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Cutaneous Graft-versus-Host Disease: A Guide for the Dermatologist

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Abstract
Graft-versus-host disease (GVHD) is defined by the aggregation of clinical and pathological manifestations in a recipient of allogeneic stem cells or bone marrow transplantation in which specific immunological as well as nonspecific phenomena lead to characteristic features. GVHD is one of the major complications after hematopoietic stem cell transplantations and responsible for posttherapeutic morbidity, mortality and decrease in quality of life of those patients. GVHD is critically induced and maintained by donor immunocompetent cells that particularly attack epithelia of fast proliferating tissues such as those from the liver, gastrointestinal tract and skin. On the basis of the time of presentation, cutaneous GVHD has been originally divided into an acute and chronic disease. The latter has traditionally been further subclassified into a more epithelial or lichenoid and a predominantly dermal or sclerodermoid form. With respect to the growing importance of this therapeutic procedure and increasing numbers of outpatients presenting with chronic GVHD, this article summarizes the updated knowledge on this disease focused for the dermatologist, and additionally it emphasizes the recent consensus documents on the various aspects of chronic GVHD of the National Institute of Health.

Evolution of Hematopoietic Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) in its current form evolved over the last 50 years from research initially aimed at treating the sequelae of radiation exposure that were feared in the 'Cold War' and Nuclear Age era [1]. Pioneering studies showed that lethally irradiated mice could survive radiation-induced bone marrow aplasia if the spleen was shielded by a lead foil or, later, after transfusion of bone marrow from mice of the same strain [2–5]. After it had initially been debated whether the protective effect was due to 'a substance of noncellular nature' (humoral hypothesis) or transplanted cells (cellular hypothesis), several laboratories subsequently used cytogenetic markers to unequivocally demonstrate that the radioprotective effect of bone marrow transplantation resulted from the replacement of the damaged hematopoietic system of the host by healthy cells from the donor [6]. These early animal studies formed the rational basis for

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the first attempts to treat human leukemias with high-dose chemoradiotherapy followed by syngeneic or allogeneic (for glossary, see table 1) bone marrow transplantation, and clinical investigators on both sides of the Atlantic Ocean soon communicated their initial promising results. For example, George Mathé published the successful use of allogeneic marrow infusions to rescue victims of accidental irradiation exposure in Europe some years later than E. Donnell Thomas from the USA, who was awarded the Nobel Prize in Physiology or Medicine together with Joseph E. Murray in 1990 for groundbreaking work on organ and cell transplantation in the treatment of human disease [7, 8]. Final proof of concept came with the first successful bone marrow transplantation from a human leukocyte antigen (HLA)-mismatched sibling donor followed by several HLA-identical sibling transplantations in children with severe combined immunodeficiency in 1968 [9].

Over the last decades, allogeneic HSCT has become an important therapeutic modality not only for the treatment of hematological malignancies (e.g. leukemias or lymphomas), but also for the treatment of nonmalignant hematological stem cell disorders (e.g. severe aplastic anemia), genetic diseases (e.g. severe combined immunodeficiency or thalassemia) and, under certain conditions, even solid tumors (e.g. renal cell cancer) [10–13]. The growing importance of HSCT is emphasized by an ongoing increase in its use over the last 20 years [14]. Today, transplanted stem cells are used from autologous, syngeneic and allogeneic sources and are collected from bone marrow, peripheral blood after mobilization or umbilical cord blood. Moreover, allogeneic HSCT is increasingly performed with success over HLA barriers due to limited numbers of HLA-matched siblings, e.g. from parents (haploidentical), HLA-mismatched siblings, and matched or mismatched unrelated donors. What has been a highly experimental therapy only a few decades ago has now emerged as standard of care for many diseases [11, 15].

Currently, the main limitations of allogeneic HSCT include treatment-related toxicity, relapse of the underlying disease (in the setting of malignant stem cell disorders) and immunological complications such as rejection, graft-versus-host disease (GVHD) and delayed immune reconstitution. GVHD in its acute or chronic form is the main cause of nonrelapse morbidity and mortality both as a direct complication (e.g. bronchiolitis obliterans) or via associated immunodeficiency and susceptibility to severe infections [16, 17]. With the increasing number of patients undergoing HSCT and the more widespread use of mismatched transplantation, the dermatologist will be more and more confronted with GVHD in her/his daily practice, and it is thus the goal of the current article to provide a concise overview of the pathomechanisms underlying GVHD, changing disease concepts, and main clinical and pathological features with focus on the skin, as well as to summarize and discuss recently published consensus documents from the National Institute of Health (NIH) on various aspects of chronic GVHD [18–25]. While some general therapeutic considerations are presented in the final chapter, an exhaustive discussion of current treatment modalities or prophylaxis of acute and chronic GVHD is beyond the scope of this article, and the interested reader is referred to recent reviews concerning these topics [26–28].

Basic Requirements of GVHD

GVHD reflects an interaction between the donor and recipient that was first described in animals by Rupert E. Billingham, who observed that mice from genetically disparate but not syngeneic donors developed a secondary lethal disease characterized by wasting, diarrhea and skin lesions following recovery from radiation-induced aplasia [29]. He realized the similarity of this 'wasting disease' with other poorly understood phenomena such as 'runt disease' in newborn mice injected with allogeneic spleen cells, the 'F_1 hybrid disease' or the 'parabiosis intoxication' [29]. In 1966, he proposed the requirements under which GVHD can occur: first, the transplanted graft must contain immunologically competent cells; second, the recipient must express tissue antigens that are not present in the transplant donor and thus can be recognized as foreign; third, the recipient must be incapable of rejecting the transplanted cells [30]. These propositions held true over time, and it is nowadays recognized that the mediators of GVHD are mature T cells that clonally expand in an antigen-specific manner after recognition of nonself HLAs expressed on the cell surface of nucleated cells in the host that is chemically or physiologically immunocompromised [31, 32].

The Increasing Significance of Chronic GVHD for the Dermatologist

Contrary to some expectations, the incidence of GVHD did not significantly decrease over the last 20 years and still develops in about half of all patients undergoing allogeneic HSCT [33]. While novel transplanta-

