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HISTOPATHOLOGIC DIAGNOSIS OF CHRONIC GRAFT VERSUS HOST DISEASE:

NIH Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-Versus-Host Disease: II. The 2014 Pathology Working Group Report

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Abstract

The 2005 National Institute of Health (NIH) Consensus Conference outlined histopathological diagnostic criteria for the major organ systems affected by both acute and chronic graft-versus-host disease (GVHD). The 2014 Consensus Conference led to this updated document with new information from histopathological studies of GVHD in the gut, liver, skin and oral mucosa and expanded discussion of GVHD in the lungs and kidneys. The recommendations for final histological diagnostic categories have been simplified from 4 categories to 3: no GVHD, possible, and likely GVHD based on better reproducibility achieved by combining the previous categories of consistent with and definite GVHD into the single category of likely GVHD. Issues remain in the histopathological characterization of GVHD, particularly with respect to the threshold of histological changes required for diagnostic certainty. Guidance is provided for the incorporation of biopsy information into prospective clinical studies of GVHD, particularly with respect to biomarker validation.

Keywords

Chronic graft-versus-host disease; allogeneic hematopoietic cell transplantation; consensus; diagnosis; pathology; histology

Background

Histopathology has played a major role in understanding the pathophysiology and aiding in the diagnosis and management of graft-versus-host disease (GVHD). Historically, the clinicopathologic classifications of both acute [1] and chronic GVHD were derived from a cohort of patients in the late 1970's [2]. Many of these early cases were untreated or had disease that was refractory to the treatment that was available at the time. Descriptions and illustrations of fully developed histological lesions of acute and chronic GVHD can be reviewed in several texts [1, 3–8]. Changes in transplant modalities e.g., reduced intensity conditioning, hematopoietic stem-cell source, and post-transplant immunosuppression therapies affect the onset of GVHD and the frequency of chronic GVHD [9].

Since the initial publication of the Pathology Working Group Report from the NIH Consensus Development Project on chronic GVHD [10], many practical issues in the surgical pathology of GVHD remain unresolved or not addressed in standard texts. It is often neither possible nor meaningful to distinguish persistent, recurrent or late acute GVHD from chronic GVHD by histology. Furthermore, uniform minimal histological diagnostic criteria for GVHD have not been established and remain a subject of study. Some advances in histological analysis have been made. Recent studies quantifying the macrophage content in skin biopsies [11] or Paneth cell loss in the intestinal crypts [12] suggest that steroid responsiveness can be predicted. A growing body of evidence indicates that in addition to damage to targeted epithelia, changes to the microvascular endothelium play a role in the pathogenesis of GVHD [13–17]. It is controversial whether the endothelium is a target of GVHD or is damaged secondarily from cytotoxic T lymphocytes or inflammatory cytokines, including tumor necrosis factor, interferon gamma and nitric oxide, as well as some of the

immunosuppressive agents. Additional studies related to the role of the endothelium are discussed in the skin and renal sections below.

In order to facilitate clinical trials of chronic GVHD, uniformly applied and interpreted criteria for histopathological diagnoses are necessary. These criteria should be shown to be reproducible by multi-institutional studies and correlated with clinical information. Ideally, chronic GVHD trials should incorporate protocol-directed biopsies, from scheduled calendar or event-driven collection procedures to allow corollary histopathological studies. This issue is further addressed at the end of this document.

Purpose of this document

The purpose of this report is to provide an update for pathologists and clinicians about the interpretation of biopsies and use of this information in the management of hematopoietic cell transplant (HCT) patients, with focus on changes since our first publication [10]. This update includes new information relevant to interpretation of histopathology, together with expanded discussions of microvascular, pulmonary and renal pathology in HCT patients. General guidance on the incorporation of biopsy pathology into clinical studies is also provided. The recommendations of the Working Group represent a consensus opinion supplemented by evaluation of available peer-reviewed literature.

Summary of changes and updates

The following list highlights the changes and new information presented in this document:

- The discussion has been updated to include specific biologic studies based on histology that shed light on the pathophysiology of chronic GVHD
- Table 1 has been updated with refinements to the histological criteria of GVHD in each organ.
- To improve inter-observer reproducibility, recommendation for final histological diagnosis has been simplified to three categories: “Not GVHD”, “Possible GVHD” and “Likely GVHD” (Table 2).
- A single liver biopsy obtained in the midst of immunosuppression can assess the severity of duct injury in GVHD, but not its trajectory.
- The highest diagnostic yield results from concurrent sampling of the upper and lower GI tract. There is no consensus on a limited biopsy strategy that can be used in preference to wider sampling.
- Histological changes in late onset colitis after cord blood transplant, not associated with an established infection, (i.e., “cord colitis syndrome”) are not histologically distinct from colonic GVHD.
- Changes of mycophenolate mofetil (MMF)-related gut injury occur in both the upper and lower GI tract.
- Intestinal Paneth cells are lost as a late occurrence in severe GI GVHD, and loss of these cells portends a poor outcome.

- Criteria for assessing the regression of dermal sclerosis after autologous transplantation for scleroderma have been defined. These can be applied to assess therapies given to reverse the dermal sclerosis of chronic GVHD.
- Reduced salivary flow and altered quantitative proteomics correlate with histological damage in minor salivary glands.
- Pathological studies of lung biopsies in patients meeting the 2005 NIH criteria for bronchiolitis obliterans syndrome (BOS) include both small airway lesions of lymphocytic bronchiolitis and constrictive obliterative bronchiolitis. Open lung biopsy can be considered in patients with typical pulmonary function tests and CT findings of BOS, when evidence of chronic GVHD is not present at other sites.
- Membranous nephropathy and minimal change disease after HCT are associated with chronic GVHD and appear to be a manifestation of GVHD. Renal biopsy is recommended for correct classification of renal injury that develops after transplantation.

Rationale for obtaining biopsies

The incidence of chronic GVHD varies widely (35–70%) among studies of allogeneic recipients, based upon the time period specified, source of hematopoietic stem cells, type of donor, and post-transplant immunosuppression. The risk of chronic GVHD is increased when the source of the hematopoietic stem cells are derived from growth factor mobilized peripheral blood [9]. As detailed in the NIH chronic GVHD Diagnosis and Staging consensus manuscript [18], biopsies are necessary to confirm the diagnosis of GVHD in situations where only distinctive clinical features of chronic GVHD are present, alternative diagnoses are entertained, clinical signs are confined to internal organs, or clinical assessment is obscured by prior changes. In these instances, histopathology should be viewed as essential for establishing diagnosis, especially if there are any atypical clinical features, confounding infections, or potential drug toxicity. Failure to obtain biopsies can result in erroneous treatment. Jacobsohn and colleagues found that 7% of patients referred to Johns Hopkins for consultation regarding chronic GVHD did not have biopsies before starting treatment and had been incorrectly diagnosed and treated for active chronic GVHD before referral [19]. The clinical appearance of cutaneous fungal infection, drug reactions and Grover’s disease can mimic chronic GVHD in the skin [20, 21].

