HISTOPATHOLOGIC DIAGNOSIS
OF CHRONIC GRAFT VERSUS HOST DISEASE

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

Author list to be determined

[NOTE TO READERS: This draft is still far from final form and requires editing for consistency and style. Content will continue to be updated both before and after the 2014 NIH GVHD Workshop. Comments from the working group have been mainly left in place.]

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Abstract
Background

Histopathology has played a major role in understanding the pathophysiology and aiding in the diagnosis and management of acute and chronic graft-versus-host disease (GVHD). Historically, the clinicopathologic classification of chronic GVHD was derived from a cohort of 20 patients in the late 1970's.[1] 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 histologic lesions of acute and chronic GVHD can be reviewed in several texts.[2-8]

More than 30 years since the initial publications of the histopathology of progressive chronic GVHD, many practical and unresolved issues in the surgical pathology of GVHD are not addressed in standard texts. It is often either not possible or not meaningful to distinguish persistent, recurrent or late acute from chronic GVHD by histology. Furthermore, uniform minimal diagnostic criteria for chronic GVHD have not been established for affected organs, and histologic grading systems have not been validated in a prospective fashion. It remains elusive whether changes in transplant modalities (e.g., reduced intensity conditioning, stem-cell source, T-cell depletion) affect the histological presentation of GVHD. In retrospective studies, the degree of inflammation or extent of epithelial damage or apoptosis has not predicted response to treatment in the gastrointestinal tract [3], skin [4] or liver, [7, 9] growing body of evidence indicates that in addition to damage to targeted epithelia, changes to the microvascular endothelium plays a role in the pathogenesis of GVHD [10, 11]. It is controversial whether this reflects GVHD targeting of the endothelium or is a secondary consequence of perturbations from the combination of inflammatory milieu and the addition of certain immunosuppressive agents. In a murine study of GVHD mesenteric resistance arteries had increased vascular strain, reduced expression of nitric oxide and electron microscopic abnormalities showing severe damage to the whole vessel wall[12]. Clinically, in contrast to patients with steroid-responsive GVHD, steroid-resistant GVHD patients had elevated levels of cytokeratin 18 and soluble thrombomodulin(TM), and decreased levels of both VEGF and angiopoietin 2[13]. Furthermore, in the steroid-resistant patients the increase of soluble TM
correlated with a loss of endothelial TM as determined by immunohistochemistry as well as survival of acute intestinal GVHD[14]. Additional studies related to the role of the endothelium are discussed in the skin and renal sections below.

A number of factors can influence or cause difficulty in histologic interpretation:

- The preparative regimen may influence histological findings.
- The timing of biopsies with respect to transplantation and treatment, including both initiation of potent immunosuppression or reduction in the immunosuppressive regimen is highly variable.
- The histology changes over time, particularly in separating old damage from ongoing or new GVHD.
- Sampling and technical factors can lead to false negative histologic assessment of GVHD.
- The interpretation and utility of serial biopsies in judging response to treatment has not been validated or standardized. Histological changes may lag behind clinical improvement.

In order to conduct clinical trials of chronic GVHD, uniformly applied and interpreted criteria for histopathologic diagnosis are necessary. These criteria should be validated by multi-institutional studies with biopsies correlated with clinical information. Ideally chronic GVHD trials should incorporate protocol-directed biopsies, from scheduled calendar or event driven collection procedures so as to allow corollary histopathologic studies.

**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 focusing on changes since our first publication[15]. Specifically, the Working group sought to 1) define and clarify the minimal diagnostic criteria for GVHD (encompassing the changes of both acute and chronic GVHD); 2) define those features that enables a specific diagnosis of GVHD; 3) develop a standardized terminology for communicating histology results; 4) distinguish active GVHD from previous irreversible changes; 5) suggest the relevant clinical data that should accompany the
biopsy; and 6) define criteria for an adequate histologic sample in various organs. The recommendations of the Working Group represent a consensus opinion supplemented by evaluation of available peer-reviewed literature. The proposed criteria and terminology are provisional and will be updated based on the results of prospective validation studies.