Table 1. Glossary

Technical term	Description
Hematopoietic cell transplantation	Superior term for transfer of hematopoietic cells within one individual or from one individual to another
HSCT	Process of infusing healthy hematopoietic stem cells into patients who have undergone previous chemo- or radiotherapy mainly for hematological disorders; stem cells are infused into the venous bloodstream from where they move to the bone marrow and form new blood cells
Stem cell sources	Depending on donor type, 3 types of stem cell transplants can be distinguished: autologous, allogeneic and syngeneic; depending on the site and mode of harvest, 3 different types of HSCT can be distinguished: BMT, PBSCT, CBT
BMT	Stem cells are collected from bone marrow by repetitive punctions of the bone marrow in general anesthesia
PBSCT	Stem cells are collected from the peripheral blood with a cell separator after mobilization of stem cells with G-CSF to the peripheral blood
CBT	Stem cells are collected from umbilical cord blood and placenta of the newborn immediately after delivery
Donor type	
Autologous transplantation	The patient's own cells are used for HSCT
Syngeneic transplantation	The cells of a twin are used for HSCT
Allogeneic transplantation	The cells from another person are used as stem cell source (bone marrow, peripheral blood, cord blood), either from the same family or from an unrelated donor
HLA-identical sibling	HLA (MHC)-matched cells from sibling
HLA-identical other family member	HLA (MHC)-matched cells from another family member
Non-HLA-identical family member	HLA (MHC)-mismatched cells from a family member
Unrelated donor	HLA (MHC)-matched or -mismatched cells from an unrelated donor
Xenogeneic	HCT from one species (e.g. human) to another (nonhuman)
Matching criteria	
Matched related/unrelated donor	HLA compatibility between donor and recipient (only in terms of HLA antigens)
Mismatched related/unrelated donor	HLA incompatibility between donor and recipient (only in terms of HLA antigens)
Genotypically identical	The same two haplotypes have been inherited by the donor and the recipient from the same parental chromosomes (siblings with the same parents)
Phenotypically identical	Both haplotypes are identical in the donor and the recipient but they are not inherited from the same parental chromosomes (unrelated donors are by definition at best phenotypically identical)
6/6, 10/10, 12/12	Degree of match between 6, 10 or 12 HLA antigens
MHC antigens; identical with HLA antigens	Molecules that are expressed by all nucleated cells (MHC class I) or by a subset of hematopoietic cells and thymic stroma cells (MHC class II) which are recognized by CD8+ T cells (MHC I) or predominantly by CD4+ T cells (MHC II)
Minor histocompatibility antigens	Peptides derived from polymorphic intracellular proteins that are presented on the cell membrane in the context of HLA class I or II molecules and that can be recognized as alloantigens by T cells of an HLA-matched/identical individual; they have a role for the GVHD and GVT effect
Conditioning regimens	Pretransplantation treatment with chemoradiotherapy to reduce tumor burden and lower immunoreactivity of the host in order to allow engraftment of the transplant
Myeloablative conditioning (traditional) regimen	Primary targets are the elimination of the tumor cells as well as induction of a state of immunosuppression in the host that allows the transplantation
Reduced-intensity conditioning	The primary target is to induce a state of immunosuppression in the host to allow transplantation; elimination of the recipient stem cells as well as of residual tumor cells is mainly performed by immunocompetent donor cells
GVHD	Immunocompetent donor cells react against tissues of the recipient
GVT reaction/effect	Immunocompetent donor cells react against malignant tumor cells of the host

BMT = Bone marrow transplantation; PBSCT = peripheral blood stem cell transplantation; CBT = cord blood transplantation; G-CSF = granulocyte colony-stimulating factor; HLA = human leukocyte antigen; MHC = major histocompatibility complex; HCT = hematopoietic cell transplantation; GVHD = graft-versus-host disease; GVT = graft-versus-tumor.

Table 2. Factors predisposing recipients of allogeneic HSCT to the development of GVHD (adapted from Schaffer [34])

Main characteristics influencing the occurrence and severity of GVHD	Risk for acute GVHD	Severity of acute GVHD	Risk for chronic GVHD	Severity of chronic GVHD
Characteristics of the donor and recipient				
HLA disparity	↑	↑	↑	↑
Unrelated HLA-matched donor (mismatched minor histocompatibility antigens)	↑	↑	↑	↑
Female (XX) donor to male (XY) recipient	↑	↑	↑	↑
Older age of donor or recipient	↑	?	↑	?
Prior acute GVHD	–	–	↑	?
Characteristics of the transplantation protocol				
More intense preparative regimen (myeloablative vs. reduced-intensity ('mini') conditioning regimens)	=	↑ ?	↑	?
G-CSF-mobilized peripheral blood rich in CD34+ cells (source and dose of hematopoietic stem cells)	↑	↑	↑	?
Unmodified (T-cell-replete) graft	↑	↑	↑	?
Less aggressive administration of prophylactic immunosuppressive agents (to prevent GVHD)	↑	↑	↑	?
T cell depletion (no GVT effect and high probability for early relapse in case of malignancy and severe infections)	↓	↓	↓	↓
Later interventions (may be intended to incite a graft-versus-malignancy effect)				
Withdrawal of immunosuppressive drugs	↑	?	↑	↑ ?
Donor T lymphocyte infusions	↑	?	↑	↑ ?

G-CSF = Granulocyte colony-stimulating factor; GVT = graft-versus-tumor; ↑ = increased risk/severity of GVHD; ↓ = decreased risk/severity of GVHD; = = equal risk/severity of GVHD; ? = unclear effect on GVHD.

tion techniques such as reduced-intensity conditioning regimens are associated with less severe acute GVHD relative to myeloablative regimens, the incidence of acute GVHD has remained unchanged (table 2) [33, 35]. By comparison, extended eligibility criteria for patients undergoing transplantation (e.g. increased upper age limit), increased HLA disparity, prolonged initial survival due to reduction of early mortality as a consequence of better prophylaxis and treatment strategies of immediate complications of transplantation (e.g. acute GVHD and infections), and increased use of peripheral blood stem cells have increased the risk for chronic GVHD [36].

The role of the dermatologists is limited in acute GVHD and mainly lies in the precise assessment of cutaneous involvement and the exclusion of other skin pathologies. In contrast, the dermatologist needs to be increasingly aware of the manyfold faces and long-term problems associated with chronic GVHD. Sustained involvement of the skin, including mucosal and adnexal sites, is common in chronic GVHD, and dermatologists have to play an active role in the long-term outpatient management and treatment of that disease, in particular because persistent cutaneous lesions are pivotal for morbidity and quality of life (QOL) of those patients [37, 38]. Furthermore, dermatologists will appropriately be obliged to have a central role in the continuous monitoring of treatment responses throughout the disease course over long periods of time based on their professional specification. The rising significance of chronic GVHD is reflected by the recent implementation of an NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD. Six working groups provided consensus statements for diagnosis and staging, pathology, biomarkers, response criteria, ancillary therapy and supportive care, and design of clinical trials in chronic GVHD [20–25]. These consensus documents summarize the current international standard criteria for diagnosis and activity assessment and provide guidance in the organ and overall scoring as well as treatment of chronic GVHD; their implications for the dermatologist will be discussed in later sections of this article.

Basic Mechanisms of Acute and Chronic GVHD

The molecular mechanisms underlying acute GVHD are much better investigated and understood than the pathogenesis of the chronic disease. Of particular note, it is still a matter of debate whether acute and chronic GVHD represent two different phases of the same disease or two independent diseases, both related to allogeneic HSCT but associated with different molecular and pathophysiological mechanisms [39]. Figure 1 illustrates the current model that includes aspects of classic alloreactivity, autoimmunity and immunodeficiency, phenomena that occur in various intensities in acute and chronic GVHD. The skin has been used in basic, animal and clinical research as a model organ for GVHD since the very beginning, which has led to a fairly good understanding of some aspects of that disease [40, 41]. Figure 2 outlines the main principles of acute GVHD in a simplified 3-step process [42, 43]. Conditioning regimens such as total body irradiation result in initial toxic epithelial injuries of highly proliferating organs such as the gut, liver and skin, thereby causing a cell-damage-induced proinflammatory cytokine milieu. These events in turn activate keratinocytes, dermal dendritic and particularly antigen-presenting epidermal dendritic Langerhans cells as well as antigen-presenting cells of the gut and liver, with ensuing increased expression of several cell surface proteins. Pivotal among these are major-histocompatibility-complex-related gene products (including HLA antigens) which also present minor histocompatibility antigens (miHA) [43–45]. The enhanced expression of HLA antigens by activated host antigen-presenting cells then activate donor T cells to increase the secretion of Th1 cytokines such as IFN-γ, IL-2 and TNF-α, eventually leading to the expansion of antigen-specific alloreactive T cells. These events are followed by the generation of cytotoxic and inflammatory cytokines, cytotoxic effector cells that dispose of Fas- and perforin-mediated killing mechanisms, large granular lymphocytes and nitric oxide. Ultimately, epithelial cell apoptosis, cell death and tissue damage are induced by the orchestrated interaction of alloreactive donor T cells, inflammatory cytokines and cells of the innate immune system (large granular lymphocytes, natural killer cells) [43, 46]. It is important to realize that even in the absence of an HLA mismatch between the donor and recipient, cutaneous GVHD can develop in the setting of miHA mismatching, as evidenced by human skin explant assays using minor-antigen-specific cytotoxic T cells (CTLs) [47]. Finally, it has been shown that isolated cutaneous GVHD can occur in skin expressing ubiquitous minor antigens (H–Y) that were recognized by their respective CTLs, but not in the setting of hematopoietic restricted miHA-specific CTLs (HA-1, HA-2) that did not attack the skin. The role of miHA in the setting of allogeneic transplantation is best exemplified by the increased incidence and severity of GVHD in male recipients of female grafts [48].

