1 The Contributions of Pathology to the Diagnosis and Management of GVHD: Caveats and Lessons Learned

Howard M. Shulman

Histologic descriptions of graft-versus-host disease (GVHD) have contributed significantly the diagnosis and management of GVHD as well as the understanding of its pathobiology. With the increasing complexities of hematopoietic stem cell transplantation (HSCT), making informed interpretations from histologic material—biopsies or autopsies—requires substantial background knowledge. The goal of this publication is to provide updated information for pathologists and clinicians with limited exposure to the HSCT setting and the nuances of histologic interpretations thereof. We illustrate the spectrum of GVHD's histopathology and some of the unresolved debates regarding its interpretation. This book's format includes clinical vignettes of classical GVHD cases as well as complex and challenging case scenarios, supplemented by both gross and histopathologic images of acute (aGVHD) and chronic GVHD (cGVHD). Through these case discussions we present insight from previous studies and experiences, describe the key points derived from the final histologic interpretation, and offer relevant information to elucidate the pathobiology of GVHD.

The classic organs targeted by GVHD are the skin, gastrointestinal (GI) tract, and liver. The principles related to histopathologic interpretation and caveats related to each of the target organs are discussed below and in the respective chapters. The contemporary diagnostic criteria and recommended format for reporting the organs involved with GVHD reflect the insights and applications of newer studies that are summarized in the two NIH histopathology consensus panels published in 2006 [1] and 2015 [2] (Table 1.1).

The cardinal feature of GVHD is apoptosis of the targeted epithelia. Criteria for defining an apoptotic epithelial cell in the skin and gut are discussed in Chaps. 3 and 8,

H. M. Shulman (✉)
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Pathology Section, Seattle Cancer Care Alliance, Seattle, WA, USA
e-mail: tigermaya@comcast.net

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Table 1.1 Criteria of the minimal and specific criteria for aGVHD and cGVHD in the organs or systems most often affected by GVHD, according to the NIH histopathology consensus panel's 2015 publication [2]

Organ or system	Minimal criteria for acute/active GVHD[a]	Specific criteria for Chronic GVHD[b]
Liver	Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular, and portal inflammation	Ductopenia, portal fibrosis, and chronic cholestasis reflect chronicity but are not specific for chronic GVHD
Gastrointestinal	Variable apoptotic criteria (≥1/piece) in crypts	Destruction of glands, ulceration, or submucosal fibrosis may reflect severe or long-standing disease but are not specific for chronic GVHD
Skin, in general	Apoptosis in epidermal basal layer or lower Malpighian layer or infundibulum / outer root sheath of hair follicle or acrosyringium / sweat ducts ± lichenoid inflammation ± vacuolar change ± lymphocytic satellitosis	
Skin lichen planus-like		Combination of epidermal ortho-hyperkeratosis, hypergranulosis and acanthosis resembling lichen planus ± lichenoid inflammation and / or vacuolar changes of eccrine units
Skin morphic (localized or diffuse)		Localized thickening and homogenization of collagen bundles throughout reticular dermis or pandermal sclerosis with overlying interface changes ± thickening and homogenization of subcutaneous septa
Skin lichen sclerosus-like		Homogenization ± sclerosis of papillary dermal collagen with overlying interface changes including melanophages in the papillary dermis and sparse lymphocytic infiltrate
Skin fasciitis		Thickening of fascial septa with adjacent inflammation ± sclerosis of subcutis
Oral/ oropharyngeal mucosa and conjunctiva	Lichenoid interface lymphocytes with infiltration of mucosa (exocytosis) and variable apoptosis[c]	
Minor salivary or lacrimal gland		Periductal lymphocytic infiltrate with infiltration and damaged intralobular ducts, fibroplasia in periductal stroma, mixed lymphocytic and plasmacytic inflammation with destruction of acinar tissue[d]

(continued)

Table 1.1 (continued)