While a biopsy can be of value in confirming the initial diagnosis of chronic GVHD and in demonstrating features of progression, assessing histological signs of activity may be difficult. The role of serial biopsies to assess the response to treatment has not been determined. In addition, the utility of screening biopsies in asymptomatic patients who are still taking immunosuppressive medications is controversial, since asymptomatic patients with positive screening biopsies are not considered to have chronic GVHD. In the context of clinical studies, a screening biopsy may serve as a useful baseline reference point.

Limitations of diagnosing GVHD by histopathology

Diagnostic interpretation by the pathologist requires integration of the clinical context with the microscopic changes. Histopathology represents a “snapshot in time” of a complex and dynamic biologic process that reflects the duration, use of immunosuppressive therapy, the possibility of more than one process, the location and the quality of the sample, and the histological preparation. Given the high prevalence of chronic GVHD in the population of interest, the positive predictive value of a positive biopsy for GVHD is high, while the negative predictive value is low [22]. As criteria for the minimal diagnostic threshold become more stringent, the sensitivity of the biopsy to detect GVHD will decrease.

A number of factors can cause a false-negative histological assessment of GVHD. Biopsies done immediately after the onset of symptoms and signs of presumptive GVHD may be falsely negative, since results may show only subtle and focal morphological changes. Tissue sampling may be suboptimal. Biopsy of an oral or gastrointestinal ulcer rather than the adjacent intact mucosa may not show the changes of GVHD. Thin needle biopsies of liver and poorly oriented gut biopsies can distort the relevant structures. Partial thickness biopsies cannot be used to assess fasciitis or fibrotic changes in the deep dermis fat. Minor salivary gland biopsies may not include enough individual glands (at least 10) that contain sufficient ducts or glandular acini to differentiate between active disease and previously damaged glandular tissue, since not all minor glands in a specimen may exhibit evidence of active disease. Suboptimally processed and sectioned biopsies may obscure key cytological features. Biopsies that are too small, and glass slides containing only limited numbers of serial sections may be insufficient for detection of focal minimal changes. GVHD may be of mild intensity or may be partially suppressed by immunosuppression. In such cases, it is difficult to demonstrate that precise minimal diagnostic criteria are uniformly applied. A false-positive diagnosis of GVHD may result from concurrent infections, drug reactions or inflammatory reactions unrelated to GVHD.

Histological criteria for the diagnosis of GVHD

Although the focus of this series is to provide consensus on the topic of chronic GVHD, differentiation of acute from chronic GVHD on biopsy material is not always possible. Features of acute GVHD may present even in organs that have defined criteria for chronic GVHD. In other sites, separable forms of acute and chronic GVHD have not been defined histologically. Therefore, the following discussion will treat the histological appearance of GVHD broadly and in the context of histological and clinical differential diagnosis. Table 1 presents the criteria necessary to diagnose GVHD (whether acute or chronic) and the features diagnostic for chronic GVHD in each involved organ system. The exact threshold at which a diagnosis of GVHD may be made with confidence remains a topic for study.

The sections below summarize consensus opinions of organ-specific pathology.

1) Liver: As both drug-induced liver damage and opportunistic infections occur frequently after HCT, the diagnosis of liver GVHD can be highly challenging. The histological diagnosis of liver GVHD is based on the identification of immune-mediated destructive damage to small bile ducts and ductules, together with cholestatic and inflammatory

changes, after considering potential confounding causes of liver injury. The bile ducts are withered, show reactive nuclear and cytoplasmic changes and may be infiltrated by lymphocytes. Unlike GVHD-associated injury of other epithelia, biliary epithelial apoptoses are infrequent. Hepatocellular apoptosis (acidophilic bodies) are more frequently observed in cases of hepatic GVHD than chronic viral hepatitis in patients without HCT [23]. The minimum amount of duct injury required to establish a diagnosis of hepatic GVHD has not been established. If the liver biopsy is done soon after the onset of liver dysfunction, characteristic bile duct changes may be absent or may affect only a minority of portal spaces [23]. Inadequate sampling may also cause a false negative result [23]. The injury reflects not only the duration of hepatic GVHD, but also the effects of therapeutic intervention, which may precede biopsy. Refractory GVHD in the liver is typically associated with chronic cholestasis, ductopenia, and less commonly a ductular reaction response [6, 24, 25], unlike other chronic cholestatic liver diseases. In such cases, it remains unclear whether a prominent ductular reaction represents a reparative effort, a secondary target of GVHD, or both [26]. A ductular reaction may be present with concomitant gut GVHD or septicemia [27], referred to as cholangitis lenta, related to the effects of interferon gamma [28].

In the liver, no clear dichotomy exists between acute and chronic GVHD. However, prolonged persistence of GVHD may result in progressive fibrosis [23]. Although rare cases of cirrhosis have been attributed to chronic GVHD [6, 24, 29], these reports are confounded by coexisting chronic hepatitis C infection. Fibrosis present at the onset of acute GVHD or within the early post-transplant period is more likely to reflect pre-transplant pathology than chronic injury from GVHD. In children with chronic liver disorders, the developing hepatobiliary tract is especially vulnerable to injury and prone to fibrosis [30].

The Lerner grading scheme for hepatic GVHD is based on the fraction of bile ducts showing injury [31]. Unlike the original corresponding Lerner grades in the skin and gut, inflammation is not used to establish the diagnosis of GVHD. Since no scoring system has yet shown consistent prognostic or predictive power, histological scoring of liver GVHD is currently not recommended.