Attention has recently been drawn to the role of regulatory T cells (T_{reg}) in acute GVHD. Naturally occurring CD4+ CD25+ T_{reg} cells, which are phenotypically best characterized by their FoxP3 expression (the forkhead/winged helix transcription factor gene that is specifically required for their thymic development), have been shown to occur at a lower frequency in the peripheral blood of patients with acute GVHD compared to patients without GVHD, an observation that may suggest their potential benefit in the prevention and treatment of that disease. More specifically, naturally occurring thymus-derived CD4+ CD25+ FoxP3+ T_{reg} cells are currently investigated for their potential to induce and maintain tolerance to allo- (and self-)antigens and to suppress allo- (and auto-)reactivity [49].

Similar to acute GVHD, chronic GVHD is also thought to be an immune-mediated process that mostly involves alloreactive cells, with donor-derived CD4+ and CD8+ T cells as main effectors [50]. As a hallmark feature, the chronic disease may present with clinical and laboratory findings that resemble various autoimmune disorders, e.g. bronchiolitis obliterans, Sjögren's syndrome, immune cytopenias and cutaneous sclerosis, suggesting that dysfunctional humoral immunity might be involved in the pathogenesis as well [51–53]. Indeed, a high prevalence of autoantibodies against a variety of antigens has been observed in these patients and is thought to be the result of autoreactive CD4+ T cells that arose in the setting of an injured thymus with impaired negative selection [54]. In contrast to acute GVHD, however, it has been suggested that chronic GVHD is primarily a Th2-type immune-mediated disease, as evidenced by increased levels of IL-4, IL-5, eosinophils and elevated expression of transforming growth factor β in lesional scleromoid skin [55, 56]. In addition, Biedermann et al. [57] hypothesized that CTL-mediated endothelial injury and subsequent loss of dermal vessels with impaired blood perfusion could be contributory to tissue fibrosis. The shift from an initially Th1-mediated acute disease to a predominantly Th2-mediated chronic immune disease has recently been evidenced in a murine sclerodermoid model by sequential global gene expression analysis. Cytokine messenger RNAs for profibrotic growth factors,

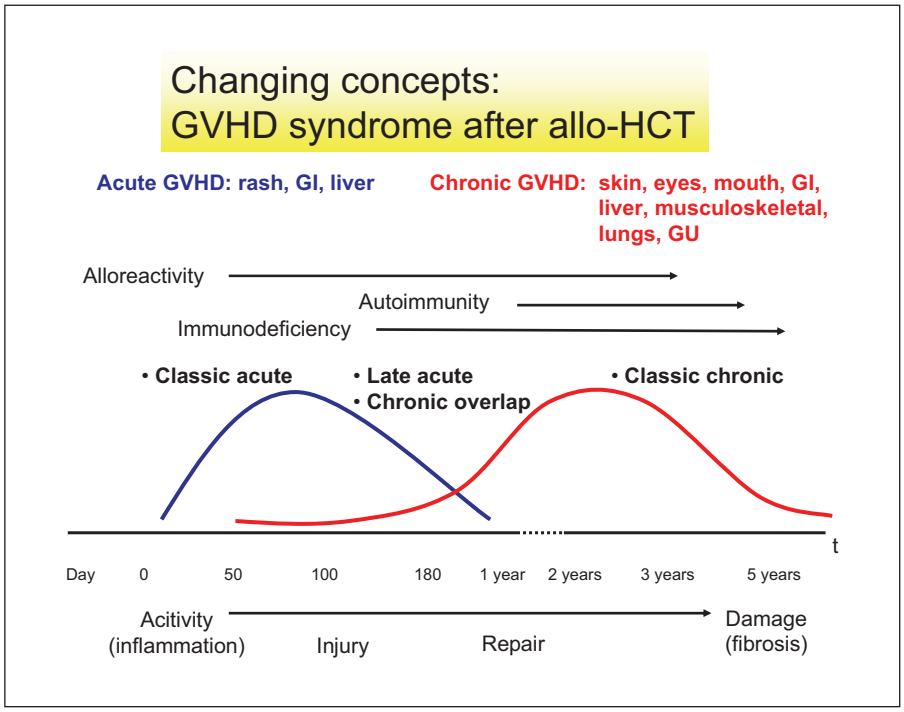

Fig. 1. The current concept of GVHD adapted from http://ccr.cancer.gov/resources/gvhd/about.asp. GI = Gastrointestinal; GU = genitourinary.