Organ or system	Minimal criteria for acute/active GVHD[a]	Specific criteria for Chronic GVHD[b]
Lung		Constrictive bronchiolitis obliterans: dense eosinophilic scarring beneath the respiratory epithelium, resulting in luminal narrowing or complete fibrous obliteration. May be preceded by lymphocytic bronchiolitis without intraluminal fibrosis[e]
Kidney		Membranous nephropathy, Minimal Change Disease
Lesions of Uncertain Pathogenesis	Central nervous system	
Lung	Cryptogenic organizing pneumonia	
Skeletal Muscle	Myositis	

[a]Conditions that result in lesser degrees of change include immunosuppressive treatment, biopsy very soon after onset of signs, suboptimal or small tissue sample, insufficient serial sectioning, confounding infection, drug reaction, or inflammatory conditions

[b]Once the diagnosis of chronic GVHD has been established or following immunosuppressive treatment, the histological manifestations of active disease may meet only minimal diagnostic criteria for activity. Different manifestations of cutaneous chronic GVHD may all be present together in one biopsy or in separate but concurrent biopsies

[c]Inflammation of the oral mucosa and within the minor salivary glands may persist from prior chemo-irradiation or prior inflammation. The distinction between acute and chronic GVHD requires the addition of distinctive oral manifestations [3]

[d]The distinction of past acinar destruction and fibrosis from ongoing chronic GVHD activity can be difficult and relies on assessing lobules that are not completely fibrotic. Acinar and periductal inflammation with features of damage to ducts, such as vacuolar change, lymphocytic exocytosis, nuclear dropout, dyspolarity or apoptosis, and resultant fibroplasia indicate chronic GVHD activity

[e]Constrictive bronchiolitis obliterans (CBO) should be distinguished from cryptogenic organizing pneumonia, which is also associated with GVHD but has a different clinicopathologic presentation and a more favorable outcome

respectively. A variety of factors are responsible for both false-negative and false-positive interpretations of GVHD. For example, skin and liver biopsies taken at the onset of clinical signs and symptoms of clinically-proven GVHD may not display the diagnostic histologic changes. Prior exposure to corticosteroids may markedly reduce the inflammatory component with variable effects on the degree of epithelia injury. The pathologist and clinician must be aware of these caveats when integrating pathologic findings disparate from clinical assessments.

Skin

Acute GVHD The basic tools needed to interpret skin biopsies include formalin-fixed tissue biopsies stained with H&E. The biopsy should ideally include some hair follicles since the progenitor regions of the follicular unit are targeted by GVHD. The histologic changes, if mild, may be infrequent or spotty. At least 4 and up to 8 serial sections should be evaluated if the tissue block permits. In routine practice, applying immunohistochemistry (IHC) staining to define the cellular phenotypes has not been shown to be a useful adjunct, except when identifying leukemia cutis (Chap. 5). The infiltrates are often sparse, and the discriminating diagnostic antibodies for T-cell subsets require research applications. In fact, Austrian investigators using research techniques to isolate and define both functional and phenotypic T cell profiles from different cutaneous GVHD lesions—acute, lichenoid, or sclerotic—have demonstrated that the different lesions display different T-cell subset patterns and that their cytokine profiles can predict the development of GVHD [4]. Of note, two studies have demonstrated that dermal macrophages may comprise the largest cellular infiltrate in aGVHD and have some correlation with steroid refractoriness [5, 6]. If malignancy is a consideration, appropriate IHC stains should be done (Chap. 5). Most skin biopsies evaluated for aGVHD consist of a 3 mm or 4 mm punch biopsy. The diagnosis of early skin GVHD is discussed in Chap. 3. The different opinions for when a skin biopsy is needed to establish aGVHD are discussed in Chap. 2. Chapter 4 describes the spectrum of cutaneous aGVHD and the differential diagnosis. Most aGVHD of the skin resolves with treatment, albeit with some residual pigmentary and atrophic changes. It should be noted that there is no clear histologic distinction between aGVHD that arises in the first several months or as a late-onset occurrence. However, the clinical implications for the latter are often severe (Chap. 6).