Special histochemical stains and immunohistochemistry may be useful in evaluating cases of hepatic GVHD and should be employed as needed. Bile duct damage and loss may be highlighted by immunohistochemical (IHC) staining of keratin 7 or 19. Immunohistochemistry or in-situ hybridization can help identify viral infection due to CMV, HSV, EBV, VZV, HHV-6 [32] and adenovirus. Routine staining for viruses in the absence of suggestive histology is not recommended, although staining may be performed if clinical suspicion of infection is high. Iron stains are useful to reveal hepatic iron overload that contributes to liver dysfunction in HCT patients, is associated with an increased rate of infections and may predispose to GVHD [33, 34]. The cellular distribution and severity of iron overload should be mentioned in the pathology report. Two unusual manifestations associated with GVHD involve hepatocyte inclusions recognized by periodic acid Schiff (PAS) staining. These include both diastase-sensitive polyglucosan-like ground-glass cytoplasmic inclusions [35, 36] and PAS-positive diastase-resistant pseudo-Lafora bodies thought to represent an unusual form of degenerative organelles and glycogen [37, 38]. Data are insufficient to determine whether IHC staining for replicative senescence by p21 [39],

determination of the Th17/T regulatory cell ratio [40], or staining for C4d provides additional information above and beyond the usual histological evaluation [41].

When evaluating a potential case of hepatic GVHD, knowledge of the clinical management (changes in immunosuppressive drugs) and serological and molecular testing for infectious agents is important for adequate biopsy interpretation. Patients presenting with an acute hepatitic onset after donor lymphocyte infusions or tapering of immunosuppressive medications may have more necroinflammatory activity and portal inflammation than typically seen in patients who are receiving immunosuppression [25, 42]. However, in the setting of rapidly rising aminotransferases in the thousands, one should screen carefully for viral inclusions and exclude infection through special studies as noted above. Infection with HBV or HCV may complicate biopsy interpretation. Chronic hepatitis C infection causes inflammation and reactive bile duct changes [6, 23, 43], but the degenerative bile duct changes of GVHD are qualitatively different from those caused by HCV. In chronic hepatitis C, the bile duct injury is usually focal and is usually associated with a lymphoid aggregate. In contrast, the duct injury in GVHD is usually present in multiple portal spaces, and the injury is usually not accompanied by significant inflammation. A potentially fatal complication, fibrosing cholestatic hepatitis from HBV or HCV, may develop in immunocompromised patients [44, 45].

Some studies have shown that serial liver biopsies provide a clearer picture of the liver disease and lead to better patient management [23, 25, 34, 46, 47]. Early biopsies done for the acute hepatitic onset of GVHD may have striking focal hepatocellular necrosis with minimal bile duct damage [25]. This presentation presumably reflects cytokine-induced bystander death of hepatocytes mediated by the Fas/Fas ligand interaction of activated T cells with hepatocytes in the sinusoids. In such cases, subsequent liver biopsies may have obvious bile duct damage. Serial liver biopsies done to evaluate persistent liver dysfunction during prolonged immunosuppression may show a further damage or loss of bile ducts, along with chronic cholestatic changes. In contrast, a single liver biopsy obtained after persistent liver dysfunction can characterize the degree of biliary damage or destruction but cannot indicate whether the process is progressive, static or recovering. Serial liver biopsies may show that progressive deterioration of presumed GVHD is actually related to a second process or may help to identify features predicting steroid-refractory disease. Lastly, assessment of response to therapy requires integration of the clinical and pathological data, especially with liver biopsies, where improvement in liver tests may precede improvement in histology by months. The extent to which improvement in clinical features correlates with repair and regeneration of bile ducts is not known. In an anecdotal case with complete ductopenia, liver tests returned to normal after one year [25]. The consensus panel encourages the integration of protocol liver biopsies when devising future biomarker studies in order to identify histological features that may be included in a multifactorial model to guide clinical management.

The literature on liver GVHD presents conflicting data regarding the relationship of the degree of biliary damage and outcome. The ominous significance of hyperbilirubinemia with poor survival has been independently validated in several studies [46, 48–51]. Therefore, it seems likely that individual histological features should be associated with the

outcome of HCT patients or predict response to therapy. Yet, because of small sample sizes, case accrual over long times periods and changes in conditioning and treatment modalities, comparisons between these histological studies are difficult [52]. For example, in one study, the extent of bile duct damage, lymphocytic infiltration of biliary epithelium, portal inflammation, and ductopenia showed no association with survival, while severe acinar inflammation and low level of hepatocellular ballooning were associated with a better outcome [53]. In another study on the acute hepatitic onset of liver GVHD, extensive destructive biliary changes regardless of the degree of inflammation were associated with an increased risk of non-relapse mortality [25]. In the collective experience of the group, the extent of bile duct damage and portal and acinar inflammation correlate with the degree of liver test abnormalities, but not necessarily with the outcome of liver GVHD. However, the degree of bile duct loss seems to be associated with decreased survival. Thus, liver biopsies may serve not only as a diagnostic tool for establishing the diagnosis of liver GVHD, but when integrated with the clinical context, may provide additional prognostic information that could help to identify patients at high-risk for fatal outcome.

2) Gastrointestinal tract: The histopathology in the gastrointestinal (GI) tract is variable during late onset acute, persistent, or chronic GVHD. Endoscopic or imaging evidence of esophageal webs remains the only uniformly accepted diagnostic feature of chronic GVHD within the GI tract [18], and histological changes are not helpful in distinguishing chronic GVHD from acute GVHD. Prolonged or incompletely treated forms of acute GVHD may leave behind extensive architectural distortion [54]. Changes of chronicity include marked architectural distortion of mucosal architecture with crypt loss, formation of cystic glands or disorganized crypts not connected with the surface, areas of atrophy alternating with partial regeneration or ulcerations, often with nuclear hyperchromasia, little associated inflammation or apoptosis, and loss of cytoplasmic mucin. Other changes of chronicity include lympho-plasmacytic inflammation, colonic Paneth cell metaplasia, lamina propria fibrosis, and rarely, submucosal or serosal fibrosis [1, 3, 7, 54].

The histological hallmarks of gastrointestinal GVHD are some combination of enterocyte apoptosis, crypt or basilar gland destruction, and mucosal denudation. The term “crypt apoptosis” is used here to refer to gastric pit apoptosis as well as crypt apoptosis in the intestines. The changes of gut GVHD are most prevalent and easiest to identify in biopsies from the large and small intestine. Apoptotic debris limited to the superficial epithelium and lamina propria is a non-specific finding and should not be used to diagnose GVHD, unless the tissue shows only surface mucosa with complete destruction of underlying crypts, as may occur with prolonged gut GVHD. However, the recognition of apoptosis is not always simple, and an ongoing study by a European pathology consortium suggests that interpretive variation can be addressed through consensus on study sets. Apoptotic bodies may appear as exploding crypts [55], as hyperchromatic karyorrhectic nuclear debris within a large clear zone, or as a small shrunken cell with eosinophilic cytoplasm and a condensed nucleus.