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 of to the definitions of GVHD.
- Table 2 has been updated to reflect results of a European consensus study that recommended reducing the diagnostic categories to three: “Not GVHD”, “Possible GVHD” and “Likely GVHD”.
- With respect to hepatic GVHD, a single liver biopsy obtained in the midst of immunosuppression can assess the severity of duct injury, but not its trajectory.
- With respect to gut GVHD, 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 the so-called cord colitis syndrome are not histologically distinct from colonic GVHD.
- The manifestations of crypt injury in GVHD have been subjected to interobserver validation by a multi-center European network, resulting in more precise definitions of crypt apoptosis and loss.
- Changes of MMF-related gut injury occur in both the upper and lower GI tract.
- Intestinal Paneth cells are lost as a late occurrence in severe GVHD and portend a poor outcome.
- Criteria for assessing the regression of dermal sclerosis after autologous transplantation for scleroderma have been defined.
• Reduction of salivary flow and quantitative proteomics correlates with histologic damage in minor salivary glands.

• Pathological studies of lung biopsies in patients meeting the 2006 NIH criteria for bronchiolitis obliterans syndrome (BOS) cast doubt on the specificity of those criteria, raising the issue of lung biopsy to confirm a clinical impression of BOS.

• Membranous nephropathy following transplant appears to be a manifestation of GVHD. Renal biopsy is recommended to correctly classify renal injury that develops after transplantation.

Areas for further study include:

• Diagnostic criteria for minimal degrees of GVHD in skin, gut and liver remain dependent on clinical context and is subject to institutional variation.

**Rationale for obtaining biopsies**

Chronic GVHD has a prevalence of $\geq 50\%$ in long-term survivors after HCT. As detailed in the Diagnosis and Scoring document,[16] biopsies are recommended to confirm 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 prior to referral.[17] For example, it is known that the clinical appearance of cutaneous mycosis, drug reactions and Grover’s disease can mimic chronic GVHD in the skin[18, 19].

Although biopsy can be of value in confirming the initial diagnosis of chronic GVHD, the role of subsequent biopsies to assess the response to treatment has not been determined. Also, 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, although in the context of clinical studies a screening biopsy may serve as a useful baseline reference point.

**Limitations of diagnosing GVHD by histopathology**

 Whereas histologic features are purely descriptive, 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 histologic 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.[20] As criteria for the minimal diagnostic threshold become more stringent, the sensitivity of the biopsy to detect GVHD will decrease. As a result, histology may not always be the gold standard for the diagnosis of low grade GVHD. However, the decision to treat GVHD is based not according to histopathologic characteristics alone but mainly according to the overall clinical assessment which includes information from the biopsy. In research studies, patients can be stratified for analysis according to the presence or absence of histologic changes.

 A number of factors can result in a false-negative histologic assessment of GVHD. Biopsies done immediately after the onset of symptoms and signs of presumptive GVHD may be falsely negative, as there may only be 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 fibrotic changes in the deep dermis fat or fasciitis. Oral labial biopsies may not include enough lobules of gland to differentiate between active disease and previously damaged glandular tissue. Suboptimally processed and sectioned biopsies may
obscure key cytological features. Biopsies that are too small and glass slides containing only limited number 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.

**Histologic criteria for the minimal diagnosis of GVHD**

Table 1 presents the minimal 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 active GVHD may be confidently made is under study. This threshold may vary depending on the quality of clinical information available to the pathologist, with higher thresholds required when only minimal information is available. 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.

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

1) Liver: As both drug-induced liver damage and opportunistic infections are frequent in HSCT patients, the diagnosis of liver GVHD can be highly challenging. Liver GVHD can appear isolated, or accompanied by other organ manifestations of GVHD. The histologic diagnosis of liver GVHD is based on the global assessment of immune-mediated destructive damage to small bile ducts and ductules,
together with cholestatic and inflammatory changes after considering clinical and serological information on potential confounding causes including exposure to cytotoxic or hepatotoxic drugs, infectious agents or sepsis, non-alcoholic steatohepatitis, or significant iron overload.