Chronic GVHD Cutaneous cGVHD has a complex biphasic pandermal histology with an early lichen planus-like inflammatory phase (Chap. 6) followed by a pan-sclerotic or morpheic phase that involves the superficial and deep layers of the skin (Chap. 7). It is important that biopsies are full thickness so the dermal adnexa and subcutaneous fat and fascia are included to aid in the evaluation. The majority of the skin biopsies from non-sclerotic skin are done with a punch biopsy. The current consensus recommendation by a panel of clinicians (82%) does not recommend performing a skin biopsy for patients with suspected cGVHD unless there are no other diagnostic features as defined in the NIH consensus' 2014 publication [7]. However, a study from a large tertiary referral treatment center for cGVHD found that 7% of their referral patients lacked confirmation of cGVHD when biopsied [8]. A European consensus panel of dermatologists, clinicians, and pathologists recommended a scalpel biopsy for sclerotic or deep fasciitis GVHD [9], though this recommendation is not uniformly followed in practice because of patients' additional discomfort, slower healing, and need for sutures. The trichrome stain may be useful in judging the degree and location of dermal sclerosis, especially when evaluating

responses to treatment, progression, or static changes. More complete descriptions of the manifestations of cGVHD are discussed in Chaps. 2, 6, and 7. Chapter 12 also discusses manifestations of cGVHD in mucosal surfaces of the oral cavity, esophagus, and anogenital region. Other organs affected by cGVHD are discussed in Chaps. 17, 18, 19, and 20.

Liver

GVHD of the liver affects 8–9% of all allogeneic HSCT recipients, mostly occurring in conjunction with gut involvement. The liver is the most difficult of the GVHD-targeted organs to assess because of the relative non-specificity of the laboratory findings, the co-existence of infection, and/or potential overlap with drug-induced liver injury (DILI). Interpretation of liver biopsies relies on somewhat empiric qualitative criteria rather than quantitative histologic criteria (Chaps. 13, 14, 15, and 16). Damage or destruction of the small bile ducts, ductitis, cholestasis, and variable inflammation are the hallmarks of liver GVHD. Chapter 13 discusses pre-transplant liver conditions that leads to post-transplant liver dysfunction which overlaps with early GVHD. Pathologists need to be aware that some benchmark histologic features used to interpret liver biopsies in a non-HSCT setting are not necessarily applicable to liver biopsies obtained in the HSCT setting. Thus, a mixed portal inflammatory infiltrate containing scattered eosinophils is not prima facie evidence of a DILI; plasma cells should not point to auto-immune hepatitis, nor should ductular reactions (proliferation), which occur in a number of necroinflammatory liver disorders, necessarily indicate biliary obstruction [10]. Likewise, the absence of perivenular endothelialitis, a hallmark of liver rejection after orthotopic liver transplantation, is an unreliable rejector of liver GVHD. Of note, biopsies obtained shortly after the onset of clinical signs of liver GVHD may only demonstrate false-negative, nonspecific hepatocyte apoptosis (councilman bodies)—which is related to cytokine-induced hepatocytolysis through the Fas-Fas Ligand (Fas-FasL) interaction—without clear bile duct damage as compared to subsequent biopsies [11] (Chap. 15). Improvement in clinical liver tests following immunosuppression (IS) is not immediately evinced by a reduction in biliary injury, and a single liver biopsy obtained while on prolonged IS can judge the severity of bile duct damage but cannot determine the trajectory.

Whether to obtain a liver biopsy is a significant decision requiring thorough understanding of the clinical context and comprehensive communication between the physician and patient. It is an invasive procedure, occasionally requiring anesthesia in a child, and carries the risk of serious bleeding or even death. The decision is based on the urgency to identify the likely cause of elevated liver tests that are not clearly explicable by the clinical context and distinguishable from concurrent possibilities, e.g. an infectious or malignant process. The interventionists should avoid using thin gauge needles as they distort the architecture and obscure the interpretation of the biliary structures, the cardinal target of liver GVHD. Transvenous forceps biopsy fragments coupled with manometric intrahepatic pressure gradient are