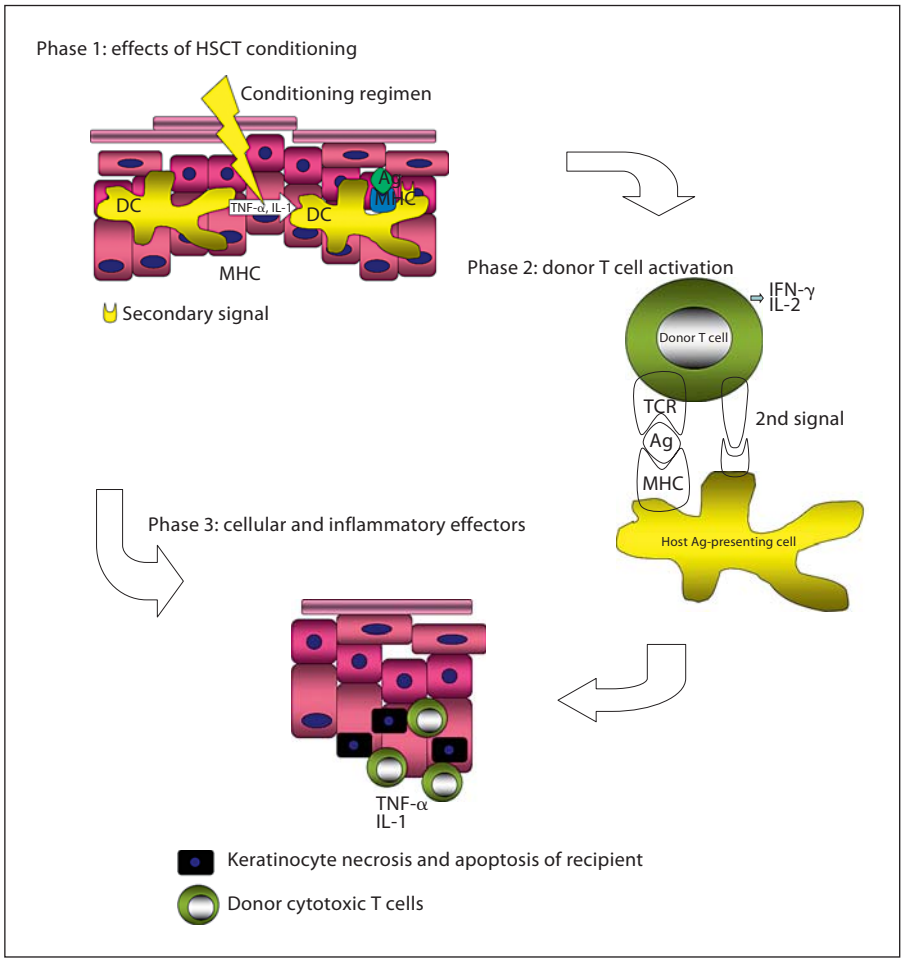

Fig. 2. Three-step model of the pathophysiology of acute cutaneous GVHD. DC = Dendritic cell; Ag = antigen; MHC = major histocompatibility complex; TCR = T cell receptor. Phase 1: the conditioning regimen results in keratinocyte injury within a proinflammatory milieu (e.g. TNF-α, IL-1) that induces antigen-presenting dendritic cells to express costimulatory molecules (e.g. CD80/86, CD40) and increased cell surface levels of major histocompatibility class I and II molecules. Phase 2: after allogeneic HSCT, antigen presentation by host dendritic cells leads to activation of donor T cells and production of Th1 cytokines (e.g. IFN-γ, IL-2). Phase 3: donor cytotoxic T cells mediate direct keratinocyte necrosis/apoptosis (by using e.g. perforins and granzymes) together with cytokines (e.g. TNF-α) and other cells of the innate immune system (for details, see text).

such as platelet-derived growth factor c, connective tissue growth factor or fibroblast growth factor, have been shown to be elevated similar to human scleroderma [41]. However, platelet-derived growth factor receptor antibodies stimulating type I collagen gene expression and myofibroblast phenotype conversion in normal human primary fibroblasts that have been found in patients with systemic sclerosis are not yet known in fibrosing variants of chronic cutaneous GVHD [58].

Similar to the acute counterpart, there is an increasing scientific interest in the potential role of T_{reg} cells in chronic GVHD [59]. However, their ability to influence chronic GVHD remains elusive. So far, it potentially turns out that the dysbalance between regulatory (tolerogenic) cells and alloreactive effector cells will enhance the risk of onset and persistence of chronic GVHD [60, 61]. Finally, there is also evidence that B cells can contribute to the development of GVHD, predominantly in its chronic form, particularly in male patients with transplants from female donors while conversely host B cells may also have the potential to attenuate GVHD by secreting IL-10 [62, 63].

Clinical Manifestations of Cutaneous GVHD

Clinically, GVHD presents either as an acute disease within days to weeks after allogeneic HSCT mainly during the inpatient phase or, alternatively, as a more heterogeneous chronic syndrome that usually occurs months to years after discharge from the hospital. Most challenging with respect to the potential differential diagnosis (e.g. viral infections, drug reactions) is a skin rash that usually occurs within the first 10–14 days after transplantation at the time of marrow engraftment but before the appearance of peripheral lymphocytes. Some authorities term this poorly characterized eruption that often occurs in the setting of multiple HLA mismatched antigens 'hyperacute type of acute GVHD' or 'early mismatch GVHD' which is generally characterized by an erythematous maculopapular eruption that occasionally becomes generalized, and is associated with high fever, hepatitis and intestinal symptoms. It remains open whether the so-called engraftment syndrome in autologous HSCT particularly following the administration of cyclosporine represents a similar phenomenon or not [64, 65]. Typically, however, acute GVHD develops between day 14 and day 42 after transplantation; the timing depends on the conditioning regimen, with a peak incidence around day 30 after myeloablative transplantation and most often later in the setting of reduced-intensity nonmyeloablative regimens or delayed engraftment of umbilical cord blood-derived stem cells. The skin can be the only target organ in acute GVHD or is often attacked before the liver and/or gastrointestinal tract. The spectrum of skin lesions after traditional conditioning regimens was recognized over 30 years ago and has been reviewed for the dermatologist previously [66,67]; these cutaneous features are still considered the most prominent characteristics of the disease. Usually, a symmetrical morbilliform or maculopapular rash with a rather sudden onset is found that predominantly involves the upper back and lateral neck but is sometimes accentuated on the palms, soles, pinnae and cheeks (fig. 3). The eruption may begin acrally but eventually shows a generalized distribution. Prominent acral erythema, a violaceous discoloration of the pinnae and folliculocentric blanching erythema with tiny macules and small papules can be suggestive of that disease. In severer cases, the exanthema can progress to a diffuse erythroderma with bulla formation, a positive Nikolsky sign and desquamation that resembles drug-induced toxic epidermal necrolysis. The mucous membranes and particularly the conjunctivae can be involved as well, and the former can be difficult to differentiate from conditioning-related mucositis [68, 69]. The skin involvement in acute GVHD is measured according to the extent of the lesions (stage 1 = 25% of body surface area; stage 2 = 25–50%; stage 3 = 50% up to erythroderma; stage 4 = erythroderma with bullae) and is included, besides liver and gastrointestinal symptoms/disease, in the prognostically relevant overall grading of acute GVHD (table 3). Approximately half of the patients with moderate to severe GVHD (grades 2–4) may die as a consequence of this treatment-related toxicity [46, 68].

Early lesions of chronic GVHD are often subtle and may include dryness of skin (xerosis), follicular prominence (keratosis-pilaris-like lesions), ichthyosis and papulosquamous lesions [68]. Psoriasiform and pityriasis-rosea-like skin changes as well as annular lesions resembling annular psoriasis, the superficial type of erythema annulare centrifugum or subacute cutaneous lupus erythematosus may be present before the more classical presentations develop, namely lichen-planus-like and sclerodermoid changes (fig. 4) [19]. Lichenoid lesions are characterized by erythematous or violaceous lichenoid papules and plaques that usually affect the dorsal aspects of the hands, forearms and trunk. They may show folliculotropism, rarely follow Blaschko's lines or appear in the dermatomal site of a previous herpes zoster infection [72]. Sclerodermoid chronic GVHD often presents with plaques