Numerous changes in transplant practice and the effects of prolonged immunosuppression have altered the severity and onset of GVHD, leading to increased willingness to diagnose GVHD based on minimal histological changes (specifically apoptosis) in the appropriate setting. Yet, defining the exact threshold of minimal histological change sufficient for the

diagnosis of GVHD remains controversial. Attempts to set a threshold of apoptosis involve a tradeoff between sensitivity and specificity. Using a threshold similar to that used for acute cellular allograft rejection in small bowel transplants (≥6/10 consecutive crypts) [56, 57] was felt by the consensus group to be too insensitive. Others have demonstrated that using the previously recommended >1–2 apoptotic bodies per biopsy piece (on average) increases sensitivity with some loss of specificity [58, 59]. The injury in GI GVHD is unevenly distributed, which increases the difficulty of establishing a threshold of histological change for the diagnosis of GVHD. A false negative diagnosis may result from a limited biopsy sampling or examination of too few serial sections. At least 8, and up to 20, serial sections should be analyzed in order to avoid missing infrequent apoptotic changes. The use of IHC markers of apoptosis (e.g. caspase 3) has been limited to research studies and has not yet found utility in routine clinical practice.

Enterocyte apoptosis is not limited to GVHD. Infections associated with apoptosis (CMV, cryptosporidia) were discussed in the earlier document [10]. Special studies may be performed to highlight infection as noted in the hepatic GVHD section above. Drug-induced gastrointestinal injury may induce enterocyte apoptosis and mimic GVHD. Since the last consensus document, numerous publications have described GI toxicity associated with mycophenolate mofetil (MMF). In the upper GI tract, one may see parietal cell ballooning, chronic active gastritis, active esophagitis and celiac-like features in the duodenum [59–64]. The presence of focal colonic ulceration, marked apoptosis, mixed inflammation and interspersed normal mucosal biopsies from sites distant to the lesions should raise the possibility of MMF-related colitis [61]. Contrary to early reports [65], the presence of increased number of eosinophils within the lamina propria and epithelium should favor a diagnosis of MMF-induced injury over GVHD [66, 67]. Additionally, loss of neuroendocrine cell nests was seen in MMF-related injury as opposed to GVHD, which spares neuroendocrine cells [67, 68]. The frequency of MMF toxicity is difficult to quantify in this patient population, due to the overlap in pathology between GVHD and MMF toxicity. In clinical practice, MMF toxicity is favored if discontinuation or dose reduction improves symptoms. With respect to other GI drug injury, mild gastric antral apoptosis has been reported with the use of proton pump inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) [69].

In the previous NIH histopathological diagnosis of chronic GVHD consensus document [10], the standard use of histological grading was not recommended, because the existing grading schemes (Lerner [31] and the Sale modification [1]) combine diagnostic criteria with the extent and chronologic stage of disease. Although severe changes (Lerner grade 4) are associated with poor survival [70], the degree of injury required for grade 1 is poorly defined and includes a broad spectrum of apoptotic activity from rare to numerous, falling just short of exploding crypts. The committee was divided as to the utility of using these grading systems in routine practice. If an institution chooses to use a grading system, the site with the greatest damage or highest histological grade should be noted because of the inherent variability in injury. Additional morphologic features that correlate with disease severity or non-relapse mortality include the loss of Paneth cells within the small intestines and crypt loss within the colon [12, 71]. Given the drawbacks of the existing histological

grading systems, we would recommend investigation into grading schemes based on the degree of apoptotic activity independent of the stage of crypt or mucosal destruction.

The cord colitis syndrome (CCS) was described as a newly entity in 2011 by Herrera et al. at the Dana Farber Cancer Institute (DFCI) [72]. This syndrome, confined to their cord blood recipients, was characterized by late-onset secretory diarrhea, chronic active colitis and multicentric granulomas, an absence of GVHD or detectable infections, responsiveness to antibiotics and lack of responsiveness to immunosuppressive treatment. The DFCI investigators used a unique non-standard definition of GVHD that excluded GVHD in colonic biopsies showing low or rare apoptosis or any features of chronicity as described above. Subsequently, two much larger studies including controls who received cord blood and non-cord blood hematopoietic stem cell transplants showed that neither the histological features of chronicity (crypt distortion, Paneth cell metaplasia, chronic active colitis) nor granulomas were confined to cord blood recipients [73, 74]. In the study by Milano et al., all of the non-cord blood allogeneic recipients with late onset secretory diarrhea and symptoms ascribed to CCS responded to anti-GVHD treatment [73]. The presence of granulomas with neutrophilia was more common in a Japanese study that included many cases with both GVHD and CMV [74]. These studies serve as a reminder that GVHD and infection are not mutually exclusive diagnoses and reinforce the need to use standardized histological definitions for GVHD.

As noted before [10], the gold standard in diagnosing GI GVHD remains unsettled among transplant clinicians, pathologists and endoscopists. Several groups require only evidence of GVHD involvement in other organs [54, 75, 76] while others use response to immunosuppressive therapy alone as an indication of GVHD [15, 77]. Involvement of one organ system by GVHD, however, does not necessarily imply coexisting GI GVHD. As noted above, infectious etiologies and drug injury may mimic GVHD. Some have proposed that the endoscopic impression alone is sufficient for a diagnosis of GI GVHD, as the extent of mucosal changes can be visualized more completely [78]. Discrepancies between upper or lower endoscopic findings, however, are well documented, as are differences between clinical, endoscopic and histological diagnoses [71, 75, 79–84]. Consensus that sampling one particular site of the GI tract is consistently better than another is lacking. The highest diagnostic yield occurs when visibly injured or erythematous regions of both the upper and lower gut are sampled [83]. On a practical level, the diagnosis of GI GVHD still requires an assessment of clinical, endoscopic and histological findings and exclusion of other causes of injury.