suitable for the evaluation of venoocclusive disease/sinusoidal obstruction syndrome (VOD/SOS) (Chap. 13), but they cause considerable crushing and distortion of liver architecture, hindering the evaluation of GVHD. There is no universal agreement on the minimum size of a liver biopsy, but the confidence in the biopsy's interpretation is related to sectioning and stain quality and the number of evaluable portal spaces (≥3). The evaluation of a liver biopsy should include staining with H&E, PAS, PAS/D, reticulin, trichrome, and immunostains for cytokeratins 7 and/or 19. When the history is suggestive, stains for infection organisms and viral agents are performed as well. The clinical approach to liver dysfunction suspicious of liver GVHD and the differential diagnoses are discussed in Chaps. 2, 14, 15, and 16. Late-occurring isolated liver dysfunction and/or ascites can be a symptom of several different viral infections, acute hepatitic onset of GVHD (Chap. 16), nodular regenerative hyperplasia, or nonalcoholic steatohepatitis (NASH). Rarely, fulminant hepatic failure from fibrosing cholestatic hepatitis (FCH) can occur with hepatitis C (HCV) [12], but more frequently occurs in patients with active hepatitis B (HBV) [13] (Chap. 15). In Chap. 16 we discuss uncommon cases of a chronic inflammatory and fibrosing hepatitis, apparently unassociated with prolonged GVHD or infection, in which patients develop cirrhosis many years after HSCT. These cases have been coined "chronic alloimmune hepatitis" (CAIH).

Gastrointestinal Tract

GVHD of the gut develops in over 50% of all allogeneic HSCT recipients [14] and is nearly always a component of clinically severe cases (Chaps. 8, 9, and 10). Non-relapse mortality is significantly greater among patients whose signs and symptoms of gut aGVHD persist or worsen despite initial prednisone therapy than among responsive patients [15] (Chap. 2). This increased non-relapse mortality in refractory patients is due to infection and the attendant immunosuppressive therapy.

There are several unresolved debates regarding the use and interpretation of endoscopic biopsies. A number of studies from different institutions disagree on the best endoscopic gut biopsy site for obtaining the highest diagnostic yield—stomach, antrum or body, duodenum, or colon/rectum [2]. However, institutions do agree that a greater number of biopsy locations improve the diagnostic yield. GVHD may have a patchy distribution even within in a single region, e.g. the colon, and concurrent endoscopic biopsies from the stomach, duodenum, and colon can display significantly different degrees of mucosal damage (Chap. 8 and 9). The tissue blocks should be serially sectioned as well.

The histologic gamut of gut GVHD ranges from infrequent scattered individual crypt cell apoptosis (Chap. 8), to widespread crypt damage (Chap. 9), to complete crypt destruction with mucosal denudation (Chap. 10). The histologic spectrum of gut GVHD does not correspond to the period of time post-transplant, but rather to the severity and duration of active GVHD. Hence there is no distinction between aGVHD and cGVHD except for visualizing esophageal web formation by endoscopy or imaging, which is designated as a feature of cGVHD.

Chapter 8 addresses the definition of an apoptotic enterocyte (crypt epithelial cell), crypt destruction, and crypt abscess. The differential of early gut GVHD includes the effects of concurrent drugs and pre-transplant chemo-irradiation conditioning regimens that cause apoptosis (Chap. 8). Chapter 9 discusses the debates regarding the minimum number of apoptotic cells to fulfill minimal diagnostic criteria. Chapter 9 also discusses two grading scales—the modified Lerner-Sale grading scheme and the Myerson apoptotic activity index—for assessing histological severity and prognostic implications [16, 17]. Chapter 10 illustrates the changes of chronic, persistent, steroid-dependent, or refractory severe gut GVHD and the immunobiology of the crypt niche and gut microbiota. Chapter 11 discusses the infectious processes that often coexist in gut biopsies and contribute to the differential diagnosis of gut GVHD.

The complex immunopathogenesis of GVHD involves the interactions of T cells, B cells, and cytokines in targeted organs. The microvascular endothelium plays a pivotal role in the trafficking of specific T cell to targeted organs (Chap. 10). It contributes to a spectrum of damage including perpetuating gut cGVHD (Chap. 10), transplant-associated thrombotic microangiopathy (Chap. 10), and some glomerulopathies associated with GVHD (Chap. 19).