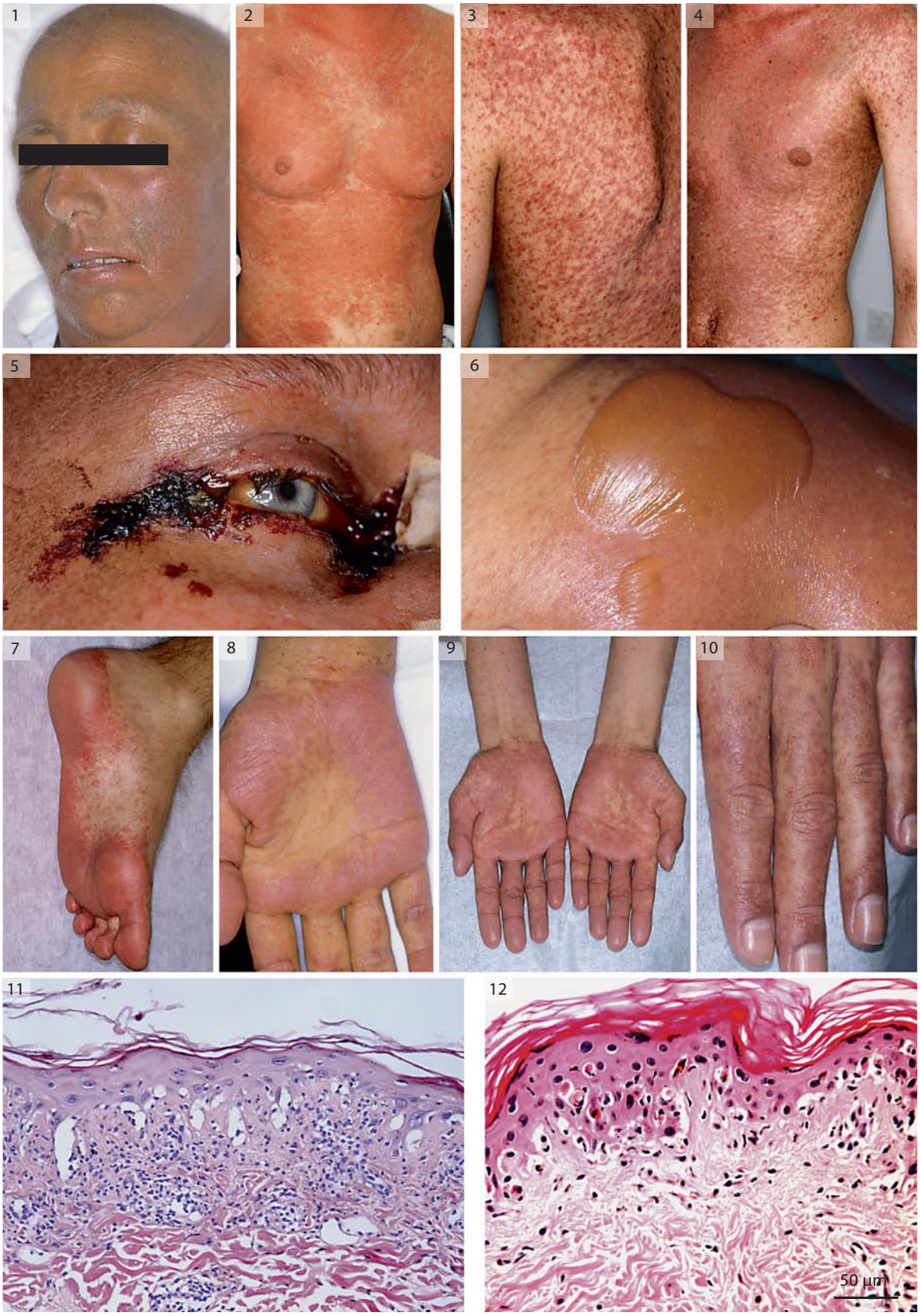

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Table 3. Consensus grading and organ extent of involvement of acute GHVD (adapted from Antin and Deeg [70] and Przepiorka et al. [71])

	Skin	Liver	Intestinal tract
Stage			
1	rash on <25% of skin	bilirubin 2–3 mg/dl	diarrhea >500 ml/day or persistent nausea
2	rash on 25–50% of skin	bilirubin 3–6 mg/dl	diarrhea >1,000 ml/day
3	rash on >50% of skin	bilirubin 6–15 mg/dl	diarrhea >1,500 ml/day
4	erythroderma with bulla formation	bilirubin >15 mg/dl	severe abdominal pain with or without ileus
Grade			
0	none	none	none
I	stage 1–2	none	none
II	stage 3	or stage 1	or stage 1
III	–	stage 2–3	or stage 2–4
IV	stage 4	or stage 4	–

Rash: use the 'rule of nines' to determine body surface area involvement. Bilirubin: range given as total bilirubin; downgrade one stage if an additional cause of elevated bilirubin has been documented. Diarrhea: volume of diarrhea applies to adults; for pediatric patients, the volume of diarrhea should be based on body surface area. Nausea: persistent nausea with histological evidence of GVHD in the stomach or duodenum. Grade IV may also include lesser organ involvement but with extreme decrease in performance status.

of dermal sclerosis that resembles morphea and eventually progresses to generalized scleroderma or, alternatively, presents with lichen-sclerosus-like features in a genital or extragenital distribution. Since its initial recognition, the spectrum of the fibrosing GVHD variants has been continuously extended, and both lichen-sclerosus-type as well as eosinophilic-fasciitis-type sclerotic lesions are now considered diagnostic features and often result in joint contractures [73]. Poikilodermatous changes can be hallmark signs of chronic GVHD and are predominantly observed on the face, lateral neck and trunk, exceptionally in addition to multiple hemangiomas. Adnexal involvement with various nail changes, different types of scarring and nonscarring alopecia, and impairment of sweating are also constant findings, as is involvement of the oral and genital epithelium in up to 80% of patients [69]. Mucous membrane involvement with dryness, atrophy, hypertrophy, lichenoid changes, lacy white plaques and erosions as well as ulcerative lesions, dental caries and periodontitis/gingivitis can all have a significant impact on nutrition behavior, sexuality and overall QOL [74, 75]. The complete clinical spectrum of skin, adnexal and mucosal involvement in chronic GVHD has recently been reviewed by the NIH Consensus Conference and is outlined in table 4 [20]. One of the most challenging aspects in the diagnosis and management of chronic GVHD is the measurement of disease activity and delineation from nonactive sequelae of a past chronic GVHD. Biedermann et al. [76] proposed a combined assessment of activation markers of circulating T cells and endothelial injury measured in skin biopsies as a sensitive and specific test to identify patients with active chronic disease.

Fig. 3. Hallmark clinical and pathological features of acute mucocutaneous GVHD: acute GVHD with characteristic edematous erythema of the head (panel 1), widespread partly confluent maculopapular rash on the trunk (panels 2–4), severe involvement of the eyes with hemorrhagic crusts (panel 5) and bullous lesions (panel 6) resembling toxic epidermal necrolysis; classical acral lesions with relatively sharp borders (panels 7–10) and prototypical histological changes showing vacuolar interface dermatitis with prominent apoptosis of keratinocytes and so-called satellite necrosis of the basal and suprabasal layer (panels 11, 12).

Impact of Histopathology in the Diagnosis and Prediction of Cutaneous GVHD

The histological criteria for the diagnosis of acute cutaneous GVHD have been established in 1974 and are still in use today [77]. Basically, acute GVHD reveals the prototypical morphological features of interface dermatitis of the vacuolar type (fig. 3). Damage to the skin epithelium has early been shown to originate in a nonrandom fashion in the tips of the rete ridges exactly at the niche where cytokeratin 15 epidermal cells, presumably epider-