Several studies have compared the ability of clinical endoscopic and histological findings to predict response to steroid treatment and risk of death [71, 78, 79, 81]. It is difficult to reconcile the differing conclusions regarding the best predictor, since some studies were based only on evaluation of the colon [71, 78, 81], while another study was confined to the upper GI tract with a focus on biopsies from the second portion of the duodenum [79]. There was general agreement that most endoscopic biopsies taken shortly after the onset of gut symptoms of GVHD were typically of lower histological grade; but those with higher-grade injury have been correlated with poor outcome. Intestinal biopsies taken after immunosuppressive treatment and showing persistent or worsening histological changes

portended a poor outcome, in agreement with endoscopic and biomarker observations [12, 70, 79]. A relevant immunohistologic study showed that the numbers of regulatory T cells in blood and gastric mucosal biopsies did not correlate with clinical or histological severity of GVHD [85].

3) Skin: Two comprehensive, richly illustrated reviews have described the gross and microscopic manifestations of cutaneous chronic GVHD [8, 86]. A consensus document specific to cutaneous GVHD biopsy performance and reporting is also available [87]. The minimal histological criteria for GVHD require apoptosis within the basilar or lower spinosum layers of the epidermis [1, 5, 31]. In cases of minor alteration, the focus should be on the interpretation of vacuolar changes and apoptotic keratinocytes, including in the adnexal epithelia, although in the case of lichenoid disease, some element of inflammation should be present to allow the diagnosis to be established with confidence [88]. The archetypical features of both acute and chronic GVHD are superficial interface dermatitis with vacuolar change predominantly in the basilar layer or a lichenoid pattern of lymphocytic inflammation with or without lymphocyte satellitosis [4, 5, 89]. As a note of caution, since no single histological feature is pathognomonic of GVHD, the pattern of inflammation should be factored into the final interpretation [90]. For example, exuberant superficial spongiotic dermatitis with marked spongiosis (intraepidermal edema) and lymphocytic infiltration into the epidermis with only a rare apoptotic keratinocyte may suggest an allergic contact dermatitis but encompasses a broad differential and likely excludes the diagnosis of GVHD. The presence of tissue eosinophils in a skin biopsy should not be considered as evidence for drug hypersensitivity, since they often occur in GVHD [91]. Lymphocyte satellitosis (lymphocytes abutting an apoptotic keratinocyte in the epidermis or appendages), when present, provides evidence that the dermatitis may be caused by GVHD. This characteristic feature is not entirely specific and can occur in drug reactions. Of note, Nishiwaki et al. noted that many cells in the dermal inflammatory infiltrate in untreated acute GVHD were actually CD163+ macrophages, rather than T-cells. Dermal macrophages present in large numbers correlated with steroid refractoriness and lower survival [11].

The histological manifestations of chronic cutaneous GVHD evolve over time, are modified by treatment, and to some extent overlap with those of acute GVHD. Severe keratinocyte dysmaturation related to conditioning with busulfan can persist for many months after HCT[92]. The histological counterparts to the clinical definitions of cutaneous chronic GVHD include several different histological patterns. The lichen-planus like eruptions (initially classified as early generalized extensive chronic GVHD [2, 4] refer to a specific constellation with epidermal thickening by acanthosis (hyperplasia) with orthohyperkeratosis (stratum corneum) and parakeratosis, hypergranulosis, a band-like infiltrate along the dermal-epidermal junction, extensive apoptosis and vacuolization of basilar keratinocytes, saw-toothed (tapered and elongated) rete ridges, plus inflammation around the adnexae. This constellation, especially when accompanied by lymphoplasmacytic inflammation around the eccrine coils, is highly specific for chronic GVHD, but has low sensitivity. In a patient with chronic skin GVHD, biopsy may show both acute and chronic GVHD, and the changes of chronic GVHD may vary from one site to another. In practice,

members of the dermatopathology subcommittee regarded a skin biopsy with the combination of epidermal compact orthohyperkeratosis, hypergranulosis and acanthosis with shortened or saw-toothed rete ridges as features that favor or are consistent with lichenoid type of chronic GVHD. Rarely, milder forms of this combination of features can occur in skin biopsies from patients with severe clinical acute GVHD. Screening biopsies from non-sun-exposed normal appearing skin, such as iliac crest, taken between day 80 –100 after transplant often contain rare isolated keratinocyte apoptotic bodies with little or no accompanying inflammation [93]. The interpretation of such findings as either non-specific or consistent with minimal (subclinical) GVHD depends on an institution's minimal diagnostic criteria.

In the initial descriptions of sclerotic or late chronic GVHD, the fibrosis that followed the lichenoid stage had a top-down progression from the papillary through reticular dermis [2, 5]. Some patients develop diffuse dermal sclerosis without an apparent inflammatory lichenoid phase. The suggested minimal criterion for the diagnosis of cutaneous *sclerotic* chronic GVHD is homogenization (sclerosis) of most of the papillary dermis or reticular dermis or subcutaneous septa. Depending on the clinical presentation, sclerotic GVHD can manifest with localized morphea-like features, diffuse sclerosis or lichen sclerosus-like features. Localized morphea-like features and diffuse sclerosis are largely confined to the reticular dermis or subcutaneous septa with little or no epidermal involvement. In lichen sclerosus-like GVHD, collagen alteration is confined to the papillary dermis, often with residual interface changes characterized by the presence of mild vacuolar alteration, melanophages and sparse lymphocytic infiltrate in the papillary dermis. In the fasciitis variant, biopsy specimens show only fibrous thickening of the fascia with adjacent inflammation without epidermal or dermal involvement [94]. Recent work has characterized angiomatous proliferations in some cases of sclerotic GVHD [86, 95]. Table 1 lists several different manifestations of chronic GVHD, all of which may be present in a single biopsy.

Following immunosuppressive treatment, a skin biopsy may contain a combination of residual changes to the damaged epidermis and appendages, any preexistent dermal sclerosis, and a reduction or absence of apoptosis and inflammation. An indication of active GVHD is residual apoptotic changes in the epidermis or appendages. During treatment, the histological significance of persistent epidermal vacuolar degeneration requires additional correlative study, as does the assessment of activity in patients who have received psoralen and ultraviolet A irradiation (PUVA) or who have established deep dermal sclerosis or morpheic chronic GVHD. Of note, additional, long-term use of steroids may also induce epidermal atrophy with loss of rete ridges. Both clinical and histological regression of dermal sclerosis occurs a year or two following PUVA therapy for chronic GVHD and autologous transplant for scleroderma (PSS) [96]. Nash et al. have developed a schema for grading the reduction in dermal sclerosis [97]. These tools can be used in future studies that seek to produce regression of dermal sclerosis. A similar, though more complicated, scheme for grading the regression of sclerosis was designed by Verrecchia et al. [98].

