatric patients who are in need of HSCT have such a donor available. Barriers to successful HLA-mismatched HSCT include increased risk of graft failure and possible induction of severe and refractory aGVHD and/or cGVHD. Even when immunosuppression is used to control the immune response and successfully prevent GVHD, delayed immune reconstitution and risk of fatal infection become major obstacles. Fortunately, the last decade has yielded significant improvements in HLA matching, with high-resolution DNA typing of HLA genes with polymerase chain reaction-based techniques replacing previous less accurate methods. More optimized matching at the HLA A, B, and C (class I proteins, expressed on all nucleated cells) and DRB1 (class II proteins expressed on hematopoietic cells) antigens, referred to as 8/8 matching, leads to better outcomes (ie, lower risk of graft failure and lower risk of GVHD).

Because of the technology improvements in HLA matching, the use of unrelated donors has increased dramatically in the past decade. However, 40% of recipients of fully matched (8/8) grafts still develop aGVHD, suggesting there are other

factors that mitigate risk of GVHD.[7] These factors are likely minor histocompatibility antigens and/or other cytokine polymorphisms (including tumor necrosis factor [TNF] alpha, interleukin-10, and interferon-γ).[7,8] The importance of these other factors is underscored by the lower risk of aGVHD for matched related donor transplantation (30%-60%) versus that for matched unrelated donor (MUD) transplantation (50%-80%).[9] UCB transplantation has been very successful despite increased use of HLA-mismatched grafts (with up to 2 alleles mismatched). On rare occasions, when an appropriate MUD or UCB donor cannot be found, a T-cell−depleted haplocompatible transplant from a parent using 3/6 matched alleles can be used, as was the case for the index case described previously.

Conditioning Regimens

Pretransplantation conditioning regimens have changed dramatically in recent years. Conditioning involves treatment with chemotherapy and/or radiotherapy to reduce tumor burden, lower immunoreactivity of the host, and allow engraftment of transplanted cells. Traditionally, myeloablative conditioning was the standard of care. In the pediatric setting, irradiation is primarily used in the treatment of acute lymphoblastic leukemia, and if mismatched UCB is used as a stem cell source. Chemotherapy-only conditioning regimens are commonly used in children with myeloid malignancies and nonmalignant disorders and commonly require the use of busulfan in combination with 1 or 2 other drugs, such as cyclophosphamide, fludarabine, and melphalan. Although high-dose chemotherapy and total-body irradiation regimens used to eradicate malignant cells as well as host stem cells allowed for engraftment of immunocompetent donor cells, these also led to considerable transplant-related morbidity and mortality. In the past 10 to 15 years, reduced-intensity conditioning (RIC; "mini-ablative") regimens have been developed to induce sufficient immunosuppression to allow engraftment but limit tissue injury. Enthusiasm for RIC stems in part from the recognition that the curative potential of HSCT for malignant indications lies in the graft versus tumor (GVT, or graft vs leukemia [GVL]) effect.[10] It is important to recognize that the reduction in nonrelapse-related mortality that has been realized with the use of RIC has not translated into improvement in overall survival because the risk of relapse-related mortality is greater in RIC than in traditional myeloablative conditioning, thus offsetting the benefit.[9]

Use of Donor Lymphocyte Infusions

DLIs have been increasingly used in both malignant and nonmalignant indications and after both myeloablative and nonmyeloablative stem cell transplantation for many reasons. As was the case for our index patient, they are used to treat and prevent infection (eg, rotavirus for our index case) and promote engraftment. They are also used to treat and prevent relapse and to establish full donor chimerism. The most significant complication of DLI is GVHD.

The occurrence of GVHD after DLI is similar to its presentation and treatment after HSCT. However, a distinctive feature of DLI is that GVT effects can occur in the absence of GVHD. Further studies are needed to try to identify GVT effector cells and tumor-specific antigens to optimize the GVT effects and minimize GVHD. Our index patient developed severe TEN-like cutaneous (Fig. 1C, D) and liver GVHD after his second DLI, which was administered to improve his engraftment and facilitate clearance of his chronic rotavirus infection.

GVHD Prophylaxis

Nearly every pediatric patient undergoing HSCT receives immunosuppressive prophylaxis against GVHD by the use of a 2-drug regimen, with either cyclosporine or tacrolimus and methotrexate or mycophenolate mofetil starting before transplant and typically continuing for up to 6 months after transplant. In addition, other newer methods, including T-cell depletion (TCD), are used to try to modulate the activated T-cells in the graft or attenuate T-cell proliferation.[11] Importantly, prophylaxis leads to lower frequency and intensity of aGVHD but does not affect posttransplant mortality or overall survival. Despite these modifications, GVHD still occurs at a significant rate and remains a major complication of hematopoietic transplantation.

Graft-Versus-Host Disease

What Is GVHD?

GVHD is an immunologic reaction that is the most frequent complication after allogeneic HSCT and comprises the primary obstacle for expanding the application of this therapy.[9] In 1966, Dr Billingham defined 3 criteria that need to be met for GVHD to occur: (1) graft contains immunologically competent cells, (2) antigenic disparity exists between host and donor tissues, and (3) incompetence of the host to reject the graft.[12] We now know that the immunologically competent cells in transplantation that Dr Billingham referred to are donor T-cells, which recognize and respond to recipient proteins (major and minor histocompatibility antigens). Dendritic cells, natural killer cells, macrophages, mast cells, and cytokines also play a major role.[7]

How Do Pediatric and Adult GVHD Differ?

In general, GVHD is more common in adults than in children and adults with GVHD usually require longer duration of therapy. Many study investigators identified increasing age of both donors and recipients to be a risk factor for development of aGVHD and cGVHD. T-cells that develop from transplanted stem cells undergo induction of tolerance in the thymus. During this process, the donor T-cells are "educated" to recognize self antigens, and clones that are self-reacting are deleted. Because thymic function decreases with age, the process of deletion of self reactive T-cell clones is not as efficient in older patients as it is in children. This might be one of the reasons for the increased incidence and prolonged course of GVHD in adults compared with children.