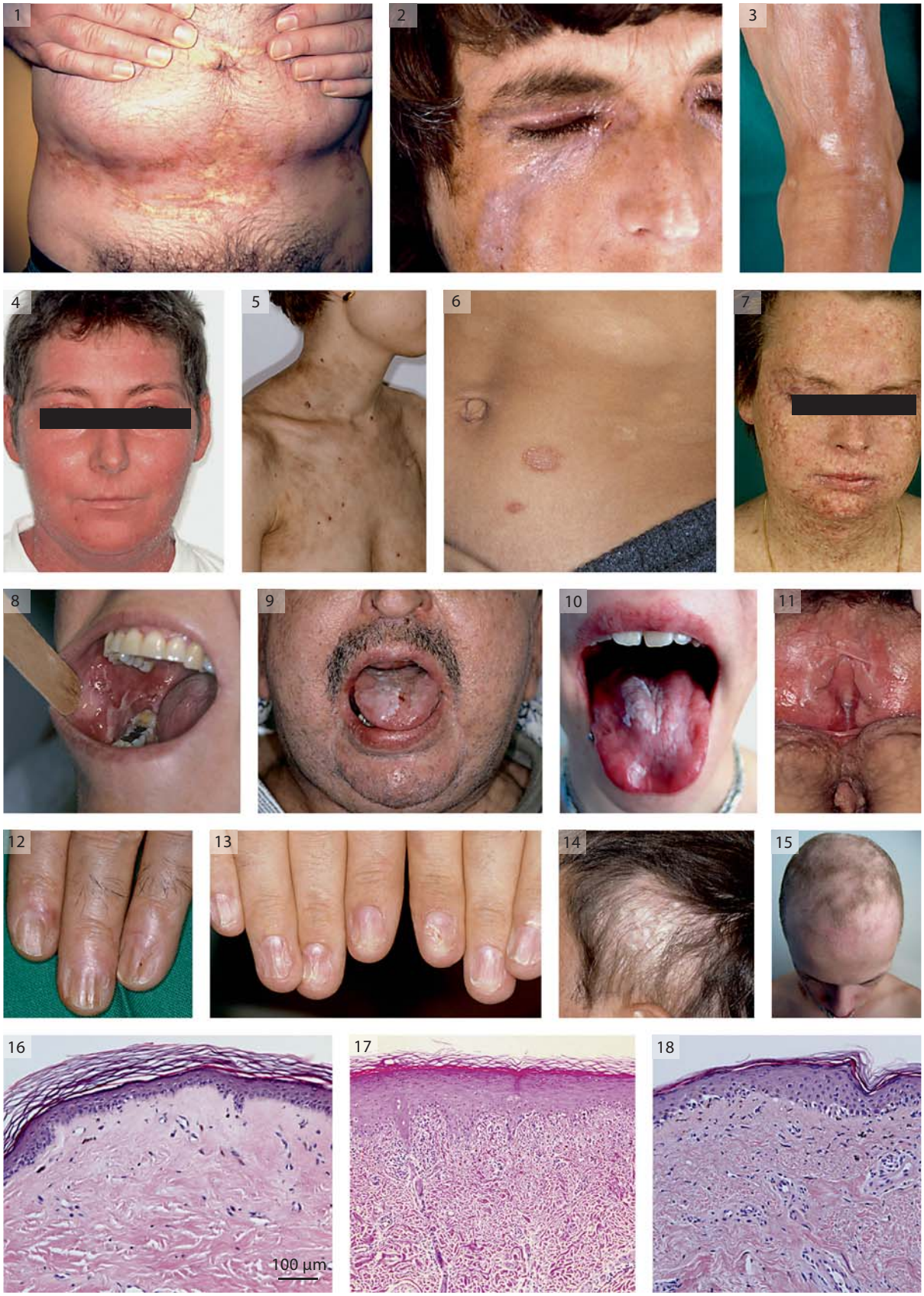

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Table 4. Diagnostic criteria for chronic GVHD of the skin, nails, scalp/hairs and mouth (adapted from Filipovich et al. [20])

A diagnosis of chronic GVHD requires – At least 1 'diagnostic' manifestation *or* – At least 1 'distinctive' manifestation *plus* confirmation of the GVHD diagnosis by biopsy/laboratory tests/imaging in the same or another organ
'Diagnostic' mucocutaneous manifestations – Lichen-planus-like lesions – Lichen-sclerosus-like lesions – Morphea-like lesions – Sclerotic lesions including deep lesions (eosinophilic fasciitis) – Poikiloderma – Oral hyperkeratotic plaques – Oral lichen-type features – Restriction of mouth opening due to sclerosis
'Distinctive' mucocutaneous manifestations – Depigmentation – New-onset scarring and nonscarring scalp alopecia or papulosquamous lesions or scaling of the scalp – Nail dystrophy, longitudinal ridging, splitting or brittle features, onycholysis, pterygium unguis and nail loss – Xerostomia, mucoceles, oral mucosal atrophy, oral ulcers and pseudomembranes
In the absence of clinical or histological signs/symptoms of chronic GVHD, the persistence, recurrence or new onset of characteristic manifestations of acute GVHD should be classified as acute GVHD, regardless of the time after the transplantation.

Fig. 4. Selected spectrum of clinical and pathological lesions in chronic cutaneous GVHD: chronic GVHD with characteristic morphea-like lesions (panel 1), sclerotic features resembling sclerodermia en coup de sabre (panel 2) and eosinophilic-fasciitis-like fibrosing lesions (panel 3) showing the 'groove sign'; head erythema with scaling in a patient with late acute/chronic overlap disease (panel 4); lichen-planus-like changes (panel 5) and pityriasis-rosea-like manifestations in 2 patients more than 6 months after HSCT (panel 6); distinctive dyspigmentation of the head with features of poikiloderma resembling xeroderma pigmentosum (panel 7); classical mucosal lesions with lichen-type features, hyperkeratotic plaques and atrophy on the buccal mucosa, tongue and genital area (panels 8–11); longitudinal ridging, splitting and pterygium formation of nails (panels 12, 13) and examples of patchy scarring (panel 14) and nonscarring (panel 15) alopecia in 2 patients after recovery from chemoradiotherapy; hallmark histological changes with superficial sclerosis and vessel rarification (panels 16), classical lichenoid-type changes (panel 17) and changes of late acute/chronic overlap disease with interface dermatitis partly of the vacuolar and lichenoid type (panel 18).

mal stem cells, are thought to reside [78]. However, while gene expression analysis in mouse models indicates that these cytokeratin-15-positive epidermal cells are early targets in GVHD, it is unclear whether this is the case in humans [40, 79, 80].

Histologically, there is a stereotypical evolution of morphological findings of subtle endothelitis and perivascular mast cell degranulation in the uppermost dermis that are suggestive of but not specific for the disease. These changes are usually followed by the vacuolization of the basal epithelial layer (grade 1), keratinocyte apoptosis and satellitosis (grade 2), up to subepidermal clefting (grade 3) and epidermal separation (grade 4). Involvement of the upper portion of eccrine or follicular structures is a distinctive finding that often occurs early in the course of the disease and can prove helpful for diagnosis. Immunohistochemistry can identify the predominance of CD8+ lymphocytes as main effectors but its value in the diagnosis of acute GVHD is rather limited [81]. On a microscopic level, there is considerable overlap of histological findings of early GVHD and various other post-transplantation diseases (e.g. viral exanthems, eruptions due to immune reconstitution, drug reactions), overall decreasing the sensitivity and specificity of a skin biopsy in a given patient. Therefore, as there are no pathognomonic histological features for GVHD, the value of a biopsy lies mainly in lending support to the clinical diagnosis of GVHD or exclusion of other diseases, depending on the clinical context. Importantly, the number of lymphocytes entering the epidermis and quantity of apoptotic keratinocytes did not prove useful as predictor of the severity of the clinical disease, in contrast to the predictive value of substantial increases in total bilirubin, diarrhea and extent of rash and overall clinical GVHD grade for potentially fatal outcome [82, 83]. Furthermore, skin biopsies of a rather unspecific rash that occurs in the early phase after transplantation have been shown to be of limited value in predicting the progression from rash (viral-induced, drug-induced or early acute GVHD) to overt acute GVHD grade 2–4 irrespective of the stem cell source (peripheral blood, bone marrow). Likewise, the histopathological assessment of normal skin before allogeneic HSCT could not predict the development of GVHD [81, 84]. So far, in patients with clinicopathological acute GVHD, there are no known morphological or immunohistochemical features predicting the risk of subsequent chronic GVHD. Only in the setting of nonmyeloablative conditioning a small retrospective study found that histologically 'proven' clinical acute GVHD with morphological features of both acute and chronic disease ('com-