The pathogenesis of the dermal sclerosis and its relationship to the dermal microvasculature was studied by Biedermann et al. [13]. They reported a correlation of dermal sclerosis in chronic GVHD with elevated concentrations of von Willebrand Factor (vWF) in the blood

and reduced capillary density identified by Ulex europeus lectin staining. Biedermann concluded that reduced vascularity was responsible for the fibrosis, and they further postulated that the presence of perivascular T cells suggested that the endothelium was the target. However, vWF multimers are a non-specific acute phase reactant, and the antigen recognized by lectin staining may be reduced in the presence of inflammation. In contrast, Fleming et al. used the specific endothelial markers CD31 and VE-cadherin and did not find a correlation between the dermal fibrosis in chronic GVHD and a reduction in dermal capillary density [99]. They further showed that the endothelial microvasculature in chronic GVHD did not have the reduced VE cadherin and vWF expression observed in capillaries from patients with systemic sclerosis. Two related studies using capillaroscopy on nailfold capillaries confirmed the findings of Fleming et al. that patients with PSS, but not chronic GVHD, had both morphologically abnormal capillaries and reduced density [100, 101].

4) *Mucosa: Oral cavity, oropharynx, eye and female genitalia*: Patients without any signs or biopsy evidence of GVHD may have chronic inflammation without apoptotic changes in the oral mucosa and minor salivary glands, as demonstrated by studies of oral labial biopsies taken at 80–100 days after HCT. These changes were attributed to chemotherapy or irradiation in the conditioning regimen [102]. The minimal histological criteria for oral chronic GVHD have remained unchanged. Mucosal changes consist of localized or generalized epithelial changes (lichenoid interface inflammation, exocytosis and apoptosis) similar to those described in cutaneous GVHD. Minor salivary glands show intralobular or periductal lymphocytic inflammation and exocytosis of lymphocytes (without neutrophils) into intralobular ducts and acini. Periductal fibrosis without generalized interstitial fibrosis is often present. Nakhleh et al. used a threshold of >3 mucosal apoptotic bodies, and for salivary changes, > 10% loss of acinar tissue or ductal epithelial cell necrosis as their minimal criteria for GVHD [103]. Horn et al. developed a histological grading system for chronic GVHD of minor salivary glands based on the degree of lymphocytic infiltration and destruction of glandular acini [104]. Soares et al. found that the most specific histological feature of oral chronic GVHD was minor salivary gland periductal lymphocytic inflammation with exocytosis, which correlated with extensive chronic GVHD and decreased survival [105]. While the reduction in glandular acinar area was greatest in patients with chronic GVHD, some reductions also occurred in those without GVHD. Moderate to intense periductal and periacinar fibroblastic stroma is evidence of previous inflammation or chronic GVHD activity, whereas dense fibrous tissue with destruction of acinar tissue and duct ectasia may be only a marker for previous non-GVHD damage, such as chronic obstructive sialadenitis secondary to trauma [106].

Persistent salivary dysfunction after treatment of chronic GVHD is related to progressive lymphocytic inflammation with absence of recovery or destruction of minor salivary secretory units [106]. Oral chronic GVHD is highly correlated with xerostomia, reduced salivary flow rates, and xerophthalmia. In one recent study, all patients with salivary dysfunction had histological damage to the minor salivary glands with mononuclear infiltration, fibrosis or atrophy [107]. Involvement of the oral mucosa did not correlate with salivary dysfunction [107]. In the future, quantitative proteomic analysis of saliva may be added as a biomarker to identify active oral chronic GVHD, especially in newly diagnosed

patients within the first 12 months after HCT [108]. Quantitative proteomic analysis of saliva from patients with and without oral chronic GVHD demonstrated altered expression in the chronic GVHD group with decreased IL-1 antagonist receptor and Cystatin B compared to the non-GVHD group. Glandular atrophy, fibrosis and inflammatory infiltrate were all associated with salivary gland dysfunction in a cohort of patients with oral chronic GVHD [107].

The diagnosis and staging document [109] and other reviews [8] provide a detailed description of the oral changes of chronic GVHD. Nonetheless clinicians and pathologists should be aware that premalignant dysplasias and oral cancers, a leading cause of secondary malignancies after allogeneic transplantation, must be considered in the evaluation [110, 111].

The same criteria described above for oral and esophageal mucosa are used for histological assessment of chronic GVHD in vulvar [112], conjunctival and lacrimal biopsies. Histopathological findings of ocular GVHD have been described in conjunctiva and in the lacrimal gland [102, 113–116]. The alterations in lacrimal gland acinar tissue resemble those in minor salivary glands with prominent infiltration of mononuclear cells around medium size ducts, and loss of acinar lobules replaced by fibrosis. While lacrimal gland biopsy is relatively invasive and may impair function, conjunctival biopsy may be obtained with little risk. Histological evaluation of conjunctiva may aid in the diagnosis and management of ocular GVHD in symptomatic patients with conjunctival disease who have normal or unchanged Schirmer's test with or without GVHD of other organs [114, 117, 118], and in cases where the diagnosis of ocular GVHD is in question. Conjunctival specimens may also be tested with the use of special stains for viral involvement when indicated. Conjunctival histological features of GVHD include lymphocyte exocytosis, satellitosis, vacuolization of the basal epithelium, and epithelial cell necrosis, similar to changes that are observed in other organs [113–117]. Other features are relatively nonspecific, including epithelial attenuation, and goblet cell depletion, and are not sufficient for the diagnosis of ocular GVHD [116]. Corneal and conjunctival pseudomembranous histological findings are clinical manifestations generally associated with acute ocular GVHD [117–119].

5) Lungs: The pathologic finding of constrictive bronchiolitis obliterans (CBO) is considered as a diagnostic feature of pulmonary chronic GVHD. CBO resembles chronic lung allograft rejection [120], systemic pulmonary Castleman's disease [121], post-infectious scarring and toxic fume exposure [122, 123]. The bronchioles show intraluminal connective tissue and chronic inflammation that develops into dense fibrotic scarring of the bronchioles, resulting in luminal narrowing. Secondary changes include distal mucostasis or aggregates of foamy macrophages. Bronchiectasis may develop late. The extent and severity of changes should be correlated with functional studies, particularly if only a single affected airway is present in the biopsy. Other causes such as infection, and chronic aspiration should be excluded [120].