Is GVHD Desirable or Beneficial?

GVHD in patients transplanted for nonmalignant indications is not desirable and does not confer any survival benefit. However, despite the morbidity it causes for patients who undergo transplantation for malignant indications, GVHD may be beneficial as it is related to GVL or GVT effect. Indeed, the risk of relapse is lower in patients with GVHD than in those without it.[13] In addition, GVHD T-cells also enhance the engraftment process, in part by destroying residual host T-cells.

Can GVHD Occur in Settings Other Than After Allogeneic HSCT?

Although GVHD is most common after allogeneic HSCT, and this is the focus of this review, it is important to recognize that GVHD can occur rarely in other scenarios. Specifically, it can occur after autologous and syngeneic transplantation, for which the pathophysiology is unclear. In addition, it can occur after blood product transfusion (if products are not irradiated), after solid-organ transplantation, and in maternal-fetal transfusion (eg, SCID disease, in which maternal cells cross the placenta and act as "graft" in an immunodeficient infant).

Classification of GVHD

Traditionally, the occurrence of GVHD after allogeneic HSCT was divided into 2 categories, acute and chronic, on the basis of whether symptoms began before or after day +100 post transplant. Indeed, aGVHD typically occurs within the first weeks after transplantation, with greater frequency and severity in nonidentical sibling or unrelated donors. cGVHD classically occurs more than 3 months after transplant and has clinical symptoms that can resemble an overlap of several autoimmune diseases. However, changes made in both the aggressiveness and timing of the transplantation procedure (conditioning and stem cell sources) and immunosuppressive prophylactic regimens have led to variability in the timing of aGVHD and cGVHD and blurred the distinctions between them. As a result, clinicians recognized that the previous classification scheme was too simplistic, and in 2005 a group at the National Institutes of Health came together to propose better definitions.[14] aGVHD and cGVHD were redefined to emphasize the central importance of distinct diagnostic manifestations differentiating the 2 entities, rather than time of onset after transplant (Table 1).[15,16]

It is now accepted that aGVHD can occur much later than 100 days after transplant, so-called "persistent, recurrent or late-onset aGVHD," precipitated by changes in immunosuppression regimens and/or DLI. If clinical and/or histologic features fail to show those of cGVHD, regardless of time after transplantation, the case should be categorized as aGVHD. Likewise, presentations consistent with an overlap of features of both aGVHD and cGVHD have been recognized, and "overlap syndrome" was also proposed as a distinct category. Both late-onset aGVHD and overlap syndrome occur with greater frequency after RIC regimens. It remains to be determined whether the new classification scheme proves to be useful in predicting survival or helping to risk-stratify patients.

The Clinical Spectrum of aGVHD

The 3 organs classically affected in aGVHD are skin, gut, and liver, in decreasing order of frequency. The severity of involvement is staged by features specific to the organ as follows: the skin is staged by the percentage of BSA involved, the gut by the volume of diarrhea, and the liver by the degree of bilirubin elevation (Table 2). The peak time of onset is from the time of engraftment (when the white cell count starts to recover) to around day +60 after transplant. As mentioned, triggers for aGVHD can be a DLI or a change/decrease in immunosuppression (both of which served as triggers for our index case). Cutaneous symptoms typically are described as pruritus or burning, and in some cases, affected areas of skin may be tender on palpation.

The morphology of cutaneous aGVHD typically starts out as a morbilliform eruption first noted on the upper back and lateral neck, malar cheeks, pinnae, palms, and soles. Periungual involvement can be a very helpful clinical clue. Folliculocentric accentuation can also be striking in the early phase of the eruption, reflecting injury to hair follicle epithelium. Sometimes, hyperpigmentation or even very subtle erythema of intertriginous sites, such as the axillae, neck, and periauricular areas are the earliest clinical findings. As the lesions become more mature, they can take on a more hyperkeratotic papular morphology.

More severe disease can progress from a primarily morbilliform morphology to a generalized, confluent erythroderma, or can occur de novo as exfoliative erythroderma. The most severe form of aGVHD is a bullous presentation with TEN-like full-thickness necrosis. Bullous involvement may favor acral sites, including the ears, face, fingers, and toes (especially periungual locations).

Accurate staging (Table 2) of aGVHD is important for determining prognosis as well as for clinical studies. Se-

Table 1 Classification of aGVHD and cGVHD from the 2005 National Institutes of Health Consensus Development Project[14]

Category of GVHD	Subcategory	Timing of Symptoms After HSCT or DLI	Presence of Acute GVHD Features	Presence of Chronic GVHD Features
Acute	Classic acute	≤100 d	Yes	No
	Persistent, recurrent or late onset	>100 d	Yes	No
Chronic	Classic chronic	No time limit	No	Yes
	Overlap syndrome	No time limit	Yes	Yes

Table 2 Clinical Staging and Histologic Grading of aGVHD by Organ System Involved[26]

	Clinical			Histology	
Stage	Skin (Body Surface Area)	Liver (Bilirubin Level, mg/100 mL)	Gastrointestinal Tract (Volume of Diarrhea, mL/d)	Grade	
I	Erythematous macules and papules, 25% BSA	2-3	500-1000	I	Focal or diffuse vacuolar change
II	Erythematous macules and papules, 25%-50% BSA	3-6	1000-1500	II	Grade I features plus necrosis of keratinocytes and lymphocytes
III	Erythematous macules and papules (>50% BSA) to generalized erythroderma	6-15	1500-2000	III	Grade II features plus focal dermal-epidermal junction separation with formation of vesicles
IV	Generalized erythroderma with bullae formation	>15	>2000; Severe abdominal pain with or without ileus	IV	Grade III features plus formation of bullae

vere aGVHD (stage III/IV) has a poor prognosis, with 25% long-term survival (5 years) for stage III disease and 5% for stage IV.[17]