Interpretation of Biopsies

The pathologist should have all relevant clinical details when making an interpretation. This includes the underlying primary diagnosis, the type of graft (allogeneic, autologous), the stem cell donor source, the number of the days post-transplant, and the use and duration of any IS in relation to the day of the biopsy. Other relevant information includes the presence of infections, viral studies, and exposure to any potentially hepatotoxic drugs. If the case is a consultation from an outside institution, this information should be provided by the patient's primary physician who will be most familiar with these details. It is important in the case of consultations that a telephone number, an email and a fax be included.

The current (2015) NIH consensus panels recommended three categories of diagnostic certainty: GVHD (unlikely or no), GVHD (possible), and GVHD (likely) [2]. A modification of this scheme was developed in the multicenter standardization of aGVHD with the additional category of "unequivocal pathologic evidence of GVHD" [18]. The clinician can then determine the pathologist's certainty with the diagnosis. In practice, a diagnosis of "consistent with" or "likely, combined with suspicious clinical findings" is used together with the treatment decisions to assign a confidence level to the attribution of symptoms to a formal GVHD diagnosis. Accompanying this designation should be a description of the amount of apoptosis and the extent or severity of the process as per the Lerner-Sale and Myerson grading scales (Chap. 9). Some histologic alterations may reflect prior static damage, e.g. skin dermal sclerosis, ulcerated gut, or marked bile duct damage or loss. Without serial sampling, such histologic changes cannot be used to assess ongoing activity or the trajectory of response to IS.

The minimum criteria for GVHD in other organs are listed in Table 1.1. In addition to the organs previously described in the 2015 NIH consensus, including the lung and muscle, the kidney is now included as a possible or likely target of GVHD and will be discussed in Chap. 19. The pathophysiology of lung and kidney damage from GVHD is not fully understood, though a recent review has documented the effects of a combination of lymphocytes and cytokines has in the genesis of GVHD [19] (Chaps. 2, 19).

In summary, the HSCT pathologists' contributions to the diagnosis and management of GVHD are part of a collaborative effort. Pathologists assess whether the GVHD changes are active, static, or progressive and/or exclude other causes, e.g. infection, drug toxicity, or malignancy. In the future, it is likely that composite biomarker panels [20, 21], especially those related to endothelial damage, will aid in predicting patient outcomes and be used to stratify high-risk patients' enrollment in research treatment protocols. Nonetheless, there will always remain a need to perform tissue biopsies, particularly for clinical manifestations of unclear etiology and to assess response treatment.

Teaching Points

1. The cardinal histologic feature of GVHD activity is apoptosis in the targeted organs' epithelia. The diagnostic threshold for minimal apoptotic activity is still controversial and may overlap with of effects from cytotoxic conditioning, infections, or adverse drug reactions.
2. The 2015 NIH consensus panels define the GVHD-related tissue changes as acute, chronic, and/or late-onset acute GVHD. There are no changes in liver or gut histology which distinguish aGVHD from cGVHD.
3. The pathologist should indicate the degree of certainty that the biopsy does or does not show GVHD or the histologic differential diagnosis. The NIH-recommended wording for stating a biopsy as positive for GVHD was "likely." In contrast to the 2015 NIH pathology consensus recommendation, a recent large international consortium on the clinical diagnosis of aGVHD recommended issuing an unequivocal diagnosis if there was no uncertainty.
4. Interpretation of tissue biopsies for GVHD should be accompanied by all relevant clinical data, especially if there is no other evidence of GVHD in other organ systems.
5. False negatives and false positives are possible with tissue diagnosis. Biopsies done at the direct onset of symptoms may not display the fully diagnostic changes. Conversely, when there is long-standing extensive damage in the gut, such as ulceration or sclerosis in the skin, it may be difficult to differentiate static damage from ongoing activity.
6. Persistent gut disease or progressive changes in cGVHD-affected tissues signify a worse outcome. The use of clinical parameters and combinatorial biomarkers will likely serve the purpose of predicting severity and outcome and will be used in the future to guide clinical trials.

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