Table 5. Histological criteria for GVHD of skin and oral mucosa (adapted from Shulman et al. [21])

Organ/lesion	Minimal criteria for active GVHD	Specific criteria for chronic GVHD
Skin, any stage	Apoptosis in epidermal basal or lower malphigian layer or outer root sheath of hair follicle or acrosyringium ± lichenoid inflammation ± vacuolar change ± lymphocytic satellitosis	
Lichen-planus-like		Combination of epidermal orthokeratosis, hypergranulosis and acanthosis with lichenoid changes ± syringitis of eccrine units ± panniculitis
Sclerotic		Collagenous deposition with thickening throughout the papillary dermis or pan-dermal collagenosis ± panniculitis
Morpheic		Sclerosis in the lower reticular dermis or along the dermal-hypodermal border ± epidermal and adnexal involvement
Fasciitis		Fibrous thickening of fascial septa with adjacent inflammation ± panniculitis
Oral mucosa	Lymphocytic infiltration of mucosa with variable apoptosis	

posite' histological features) may predict the subsequent development of chronic GVHD [85]. In daily routine, the best way to provide an accurate diagnosis of acute GVHD may encompass a combined approach that includes mucocutaneous assessment, histopathological evaluation and exclusion of other diseases in the hands of experienced physicians and that sometimes requires repeat evaluations for an adequate diagnosis.

The histopathology of chronic GVHD has traditionally been divided into an epidermal (lichen-planus-like) and a dermal (sclerodermoid) type (fig. 4). Subtle histological features that are mainly regarded as hallmark changes in acute GVHD, e.g. satellitosis and vacuolization of the basal layer, may also be found in both chronic types, but are far more predominant in the epidermal type, as is adnexal involvement, e.g. cytotoxic acrosyringitis or folliculitis. However, spongiotic alterations may be found, and marked thickening of the stratum corneum, stratum granulosum and stratum spinosum together with a band-like infiltrate and perifollicular fibrosis are indicative features of lichen-planus-like chronic GVHD, whereas thickening, homogenization and compaction of the collagen bundles are hallmark findings in the sclerodermoid type.

The NIH Pathology working group has recommended to confirm clinically suspected chronic GVHD by histology; however, the role of subsequent biopsies to assess the response to treatment has not yet been determined [21]. The group emphasized that histological interpretation requires the consideration of the clinical context to minimize false-negative and false-positive diagnoses (e.g. drug reactions, concurrent infections or inflammatory reactions unrelated to GVHD). The proposed histological criteria for skin and oral mucosa now differentiate between minimal criteria for active disease and specific criteria for chronic GVHD (table 5) and, together with clinical, laboratory and radiographic information, are an integral part to yield 4 diagnostic categories, i.e. 'no GVHD', 'possible GVHD', 'consistent with GVHD' and 'definite GVHD'. Importantly, the diagnosis and staging committee recommended that keratinocyte apoptosis without other features of chronic GVHD found on day 80–100 screening skin biopsies does not indicate chronic GVHD and in that particular situation should not be taken to predict that alloreactive T-cell-induced flares may follow cessation of immunosuppressive therapy [20].

New Classification Systems for GVHD

Whereas the term acute GVHD has historically been used to describe a syndrome of dermatitis, cholestatic hepatitis and gastroenteritis that developed within the first 100 days after allogeneic HSCT, chronic GVHD has traditionally been used to describe a syndrome that occurred after day 100 and often contained features of auto-

Table 6. Categories of acute and chronic GVHD (adapted from Filipovich et al. [20])

Category	Timing of symptoms after HSCT or DLI	Presence of acute GVHD features	Presence of chronic GVHD features
Acute GVHD			
Classic	≤100 days	yes	no
Persistent, recurrent or late onset	>100 days	yes	no
Chronic GVHD			
Classic	no time limit	no	yes
Overlap syndrome	no time limit	yes	yes

DLI = Donor lymphocyte infusion.

immunity. This somewhat arbitrary time delineation was of high clinical value in the early era of myeloablative conditioning regimens in the setting of bone marrow or peripheral blood HSCT. Nowadays, in the era of increased numbers of haploidentical, cord blood, nonmyeloablative and unrelated transplants, so-called stem cell boosts and donor lymphocyte infusions, disease manifestations that were commonly observed in acute GVHD, can often develop after day 100 following transplantation, whereas, conversely, signs and symptoms of chronic GVHD may be present shortly after donor lymphocyte infusion. This paradigm shift is respected in the new categories of acute and chronic GVHD that were proposed by the recent NIH consensus conference and are outlined in figure 1 and table 6 [20]. Moreover, the distinction between limited and extensive chronic GVHD [86] that was introduced in 1980 on the basis of a retrospective observational study of only 20 patients is now proposed to be displaced by an organ scoring system (table 7). This proposed global scoring system reflects the clinical effects of chronic GVHD on the patient's functional status and finally classifies patients as having mild, moderate or severe chronic GVHD [20, 86]. In the absence of clinical or histological features suggestive of chronic GVHD, the new onset, persistence and recurrence of characteristic manifestations of acute GVHD should now be considered as acute GVHD regardless of the time after transplantation [20]. The lack of standardized criteria for quantitative measurement of therapeutic response in clinical trials remained a major problem and obstacle for the efficient development and introduction of new agents in this disease [23]. The proposed organ scoring and response criteria address this issue at least in part, but it remains to be demonstrated in the future whether the course of the disease and the influence of drugs can be more precisely recorded.

Basic Preventive and Therapeutic Approaches to (Cutaneous) GVHD

Two fundamentally different approaches for the prevention of GVHD have been established: immunosuppression with cyclophilin inhibitors such as cyclosporine or FK506 with or without methotrexate or T cell depletion. Both approaches have their specific advantages and disadvantages. Cyclophilin inhibitors interfere with the activation and expansion of donor T cells and are very effective in the prevention of GVHD but need to be taken over a long period of time and are associated with significant side effects, such as nephrotoxicity. In contrast to standard transplantation, ex vivo graft manipulation such as T cell depletion has lower organ toxicity but is associated with higher incidences of graft failure or graft rejection, delayed immune reconstitution particularly of the CD4+ lymphocytes, decreased functional recovery of T cells and, importantly, impaired recovery of T cell repertoire diversity. While increased incidences of bacterial or fungal infections have not been documented, there is an enhanced risk of posttransplantation lymphoproliferative disease and leukemia relapse after transplantation of manipulated compared to unmanipulated stem cells, and the probability of reactivated viral infections such as cytomegalovirus appears to be increased [87, 88]. Newer preventive strategies including cytokine-based approaches, e.g. antithymocyte globulin, anti-TNF-α antibody (infliximab), anti-TNF-α receptor (etanercept) or anti-IL-2 receptor antibody (dacalizumab) to neutralize the conditioning-induced cytokines of the afferent phase of acute GVHD before infusion of the graft, are not yet firmly established in clinical use and may have a predominant role in the treatment of steroid-refractory disease [88]. Other immunosuppressive drugs such as mycophe-