The primary differential diagnoses for the morbilliform morphology of aGVHD include viral exanthems, morbilliform drug eruptions, eruption of lymphocyte recovery, and engraftment syndrome.[1] Eruption of lymphocyte recovery is a morbilliform eruption that occurs 1 to 2 weeks after the chemotherapy-induced nadir. The eruption occurs with a fever, both of which are typically transient and resolve within a few days.[1] Engraftment syndrome is a clinical constellation of a morbilliform eruption very similar to GVHD that occurs within the first 14 days after transplant, associated with a fever, neutrophil count > 500 for more than 2 consecutive days, and pulmonary infiltrates/edema that is not cardiogenic in origin. Some authorities think that engraftment syndrome represents a hyperacute phase of GVHD.[1] In severe cases (stage III and IV disease), the differential diagnosis of aGVHD includes TEN. It can sometimes be very difficult to distinguish between TEN-like GVHD and drug-induced TEN.

aGVHD Pathophysiology

There have been numerous excellent reviews published on the pathophysiology of aGVHD, which describe 3 distinct phases.[7,18-20] To briefly summarize, in phase I, chemotherapy and/or radiation cause nonspecific tissue damage (especially in the gut), and toxins, such as lipopolysaccharide, enter the bloodstream. Proinflammatory cytokines (such as TNF-alpha and interleukin-1) are produced by residual recipient antigen-presenting cells (APCs) and are present at increased levels in the blood. In phase II, activated host APCs present alloantigens to donor T-cells infused with the stem cell graft. These donor T-cells proliferate in response to the cytokines. In phase III, also known as the effector phase, the stimulated and clonally expanded donor T-cells cause damage to host epithelial tissues (skin, gut, and liver), as well as thymus. The effector phase is also thought to play a role in GVT effects whereby host targets are residual malignant cells. Enhanced

DC activation is thought to occur in more advanced malignant disease, more intense conditioning regimens, and viral reactivation, all of which increase the likelihood of aGVHD.[7]

There has been a great deal of attention about the role of FoxP3^{+}/CD4^{+}/CD25^{+} regulatory T (Treg) cells and their potential to protect against and attenuate the severity of GVHD, without loss of donor T-cell–mediated GVL effect, both in animal models[21] and in humans.[20] Edinger et al[21] have shown in mice that Tregs suppress autoreactivity in the skin, and if Tregs are either depleted from the donor graft, or knocked out in recipients, aGVHD and cGVHD are promoted. Some investigators[22,23] have tried to determine correlations among expression of FoxP3^{+} on CD4^{+}/CD25^{+} Tregs in skin biopsies of patients with aGVHD and disease severity and treatment response. Data are mixed at this point, and additional work is needed to clarify this complex physiology.

Risk Factors for aGVHD

Risk factors include HLA disparity (major and minor antigens), older age of recipient, donor-recipient gender mismatch, source/dose of stem cells, greater number of T-cells in the donor graft, and DLI. aGVHD occurs in 35% to 45% of recipients of fully-matched (10/10) sibling donor grafts, in 60% to 80% of recipients of one-antigen HLA-mismatched unrelated-donor BM or PBSC transplantation grafts, but in only 35% to 65% of recipients of 2-antigen mismatched UCB transplants.[7] In addition to the aforementioned factors, in recent research,[24,25] investigators have implicated viral HHV-6 reactivation in potentiating aGVHD, particularly in children. HHV-6 reactivation was demonstrated by multivariate analysis to be associated with greater nonrelapse mortality. The authors argue that monitoring HHV-6 viral load after transplant and treating aggressively may be helpful.

Factors to Consider in Making the Diagnosis of aGVHD

GVHD is a clinical diagnosis first and foremost. Clinical factors to consider are (1) history, including type of transplant,

degree of mismatch, use of prophylaxis, timing of symptom onset relative to transplant, organ systems involved, (2) risk factors, (3) morphology, and (4) evolution of the clinical examination. Although some authors argue against the utility of skin biopsy in diagnosing aGVHD, we believe there *is* a role for skin biopsy in these patients.

Histopathology of aGVHD

The classic histopathology of aGVHD is typified by an interface dermatitis with epidermal injury out of proportion to the degree of inflammation observed. One sees single necrotic (apoptotic) keratinocytes present in both the epidermis and appendages (hair follicles and eccrine ducts). Lerner et al[26] proposed microscopic grading criteria in 1974 which continue to be used (Table 2). Numerous studies have demonstrated that no single or combined feature (eg, apoptotic keratinocytes in epidermis and appendages, basal cell vacuolization, lymphocytes adjacent to necrotic keratinocytes [also known as satellitosis]) is predictive of clinical GVHD.[27]

Unfortunately, there have been no definitive studies on the sensitivity and specificity of skin biopsy in this setting. Some authors have argued that if the pretest probability is high on the basis of history and clinical features, one should treat regardless of what the biopsy shows.[28,29] Zhou et al[28] found that positive biopsy results did not correlate with GVHD severity; positive biopsy results did not correlate with likelihood to be treated; and that greater clinical grading was correlated with likelihood to be treated. Kuykendall and Smoller[30] argued that skin biopsy in the initial 3 weeks after transplant is not helpful diagnostically. Although the biopsy may be nonspecific, and cannot always be used to distinguish between the various clinical entities being considered (ie, GVHD, viral exanthems, drug eruption, eruption of lymphocyte recovery, and engraftment syndrome), it is generally accepted that a biopsy facilitates clinical decision making. The rationale is that supportive evidence either in favor or opposed to the diagnosis of GVHD can be crucial in determining whether prolonged aggressive immunosuppression is warranted, particularly given that this is an extremely vulnerable patient population and immunosuppression can lead to greater risk for complications, including infection and delayed immune reconstitution. In addition, because of variable time of onset of aGVHD in today's HSCT recipients, a biopsy can provide important immediate diagnostic assistance and can serve as a baseline for future clinico-histopathologic comparison if additional biopsies are required during the course of disease and recovery.