The 2014 NIH chronic GVHD diagnosis and staging document states that open lung biopsy may be considered if the characteristic pulmonary function tests and CT findings of bronchiolitis obliterans syndrome (BOS) are not accompanied by a distinctive manifestation

of chronic GVHD in another organ system in a patient without prior diagnosis of chronic GVHD [109]. Two recent studies point out that the clinical syndrome of BOS [124] encompasses several entities. The study by Holbro et al. based on 33 open lung biopsies for suspected histological CBO reported discrepancies between the histological findings and the NIH consensus criteria [125]. Half of the fourteen biopsies with histological CBO did not meet the clinical consensus criteria for BOS. In addition, of the 9 biopsies with lymphocytic bronchiolitis (LB), lymphocytic inflammation around and infiltrating small airways without subepithelial fibrosis, three met the NIH consensus for BOS. LB may represent an earlier stage in the final common pathway towards the development of histological CBO from several different disorders including viral infection as suggested in a recent review [126]. Although CBO and LB had similar pulmonary function tests and clinical manifestations, the patients with LB fared considerably better with treatment and had improved survival compared to those with CBO. Many of the discrepant cases with clinical BOS had infection without histological CBO. A study by Gazourian et al. also found a variety of pulmonary histopathological changes in the autopsy lungs of 35 patients who lived at least one year, 80% of whom had chronic GVHD [127]. Airway disease was present in 33 cases, and clinically unrecognized interstitial fibrosis and pulmonary veno-occlusive disease were seen in 8 and 12 patients respectively. Thus, the clinical term of BOS may more accurately reflect the variety of pulmonary pathologies associated with PFT airflow disturbances. A recently described CT methodology termed parametric response mapping (PRM), quantifies normal parenchyma total lung volume, functional small airways, emphysema, and parenchymal disease as relative lung volumes. PRM was able to identify BOS, even in the presence of concurrent infection [128].

Cryptogenic organizing pneumonia (formerly termed idiopathic bronchiolitis obliterans organizing pneumonia, BOOP) is associated with both acute and chronic GVHD. COP is a pathologic process defined by plugs of granulation tissue that fill the lumens of the distal airways in a patchy distribution, extending into the alveolar ducts and alveolar sacs, and associated with chronic interstitial inflammation [129]. COP should be distinguished from CBO because COP has a different clinicopathologic presentation and a more favorable outcome.

6) Kidney: Acute and chronic kidney diseases occur frequently after HCT. Acute renal failure occurs in 30–50% of patients, and CKD occurs in up to 60–70% of patients. An accumulating body of evidence has linked chronic GVHD with several kidney disorders, including nephrotic syndrome (NS) with or without renal insufficiency [130], membranous nephropathy [130–132], transplant associated microangiopathy (TAM) and chronic kidney disease (CKD). More recent studies measuring urinary cytokines [133] and elafin [134] indicate that most of the renal conditions associated with GVHD are secondary to the cytokines within the inflammatory milieu and not due to toxicity from pre-transplant conditioning or toxicity from calcineurin inhibitors. Nonetheless, the toxicity of CSP may be potentiated by cytokine effects from the presence of a chronic inflammatory state [135]. For a more comprehensive review, the reader should refer to Chang et al. [130].

Nephrotic syndrome may occur with or without renal insufficiency (reviewed in [136]). Patients usually present with proteinuria, edema and hypoalbuminemia. Most case reports

demonstrated membranous nephropathy with subepithelial glomerular deposits, and it is postulated that these deposits represent antigen–antibody complexes. It is unknown whether these deposits are directed against endogenous antigens expressed in the kidney, thus representing a direct immunologic attack by GVHD against the kidney, or if they are derived from antigens expressed elsewhere, such that the kidney injury occurs through indirect bystander mechanisms. Cases of minimal-change disease (MCD) have also been described [136]. Based on published case reports, membranous nephropathy occurs in 61% of NS cases, and MCD occurs in 22% of NS cases [137]. Both MCD and membranous nephropathy occur later after transplant at 8 and 14 months, respectively, and tend to occur within 1–5 months after the onset of GVHD or the tapering of immunosuppression during treatment for chronic GVHD. Others have reported cases of diffuse proliferative glomerulonephritis, antinuclear cytoplasmic antibody-related glomerulonephritis, focal segmental glomerulosclerosis, and IgA nephropathy [138–142] occurring after HCT. The development of each of these diseases appears to be associated with chronic GVHD that is unmasked when immunosuppression is tapered, similar to the development of acute hepatitic liver GVHD. Chronic kidney failure is also associated with GVHD, even after infection and toxicity from pre-transplant conditioning are excluded [131]. In an autopsy study of renal pathology in six autologous and 20 allogeneic HCT patients, renal tubulitis identical to that seen in renal allograft rejection was present in 67% of patients, whereas a later study found only 12% of patients had tubulitis associated with more severe forms of GVHD [143, 144]. Tubulitis may account for some of the renal dysfunction in patients with chronic GVHD.

Renal biopsies are needed to clarify these findings and guide therapy, especially in patients with proteinuria presenting at day 80–100 after HCT.

7) Other sites: Several other sites of chronic GVHD are less commonly involved or biopsied. Myositis is a phenomenon that is clearly associated with chronic GVHD. A comprehensive description with comparison to other myositis entities has not been made. The changes in skeletal muscle range from mild perimysial lymphocytic infiltrates to extensive endomysial inflammation with necrosis and regeneration of fibers. Clinical presentations and pathologic changes resembling both polymyositis and dermatomyositis have been reported [145, 146].

Biopsies may be useful in the evaluation of other rare manifestations that may be related to chronic GVHD. These syndromes include inflammatory neuropathies and synovitis. Chronic GVHD has been reported to cause obliterative coronary artery changes resembling transplant atherosclerosis [147]. Autopsy studies of lymphoid organs of patients with chronic GVHD have demonstrated profound lymphoid depletion with loss of or only rudimentary residual primary germinal centers [2].

Standardized reporting of GVHD in the pathology reports

In the prior document [10], we proposed terminology that can be used to qualify the certainty of a histological diagnosis of GVHD from any particular site (Table 2 in ref [10]). This schema separates the objective histological findings in the microscopic description

from the subjective global interpretation and allows the diagnosis to be expressed as a continuum rather than “yes” or “no.” This approach has been subjected to study for reproducibility by a European consortium [148]. Based on this study, we recommend reducing the categories for the diagnosis of GVHD from four to three: Not GVHD, Possible GVHD and Likely GVHD (Table 2). The category of “Likely GVHD” combines the prior categories of “consistent with” and “unequivocal” into a single category (synonymous with probable, favor or suggestive). The pathologist should add these qualifiers, as needed in the final diagnosis. In line with this update, the 2014 NIH Diagnosis and Staging document considered a biopsy read as “likely GVHD” sufficient to establish the diagnosis of chronic GVHD if accompanied by at least 1 distinctive clinical feature of chronic GVHD [109].