Table 7. Organ scoring and global assessment and severity of chronic GVHD as proposed by the 2005 NIH Consensus Development Project (adapted from Schaffer [34])

	Score 1	Score 2	Score 3
Skin	<18% of BSA and no sclerotic features	19–50% of BSA or superficial sclerosis (able to pinch)	>50% of BSA or deep sclerosis (unable to pinch) or impaired mobility, ulceration or severe pruritus
Mouth	Mild signs/symptoms not limiting oral intake	Moderate signs/symptoms with partial limitation of oral intake	Severe signs/symptoms with major limitation of oral intake
Eyes	Mild dry-eye symptoms (using eyedrops ≤3 ×/day) or asymptomatic but signs of keratokonjunctivitis sicca	Moderate dry-eye symptoms partially affecting ADL (using eyedrops ≥3 ×/day or punctuate plugs), no visual impairment	Severe dry-eye symptoms significantly affecting ADL or unable to work or loss of vision
GI tract	Symptoms without significant weight loss	Symptoms with weight loss of 5–15%	Symptoms with weight loss >15%, requiring nutritional supplementation or need for esophageal dilatation
Liver	Bilirubin, AP, AST or ALAT <2 × of normal upper limit	All 2–5 × of normal upper limit or bilirubin >3 mg/dl	All ≥5 × of upper normal limit
Lungs[1]	Mild symptoms (SOB after 1 flight of steps); FEV_1 60–79% or LFS 2	Moderate symptoms (SOB after walking on flat ground); FEV_1 40–59% or LFS 6–9	Severe symptoms (SOB at rest or requiring supplement O_2); FEV_1 ≤39 or LFS 10–12
Joint/fascia	Mild tightness of arms or legs, mildly decreased ROM and not affecting ADL	At least 1 of the following: tightness of arms or legs, joint contractures, erythema due to fasciitis, moderately decreased ROM and mild-moderate limitation of ADL	Contractures with significantly decreased ROM and significant limitation of ADL
Genital tract	Mild signs/symptoms and no effect on coitus/minimal discomfort on examination	Moderate signs/symptoms and mild dyspareunia/discomfort with examination	Advanced signs (strictures, labial fusion or severe ulceration) and severe pain with coitus/inability to insert vaginal speculum
Global assessment of chronic GVHD	Mild 1–2 organs (except the lung) with a maximum organ score each of 1	Moderate ≥1 site with an organ score of 2 or ≥3 sites with an organ score of 1 or lung score of 1	Severe Any organ score of 3 or lung score of 2

ADL = Activities of daily living; ALAT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; BSA = body surface area; FEV_1 = forced expiratory volume in 1 s; GI = gastrointestinal; LFS = lung function score (includes FEV_1 and diffusion capacity of the lung for CO); ROM = range of motion; SOB = shortness of breath.

[1] When a discrepancy exists between symptoms and pulmonary function test scores (FEV_1/diffusion capacity of the lung for CO), the higher score should be used.

nolate mofetil as prophylactic treatments alone or in combination with established drugs are under active investigation but have not yet been proven to be effective [28].

The first-line treatment of significant acute (≥grade II) or chronic GVHD consists of high doses of systemic corticosteroids [46, 54]. The standard therapy of acute cutaneous GVHD below 50% of body surface involvement (<grade II) includes topical application of potent corticosteroids or tacrolimus [89]; by comparison, their application in chronic GVHD in addition to systemic therapy, especially at mucosal sites, is not established. Unfortunately, there is still no effective treatment for corticosteroid-refractory acute GVHD. A variety of agents has been investigated and will be tested in future, including chemical immunosuppressants such as mycophenolate mofetil, sirolimus or thalidomide, broad antilymphocyte antibodies (antithymocyte globulin, anti-CD3 such as OKT3 and visilizumab) and more specific agents directed against activation or adhesion molecules (anti-

CD25, anti-IL-2 receptor, anti-CD147, anti-CD52) or cytokines such as anti-TNF-α antibody (infliximab) [26, 50, 54, 62, 88, 90, 91]. Extracorporeal photopheresis has been successfully applied in chronic GVHD, particularly in cutaneous GVHD, and will need to be tested as first-line treatment of specific subgroups in steroid-refractory GVHD [92]. Furthermore, other light sources such as psoralen preparations and direct exposure to UVA have been proven to be effective in some patients with isolated cutaneous GVHD [93]. Standard treatment of chronic GVHD includes cyclosporine and prednisone. Treatment regimens combining prednisone with cyclosporine or tacrolimus with mycophenolate mofetil are under current investigation. Other approaches including anti-TNF-α and anti-CD20 antibodies, pentostatin and sirolimus are currently studied as well [39]. Supportive care, accurate anti-infective treatment, balanced nutrition and topical emollients for the skin, mucous membranes and eyes are established in patients with both acute and chronic GVHD [89]. Furthermore, there are early reports indicating some efficacy of mesenchymal stem cells in steroid-refractory severe acute GVHD. Whether these beneficial effects will persist over time in these patients and whether they have a role in the treatment of chronic GVHD needs to be shown [94, 95].

Outlook

Allogeneic HSCT will remain a therapeutic cornerstone for an increasing number of diseases, with currently over 12,000 transplantations performed worldwide per year, and estimates of ongoing annual growth rates of 10–20% [96]. Global research goals to enhance treatment outcome and QOL of those patients incorporates extensive laboratory and clinical investigations, in particular to decrease the toxicity of conditioning regimens, advance donor-recipient typing and graft tolerance, reduce graft rejection, decrease GVHD and increase the graft-versus-tumor (GVT) effect in the setting of malignancy. In particular, research activity in the field of biomarkers, pathobiology, response criteria and therapy are key areas that will be of critical importance to encounter the increasing significance of chronic GVHD [22, 97]. Based on its multiorgan involvement with its features of both allo- and autoimmunity (or 'altered immunity'), chronic GVHD will require a multidisciplinary approach that importantly includes the dermatologists. Similarly, a site-specific long-term management of active disease and sequelae will require the collaboration of various specialists, not only physicians, but also non-physician medical workers such as physiotherapists, psychologists and social workers. Effective coordination of information between patient, physicians and therapists will be facilitated by a simple and precise organizational and administrative algorithm. Despite these challenges, improvement of QOL and survival, which are both influenced by the occurrence and severity of chronic GVHD, remain the primary aims for patients after HSCT [38, 98]. Unfortunately, we are not yet capable of separating T-cell-mediated harmful GVHD and beneficial GVT effects: as a result, graft manipulation such as T cell depletion not only abolishes the undesirable GVHD, but also minimizes beneficial GVT effects in the setting of malignancy. Missing alloreactive immunocompetent T cells thus increase the risk for disease relapse as well as severe infections at least during the first few years after transplantation. Therefore some degree of clinical chronic GVHD is currently embraced to decrease the chance for disease relapse accepting some decrease in QOL, rather than totally eliminating GVHD [38, 98, 99]. The ultimate goal is to find the optimal risk-benefit compromise and to accept mild chronic GVHD while maintaining the best possible QOL. Current and future investigations to enhance the GVT effect while decreasing GVHD will be ultimate to decrease tumor relapse and GVHD while enhancing survival and provide allogeneic HSCT to a broader patient collective.

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