Clinicopathologic Correlation Case Studies for aGVHD

Table 3 and the associated figures 1, 2, and 3 illustrate 3 cases (including our index case) of aGVHD with clinicopathologic correlation and the take-home points for each case.

The Clinical Spectrum of cGVHD

cGVHD is an immunologic complication that develops after allogeneic HSCT because of inappropriate T-cell auto- and

Table 3 Clinicopathologic Correlation Case Studies for aGVHD

Case	Age/ Gender	Primary Diagnosis	Source of Transplant Cells	Skin, GI, Liver Involved?	Onset	Skin Examination	Skin Biopsy Result	Treatment	Take-Home Points	Figures	Outcome
1	7-yr-old M	Relapsed ALL	MUD (9/10) PBSC	Skin, GI	Day +33	Morbilliform eruption on face, ears, chest, extremities	VIC with adnexal involvement	Steroids, higher dose of CsA	Classic morbilliform aGVHD, concordant pathology	2 A, B	Resolved with no sequelae
2	15-yr-old M	Myelodysplastic syndrome-AML	MUD (9/10) PBSC	Skin, GI	Day +30	Folliculocentric papules with subtle erythema on neck, cheeks, upper chest	Follicular plugging, VIC, extending down HFE	Sirolimus, CsA (steroids not used because of active CMV infection)	Correlation of KP-like follicular papules clinically and follicular involvement on path	3 A, B	Progressed to cGVHD at day +106
3 (index case)	11-mo M	SCID	Haplocompatible (3/6) PBSC	Skin, liver	Day +102	Rapid progression from morbilliform eruption to TEN-like picture	VIC, and adnexal involvement, progressing to full thickness necrosis	Steroids, TAC, infliximab, daclizumab, IVIG, mesenchymal stem cells	Difficulty in distinguishing TEN-like GVHD from drug-induced TEN	1 A-D	Slowly improved, maintained on topical steroids bid and TAC

ALL, lymphoblastic leukemia; AML, acute myeloid leukemia; bid, twice daily; CsA, cyclosporine A; HFE, hair follicle epithelium; IVIG, intravenous immunoglobulin; MUD, matched unrelated donor; PBSC, peripheral blood stem cells; SCID, severe combined immunodeficiency disease; TAC, tacrolimus; VIC, vacuolar interface change.
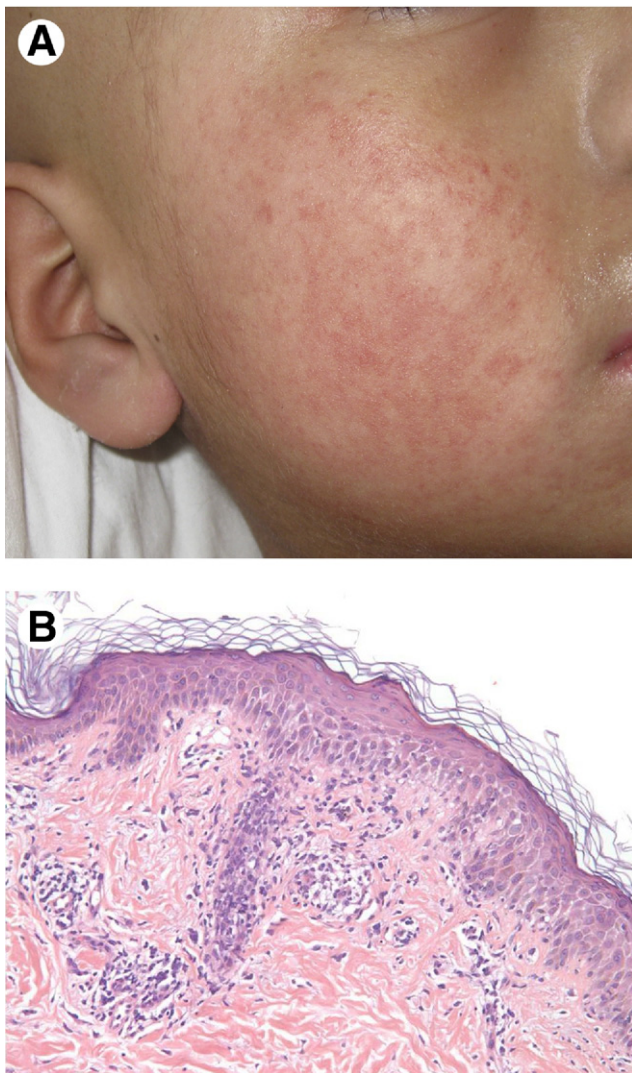
Figure 2 Erythematous papules on the malar and midcheek, with macular erythema of the earlobe with fine desquamation. Histopathology showing vaculolar interface dermatitis with junctional and periadnexal involvement (magnification ×20).

papules, typically affecting the dorsal hands and feet, extensor forearms, and trunk. The sclerodermoid form has been divided into 4 subtypes depending on the level of involvement (dermis, subcutaneous tissue, and/or fascia): lichen sclerosus-like (LS), morpheaform/sclerodermatous plaques with or without joint contractures, panniculitis, and eosinophilic fasciitis.[16,31-33]