Characteristic bile duct changes may be absent or may affect only a minority of portal spaces, if the liver biopsy is done soon after the onset of liver dysfunction related to GVHD or the specimen is suboptimal in terms of portal tracts available for evaluation[21]. The histologic picture reflects the duration of active liver GVHD, the anti-inflammatory effects of immunosuppression, and the anti-cholestatic and cytoprotective influence of ursodeoxycholic acid. Several studies indicate that only 50-60% of clinically suspected cases of liver GVHD could be confirmed histologically. Some studies have shown that more frequent or serial liver biopsies provide a clearer picture of the liver disease status and lead to better patient management[9, 21-24]. There is no clear dichotomy between acute and chronic GVHD in the liver as degenerative and inflammation-associated biliary changes are present in both situations. Long-term persistence of GVHD results in increases in portal and bridging fibrosis, and reduction or loss of small interlobular bile ducts (ductopenia)[21]. Although rare cases of cirrhosis have been attributed to chronic GVHD,[6, 25, 26] these reports are confounded by coexisting chronic hepatitis C virus infection. In young pediatric patients with chronic liver disorders, the developing hepatobiliary tract is especially vulnerable to injury and prone to fibrosis[27].

In some patients a prominent ductular reaction may be present. In these cases it remains unclear whether it represents a reparative effort or a secondary target of GVHD or both[28]. A ductular reaction is typically present when there is concomitant gut GVHD or sepsis. In the latter case, the presence of ductular bile plugs indicates sepsis-associated cholangiolitis lenta[29].

Hepatic iron overload has been shown to contribute to liver dysfunction in HSCT patients and was associated with an increased rate of infections and may eventually predispose to the development of liver GVHD[24, 30]. As the cellular distribution and severity of iron overload have differential diagnostic implication, both should be mentioned in the pathology report.
The ominous significance of hyperbilirubinemia has been independently validated in several studies\[22, 31-33\]. Therefore, it seems likely that individual histological features would be associated with the outcome of HSCT patients or predict response to therapy. The literature on liver histology, present conflicting data particularly regarding the degree and frequency of biliary damage (vacuolization, anisonucleosis, cell drop-out, etc.) and its relationship to outcome. Most of these studies are based on small samples whose clinical context and case variables were obtained over a broad range of time, are not easily comparable to other studies as both conditioning and treatment modalities have changed\[34\]. For example, in one study the extent of bile duct damage, lymphocytic infiltration of BEC, portal inflammation, and ductopenia detected in a given biopsy showed no association with patient’s survival, while severe acinar inflammation and low level of hepatocellular ballooning were associated with a better outcome\[35\]. In another study on the acute hepatitic onset of liver GVHD extensive destructive biliary changes regardless of the degree of inflammation were associated with non-relapse mortality\[9\]. In the collective experience of the group, the extent of bile duct damage, portal and acinar inflammation are correlated with the degree of liver test abnormalities, but not necessarily the outcome of liver GVHD. However, the degree of ongoing biliary damage seems to be associated with overall survival in HSCT patients in general. Thus, liver biopsy may not only serve as a diagnostic tool for establishing the diagnosis of liver GVHD, but may provide additional prognostic information that could help to identify patients at high-risk for fatal outcome.

The minimum diagnostic threshold relies on a global assessment of characteristic withered interlobular bile ducts with nuclear and cytoplasmic alterations, with or without lymphocytic BEC infiltration (Figure 1). While these changes may not be present in every portal space, they should be representative of the overall picture. Liver biopsies vary greatly in the number of portal spaces that can be evaluated. The greater the number, the more accurate the overall assessment. Unlike some other epithelia, BEC apoptoses are infrequent and hepatocellular apoptoses are more likely associated with viral infections than liver GVHD. Refractoriness or a delay in starting treatment is associated with
greater loss of bile ducts and a longer time to recovery[9]. Refractory GVHD in the liver usually produces a picture of chronic cholestasis with ductopenia, and rarely a picture of ductular reaction[6, 9, 26].

Qualitative and quantitative assessment of bile duct damage or duct loss by immunohistochemical (IHC) staining of cytokeratin 7 or 19 is useful. Data are insufficient to determine whether IHC staining of replicative senescence by P21, [36] determination of the Th17/T regulatory cell ratio, [37] or staining for C4d provides additional information above and beyond the usual histologic evaluation[38].

The Lerner grading scheme categorizes the frequency of bile ducts in portal spaces. It can also be considered as a staging system of liver GVHD as it categorizes the level of ductopenia[39]. 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, scoring of liver GVHD is currently not recommended.