In addition to the diagnosis of GVHD, the pathology report should convey at least qualitative information on the severity of the injury as well as any additional findings of note. If a semi-quantitative grading system is used, references to the particular system should be recorded. A common understanding regarding the application of grading systems between the pathologists and the clinical staff should be established. No new recommendations regarding data collection and formal communication from clinicians to pathologists are made. These forms can be found in the previous document [10].

Areas for further study and notes on clinical studies incorporating pathology

Diagnostic criteria for minimal degrees of GVHD remain variably defined, subject to institutional variation. Examples of minimal criteria to diagnose GVHD for which further study is needed include the number of apoptotic bodies required in a skin, oral mucosal or gastric biopsy, the need for apoptosis when lymphocytic exocytosis is present in a skin or mucosal biopsy taken immediately after the onset of symptoms, the amount and location of inflammation in the minor salivary glands required for the diagnosis, the extent or degree of interlobular bile duct changes and portal infiltration in liver injury, and whether inflammatory peribronchiolar changes are a precursor to constrictive bronchiolitis obliterans.

This problem of minimal criteria may not have a discrete solution. When histological changes deviate only slightly from normal, histological “noise” may be interpreted as a signal indicating disease. In other areas of pathology, this problem is addressed by introducing a category of borderline change that has clearly defined boundaries. The clinical response to such a diagnosis might be to withhold therapy, watch closely and resample if necessary, or proceed with therapy if the clinical suspicion is high and initial sampling is limited. Because apoptotic cell injury is a characteristic of both skin, oral mucosal and GI GVHD that may be seen at any stage, the panel discussed developing grading schemes based on the extent of apoptotic change. Ideally, such a system would be reproducible and simple to apply, would set thresholds for borderline and definite GVHD, and thereby help guide clinical decision-making.

Initial studies could have a retrospective design, with blinded re-evaluation of biopsies according to pre-defined criteria and with adequate follow-up information on any

therapeutic intervention and clinical course. Case selection should focus on mild disease in order to define thresholds for therapeutic intervention more clearly. Multi-institutional studies are needed in an effort to decrease practice variation and increase the applicability of any findings. Prospectively designed studies would allow testing of proposed thresholds according to standard treatment protocols. Prospective studies could also serve to collect biosamples in parallel with biopsies for biomarker discovery. Ideally, standardized collection of biopsy data should be part of any prospective study, even if the study is not specifically designed to address a diagnostic issue.

Pathologists should be involved in the early stages of any study design. Observer variability must be minimized, possibly through on-line image atlases and consensus reviews. Sampling strategies, particularly with respect to evaluation of GI GVHD, should be standardized as much as possible. Although it is difficult to enroll patients in studies that have histological entry criteria, tissue-based diagnosis should be encouraged whenever possible, so that protocols designed to evaluate therapies are based on the most complete diagnostic information and biomarker discovery can be founded on histological findings.

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APPENDIX: NATIONAL INSTITUTES OF HEALTH CONSENSUS-DEVELOPMENT PROJECT ON CRITERIA FOR CLINICAL TRIALS IN CHRONIC GVHD STEERING COMMITTEE

Members of this committee included: Steven Pavletic, Georgia Vogelsang and Stephanie Lee (project chairs), Mary Flowers and Madan Jagasia (Diagnosis and Staging), David Kleiner and Howard Shulman (Histopathology), Kirk Schultz and Sophie Paczesny (Biomarkers), Stephanie Lee and Steven Pavletic (Response Criteria), Dan Couriel and Paul Carpenter (Ancillary and Supportive Care), Paul Martin and Corey Cutler (Design of

Clinical Trials), Kenneth Cooke and David Miklos (Chronic GVHD Biology), Roy Wu, William Merritt, Linda Griffith, Nancy DiFronzo, Myra Jacobs, Susan Stewart, and Meredith Cowden (members).

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Table 1

Histological Criteria for GVHD by Organ System

Organ or system	Minimal criteria for acute/ active GVHD*	Specific criteria for Chronic GVHD†
Liver	Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular and portal inflammation	Ductopenia, portal fibrosis, chronic cholestasis reflect chronicity but are not specific for chronic GVHD
Gastro-intestinal	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 Malphigian 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 orthohyperkeratosis, hypergranulosis and acanthosis resembling lichen planus ± lichenoid inflammation and / or vacuolar changes of eccrine units
Skin morpheic (localized or diffuse		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		Homgenization ± 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‡	
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§
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¶
Kidney		Membranous nephropathy, Minimal Change Disease
Lesions of Uncertain Pathogenesis		
Lung	Cryptogenic organizing pneumonia	
Skeletal Muscle	Myositis	

*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.

†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

‡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 [18].

[§]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.

[¶]Constrictive bronchiolitis obliterans (CBO) [120] should be distinguished from Cryptogenic organizing pneumonia, [129] which is also associated with GVHD but has a different clinicopathologic presentation and a more favorable outcome.

Table 2

Recommendation for Final Diagnosis Categories

Category	Definition	Examples	Comments
Not GVHD	No evidence for GVHD		
Possible GVHD	Evidence of GVHD but other possible explanations	• Obvious CMV enteritis with inclusions near the apoptotic changes • Focal colonic ulcers with marked apoptotic cryptitis and destruction of crypts associated with use of MMF • Co-infection with known active viral hepatitis • Clinical features which suggest or favor a drug reaction	Indicate possible alternate diagnoses and reasons for suspicion
Likely GVHD	Clear evidence of GVHD without a competing cause of injury OR Clear evidence of GVHD with mitigating factors OR GVHD most likely diagnosis but relevant clinical information is limited OR GVHD is validated by sequential biopsy or by absence of competing diagnosis	• Abundant epithelial apoptosis without clinical or histological evidence of drug injury or infection • Evidence of CMV yet abundant apoptotic epithelial changes that are not associated with CMV infected cells by immunostaining • Single or rare apoptotic epithelial changes without other features of active GVHD and no alternative explanations • Limited sample or minimal or focal findings • Proximity to recent chemotherapy or radiotherapy	Included old categories of “Consistent with” and “Unequivocal” GVHD