LS lesions occur on average 300 days after transplantation and appear as hypopigmented plaques with atrophy, scale, and follicular plugging. Lesions of LS tend to occur on the neck and upper to midtrunk but can also occur on the ex-
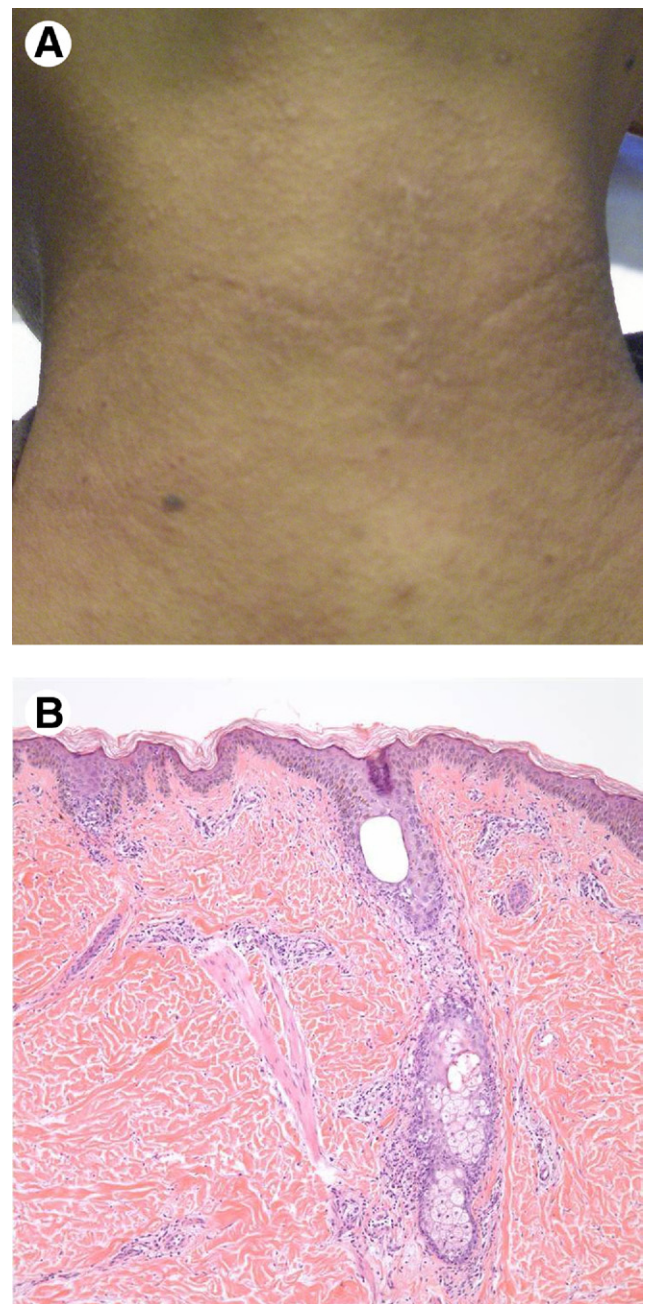
Figure 3 (A) Follicular papules with subtle erythema distributed across the nape of the neck. (B) Histopathology showing acute vacuolar interface reaction, extending down the hair follicle with overlying follicular plugging (magnification ×10).

alloreactivity and B-cell dysregulation. It is the cause of significant morbidity and mortality after allogeneic transplant. It typically involves the skin, intestine, liver, eyes, mouth, and lungs but can involve any organ, including the esophagus, GU tract, musculoskeletal system, BM, heart, and kidneys.[14] New criteria for diagnosis have been defined (Table 4), and a new clinical scoring system for each organ involved and guidelines for global assessment of severity has been developed.[14] The onset is typically greater than 4 months after transplant but can vary. In approximately one third of cases, cGVHD occurs as a progression of aGVHD (progressive form), in one third of cases as a recurrence after a disease-free interval from aGVHD (quiescent form), and in one third of cases, de novo, without a history of aGVHD.

Cutaneous presentations of cGVHD are much more protean compared with those of aGVHD. The 2 primary morphologic variants of cGVHD include lichenoid and sclerodermoid. Lichenoid lesions are erythematous to violaceous flat-topped

Table 4 cGVHD Features per NIH Consensus Development Project, 2005: Diagnosis Requires at Least 1 Diagnostic Finding or 1 Distinctive Finding Plus Biopsy/Laboratory/ Imaging Test

Diagnostic
Lichen planus-like lesions
Lichen sclerosus-like lesions
Morphea-like lesions
Deep sclerotic lesions or fasciitis
Poikiloderma
Distinctive
Depigmentation
New-onset alopecia or papulosquamous lesions of scalp
Nail dystrophy
Xerostomia, mucoceles, oral mucosal atrophy, or oral ulcers

tremities, favoring catheter sites.[31] Morpheaform lesions are characterized by circumscribed, firm hyperpigmented plaques that tend to occur on the lower trunk and proximal extremities. The key to making this diagnosis is palpation of the skin, because it is easy to miss the indurated quality of the lesions by inspection alone. The isomorphic response or Koebner phenomenon has been described in patients with morpheaform cGVHD with lesions localizing to the waistband or brassiere-band areas of the torso.[34]

Ulceration is a particularly debilitating and painful complication of sclerodermoid cGVHD, and has been postulated to confer a worse prognosis.[35] Eosinophilic fasciitis-like cGVHD is the rarest form, occurring in less than 5% to 6% of patients with cGVHD, typically occurring with later onset compared with LS-like lesions (average of 700 days posttransplant). Sixty percent of patients with eosinophilic-fasciitis cGVHD have peripheral eosinophilia at the time of symptom onset.[31] It is most common on the extremities and is characterized by induration with a rippled skin appearance, sometimes leading to a "groove sign" demarcating muscle/fascial bundles or along the path of superficial vessels.[31] Patients complain of acute-onset edema and pain, sometimes with cramping and weakness. The use of magnetic resonance imaging is being studied as a potential method of assessing subcutaneous and fascial disease activity as an adjunct to incisional biopsy.[36] Importantly, Schaffer et al[31] reported that LS morphology (which was previously underemphasized in the dermatology literature) occurred as the initial presentation of cutaneous cGVHD in 6 of their 6 cases with sclerodermoid GVHD. Subtle LS-like lesions may go unnoticed and evolve to morpheaform plaques by the time the patients present for evaluation. Some authors have argued that lichenoid GVHD is a precursor to sclerodermoid GVHD, but others have contended that they occur independently.

The clinical differential for lichenoid cGVHD lesions includes idiopathic lichen planus, lichenoid drug eruption, and pityriasis lichenoides chronica. Distribution of lesions can be helpful to distinguish lichenoid cGVHD from idiopathic LP because the latter favors the dorsomedial forearms, shins, and genitalia, which are distinct from

the common sites of lichenoid cGVHD (dorsal hands and feet, forearms, and trunk). In addition, the morphology of lichenoid cGVHD tends to be less angulated than that of idiopathic LP. The clinical differential for sclerodermoid spectrum cGVHD includes LS, morphea, scleroderma, atrophoderma of Pasini and Pierini, and discoid lupus erythematosus.

Other described presentations of cGVHD include poikiloderma, xerosis, keratosis pilaris, ichthyosis, seborrheic dermatitis, eczematous dermatitis, and papulosquamous eruptions mimicking pityriasis rosea or psoriasis. Alopecia (both scarring and nonscarring), pigmentary changes with or without mottling, and oral involvement, including xerostomia, mucositis, ulceration, and reticular or papular lichenoid lesions may occur. Nail changes range widely but periungual inflammation, pterygium, longitudinal ridging, hyperkeratosis, and fragility have all been described. Importantly, cGVHD can have features that resemble many autoimmune diseases, including scleroderma, Sjogren's syndrome, biliary cirrhosis, bronchiolitis obliterans, and immune cytopenias, including thrombocytopenia. Sixty percent of patients with cGVHD demonstrate autoantibodies to nuclear antigens similar to those found in autoimmune diseases.[37,38]

cGVHD Pathophysiology

The pathophysiology of cGVHD is poorly understood relative to that of aGVHD and it is likely that many biological mechanisms play a role in pathogenesis, thus explaining the diverse and protean manifestations of this disease. cGVHD is a T_h2 dominant disorder, the ultimate endpoint of which is often fibrosis of the affected organs.[7] Although aGVHD involves mainly alloreactivity, there are thought to be 3 main mechanisms involved in cGVHD pathogenesis: (1) T-cell allo-reactivity directed against recipient antigens, (2) T-cell auto-reactivity, and (3) B-cell dysregulation.[18] Martin proposed 4 theories that are implicated in cGVHD pathophysiology, including: (1) defective negative selection of auto-reactive T-cells attributable to thymic damage, (2) aberrant production of transforming growth factor-beta and activation of platelet-derived growth factor (PDGF) receptor, (3) autoantibody production, and (4) deficiency of Treg cells.[39] Transforming growth factor beta and PDGF have been implicated in the development of skin fibrosis in cGVHD. Activating antibodies targeting the PDGF receptor were reported in a group of patients with extensive cGVHD, suggesting that targeted inhibition of PDGF receptor signaling with therapies, such as imatinib may inhibit the fibrotic process associated with sclerotic cGVHD.[40,41] The reader is directed to a few excellent review articles for additional information on cGVHD pathophysiology.[18,39]

Histology of cGVHD

The histology of lichenoid cGVHD can resemble lichen planus with chronic interface dermatitis, "saw-toothing" of epidermal rete, and hyperkeratosis. LS-like cGVHD shows epidermal atrophy with vacuolar basal alteration, a subepidermal zone of homogenized collagen with loss of elastic fibers, and a band-like

Table 5 Clinicopathologic Correlation Case Studies for cGVHD

Case	Age/Gender	Primary Diagnosis	Source of Transplant Cells, Trigger	Organs Involved?	Onset	Skin Examination	Skin Biopsy Result	Treatment	Take-Home Points	Figures	Outcome
3 (index case)	11-mo M	SCID	Haplocompatible (3/6) PBSC	Skin, liver	Day +208	Lichenoid papules on proximal extremities, dorsal hands and feet; urticarial plaques on trunk	VIC, with adnexal involvement	Sirolimus, steroids, topical steroids bid	Overlap features of cGVHD lichenoid morphology clinically and aGVHD histopathologically	1 E-F	Multiple admissions for failure to thrive, chronic cutaneous and liver GVHD
4	16-yr-old M	High-risk AML	MUD (9/10) PBSC trigger: taper of TAC 1 wk prior	Skin, GI, mouth, eyes Hx of aGVHD (case 2, Table 3) ST with sirolimus, CsA	+4 mo initially; time of bx + 18 mo	Hyperpigmented thin plaques on trunk, proximal extremities; lichenoid papules on back and tongue	Hypergranulosis, VIC, melanophages, dermal sclerosis	MMF s/p steroids, TAC, sirolimus, rituximab (day + 106)	Lichenoid chronic clinical morphology with overlap acute and chronic features histopathologically	4 A, B	Lichenoid papules cleared; hyperpigmented plaques thinned to patches, now asymptomatic
5	24-yr-old M	Aplastic anemia	MUD (8/8) PBSC	Skin, GI, eyes, nails Hx aGVHD day + 45, persistent despite steroids, TAC; ST with daclizumab	+13 mo initially (with ulceration); time of bx + 6 years	Severe dyspigmentation; poikiloderma, atrophoderma on arms, chest, back; hyperpigmented plaques face and trunk; indurated plaques abdomen, thighs	Multiple biopsies: dermal sclerosis with thickened collagen bundles in both papillary/reticular dermis	None (per patient request); s/p treatment +13 mo: MMF, MTX, steroids, TAC, PUVA	Clinical spectrum of sclerodermoid cGVHD w/ LS-like, morphea-form and atrophoderma; path shows sclerodermoid GVHD	5 A, B	Being monitored closely for progression of signs and symptoms

bid, twice daily; bx, biopsy; CsA, cyclosporine A; MMF, mycophenolate mofetil; Mos, months; MUD, matched unrelated donor; PBSC, peripheral blood stem cells; PUVA, psoralens plus UVA phototherapy; ST, successfully treated; S/p, status-post; TAC, tacrolimus; VIC, vacuolar interface change.
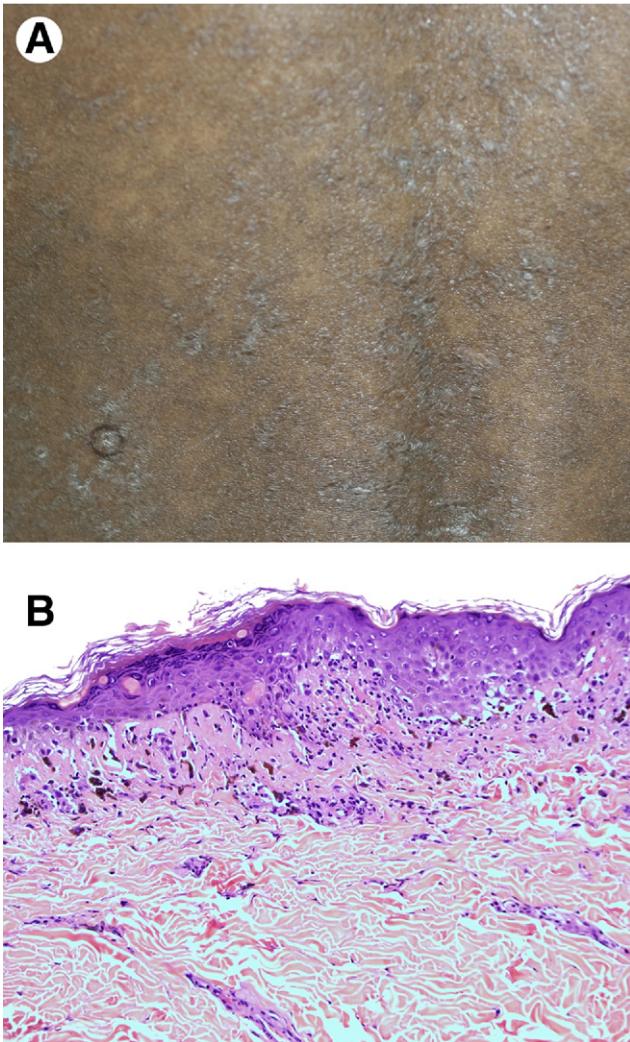
Figure 4 (A) Lichenoid papules on the back. (B) Histopathology showing vacuolar interface changes, attenuated rete ridge pattern, hypergranulosis, perijunctional lymphohistiocytic infiltrate with admixed melanophages, and papillary dermal fibrosis and sclerosis (magnification ×40).

lymphocytic infiltrate underlying the homogenized collagen. The histology of the sclerodermoid form consists of dermal fibrosis in the papillary dermis, which can extend to the subcutaneous fat. Distinguishing sclerodermoid cGVHD from conventional scleroderma is difficult, but sclerosis that is more prominent in the papillary compared with the reticular dermis can favor sclerodermoid cGVHD.[42] Ultimately, in longstanding lesions, sclerotic collagen and adnexal atrophy are observed.

Risk Factors for cGVHD

The 2 most important risk factors for cGVHD are older recipient age and history of aGVHD.[43] All the risk factors for aGVHD also apply. A multivariate analysis showed that increased CD3^{+} T-cell dose in the donor graft as well as peripheral eosinophilia are associated with an increased risk of sclerotic cGVHD.[44] Notably, strategies that have decreased the frequency and severity of aGVHD (such as UCB transplantation) have not as clearly affected the incidence of cGVHD.[45]

In addition, strategies that have not significantly altered aGVHD rates (such as PBSC transplantation) appear to have increased the incidence of cGVHD.[46]

Clinicopathologic Correlation Case Studies for cGVHD

Table 5 and the associated figures 1E-F, 4, 5 illustrate 3 cases (including our index case) of cGVHD and the main teaching points garnered from each clinicopathologic correlation.

Immunomodulatory Therapy for Prevention and Treatment of aGVHD

GVHD prophylaxis is used in nearly all pediatric transplant cases, and as mentioned previously, standard strat-
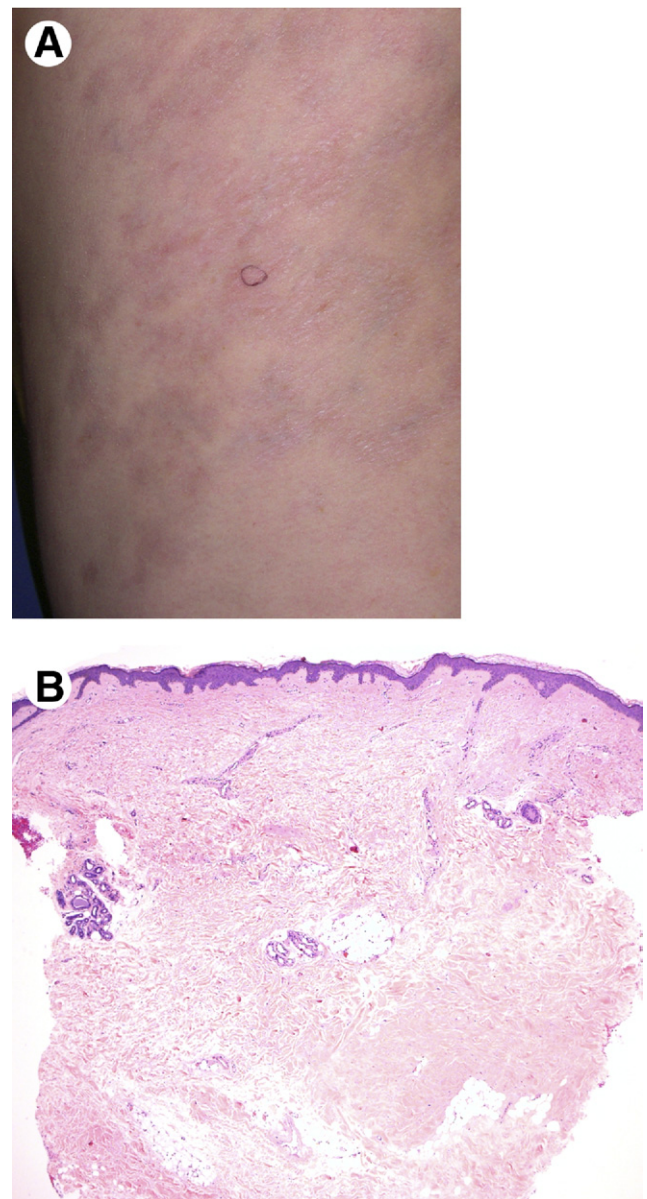
Figure 5 (A) Left flank showing changes of lichen sclerosus overlying indurated plaques adjacent to areas of atrophoderma. (B) Histopathology showing significant dermal sclerosis with minimal lymphocytic inflammation (magnification ×10).

egies for prophylaxis use 2 drug regimens with either cyclosporine or tacrolimus (FK-506) and methotrexate or mycophenolate mofetil.[11] These agents target phase II of aGVHD pathophysiology, specifically limiting donor T-cell activation by alloantigen presentation by host APCs. Other approaches to prevent GVHD include the use of in vivo TCD, which can be accomplished by the use of antithymocyte globulin (ATG) and alemtuzumab (Campath), which is an anti-CD52 monoclonal antibody that eliminates activated T-cells. Low-dose ATG has been shown to effectively lower rates of aGVHD and improve nonrelapse mortality and infection rate.[47,48] In vivo TCD is used for nearly all pediatric MUD transplants. Efforts at ex vivo TCD have been limited by graft failure.

First-line treatment of isolated cutaneous aGVHD that is less than 50% BSA (stage I-II) is mid- to high-potency topical steroids. If the intestine or liver are involved, or cutaneous involvement exceeds 50% BSA, the standard first-line treatment is intravenous methylprednisolone. Although the doses of methylprednisolone vary by institution, the standard starting dose is 2 mg/kg/d.[49] Roughly 50% of patients respond to single-agent corticosteroid treatment.[49] The use of doses greater than 2 mg/kg/d have not been shown to improve response rate.[50] For those patients who respond to steroid monotherapy, steroids are continued for 1-2 weeks followed by a slow taper. Outcome of aGVHD is correlated with initial stage at presentation, and better outcomes in aGVHD are correlated with improved response to initial treatment.[51]

There is no standard established treatment for steroid-refractory cases. Depending on what agents were being used for prophylaxis, cyclosporine and/or tacrolimus may be added. Monoclonal antibodies, chemotherapeutics with immunomodulatory properties, biologics, and cellular therapies have been increasingly used and demonstrated to have high response rates. There have been many excellent review articles summarizing the use of these newer agents to treat aGVHD.[52,53] Biological therapies include polyclonal antibodies (ATG), monoclonal antibodies (daclizumab, inolimomab, alemtuzumab, OKT3), biological toxin-conjugate (denileukin diftitox), and TNF-alpha blockade (infliximab, etanercept). Chemotherapeutic interventions include mycophenolate mofetil, calcineurin inhibitors, and sirolimus. Phototherapy methods include psoralen + UVA (PUVA) and extracorporeal photopheresis. Cellular therapy with MSCs is an exciting treatment advance in the setting of steroid-refractory intestinal GVHD and appears to be helpful for cutaneous aGVHD as well.[53,54] MSCs are a heterogeneous population of cells that provide growth factors, cell to cell interactions and matrix proteins that have an immunomodulatory role as well as supportive role for hematopoietic cells. Although their mechanism of action is incompletely understood, MSCs are thought to actively home to and repair tissues damaged by activated T-cells.

Although many of these treatments show promise in aGVHD, the clinical outcomes and long-term survival for steroid-refractory aGVHD remain poor, with high incidence of infections. The goals of treatment now are focused on inducing remission early, quickly, and effectively, and then safely tapering immunosuppressive therapies as soon as possible to minimize infection.[52]

Treatment of Cutaneous cGVHD

Adequate treatment of cGVHD has been limited by an incomplete understanding of disease pathophysiology. LS and morphea-like sclerodermoid cGVHD often respond to mid-to high-potency topical steroids. Sclerotic cGVHD with deep fibrosis is poorly responsive to available topical therapies, but oral corticosteroids or phototherapy (i.e., PUVA) can induce softening. Calcineurin inhibitors (cyclosporine and tacrolimus), extracorporeal photopheresis, rituximab, daclizumab, intravenous immunoglobulin, thalidomide, Plaquenil, etretinate, mycophenolate mofetil, and imatinib have all been used with variable success as adjuvant therapies for sclerodermoid GVHD that is refractory to steroids and cyclosporine. Choice of treatment depends on which other organs are involved and other individual patient factors. The provision of supportive care, including infectious prophylaxis, nutritional assessments, symptom management, physical and occupational therapy to maintain functional range of motion, and identifying support groups, is equally important as providing pharmacologic immunosuppression.

Conclusions

Despite improvements in hematopoietic transplantation techniques, immunomodulatory prophylaxis, and treatment approaches, GVHD remains the cardinal complication in allogeneic HSCT with significant associated morbidity and mortality. Our understanding of disease pathophysiology has improved dramatically, but, unfortunately, outcomes remain suboptimal. Early recognition of aGVHD and cGVHD is of critical importance for undertaking necessary measures to prevent progression to life-threatening stages. Dermatologists play a key role in diagnosing this disease and providing guidance for treating the cutaneous manifestations. We owe it to these critically ill patients to work closely with our pediatric bone marrow transplant colleagues and other interdisciplinary team members to coordinate a personalized diagnosis and treatment regimen for each patient. It is also crucial for dermatologists to continue research in this area to improve treatments and outcomes for patients with these complex and challenging conditions.

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