When dealing with a hepatitic damage, knowledge on the clinical (especially tapering of immunosuppressive drugs) and serological (i.e., infectious agents) is a prerequisite for adequate biopsy interpretation. Patients presenting with an acute hepatitic onset after donor lymphocyte infusions or tapering of immunosuppressive medications have more necroinflammatory activity and portal inflammation than typically seen in patients who are receiving immunosuppression.[9, 40] However, in the setting of rapidly rising aminotransferases in the thousands, viable hepatocytes adjacent to necrosis should be carefully evaluated for virus-induced cytopathic effects (e.g. Cowdry type bodies). In addition, immunostaining for viruses such as adenovirus and herpes viruses should be performed if rapid shell vial centrifugation culture is not available. Importantly, fibrosing cholestatic hepatitis from HBV or HCV may develop in immunocompromised patients, [41] while the usual manifestation of HCV is a mild self-limited elevation of serum aminotransferases during tapering of immunosuppression[42].
Although HCV causes inflammation and reactive bile duct changes,[6, 21, 43] the withered degenerative bile duct changes of GVHD are qualitatively different from those caused by HCV (Figure 1A and B). Principally all viral agents can be detected in FFPE tissue using PCR or RT-PCR assays. In addition, HHV6 may cause liver dysfunction. When the serum copy numbers are elevated, in situ hybridization may detect HHV6 (or other viruses) in the liver biopsy. Routine immunostaining for CMV is not recommended.

There are several observations gleaned from studies which included serial biopsies. The first: liver biopsy obtained soon after onset of liver dysfunction may have minimal non-diagnostic changes. Early biopsies done for the acute hepatic onset of GVHD may have striking hepatocellular (spotty) necrosis with minimal bile duct damage. This is presumably a reflection of cytokine induced bystander death of hepatocytes mediated by the Fas/Fas ligand interaction of activated T cells in the liver sinusoids. In such cases subsequent liver biopsies may have many plasma cells, and scattered eosinophils in the portal space and obvious bile duct damage. Serial liver biopsies done for persistent liver dysfunction while receiving prolonged immunosuppression may show a further damage or loss of bile ducts along with chronic cholestatic changes. In such cases the liver histology typically lags behind the improvement in the liver tests. In contrast, a single liver biopsy obtained after persistent liver dysfunction despite immunosuppressive treatment can collate the degree of biliary damage or destruction but cannot indicate if the process is progressive, static or recovering. Serial liver biopsies may identify that progressive deterioration of presumed GVHD is related to a second process or may help to identify features predicting a 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 proceed 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.[9] 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.

2) Gastrointestinal tract: The histopathology in the gastrointestinal (GI) tract during chronic GVHD is variable. The changes encompass the onset of late occurring acute GVHD or chronic and persistent mucosal damage with fibrosis of chronic GVHD. Endoscopic or imaging evidence of esophageal webs remains the only uniformly accepted diagnostic feature of chronic GVHD within the GI tract[16] although the histological changes are non-specific. Prolonged or incompletely treated forms of acute GVHD may leave behind extensive architectural distortion[44]. 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 and/or ulcerations, often with little associated inflammation or apoptosis, Other changes of chronicity include, lympho-plasmacytic inflammation, colonic Paneth cell metaplasia, lamina propria fibrosis and rarely submucosal or serosal fibrosis [2, 3, 7, 44].

The histologic hallmarks of gastrointestinal GVHD are some combination of enterocyte apoptosis, crypt or basilar gland destruction, and mucosal denudation. The term "crypt apoptosis" will used as a general term for 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 there is only surface mucosa with complete destruction of underlying crypts as may occur with prolonged gut GVHD. However, the recognition of these findings may not be uniformly interpreted. After circulating a set of intestinal biopsies, a consortium of pathologists from five German institutions proffered diverse interpretations from the same images. In a follow-up study with additional review consensus was reached on more precise definitions as illustrated in the study set of photomicrographs. For example, apoptotic bodies may appear as exploding crypts, [45] with a large clear zone surrounding hyperchromatic karyorrhectic nuclear debris, or as a small
shrunken cell with eosinophilic cytoplasm with a condensed nucleus. The aim of the European study was to provide more consistent application of histologic features for diagnostic and research studies.

As noted before[15], 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[44, 46, 47] while others use response to immunosuppressive therapy alone as an indication of disease[11, 14]. However, we advise caution as involvement of one organ system by GVHD doesn’t necessarily imply coexisting GI GVHD. Diagnostic considerations would also include infectious etiologies and medication related injury. Additionally, by narrowing the scope of patients to only those who respond to therapy, those who are refractory or unresponsive are potentially excluded from further study. 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[48]. However, discrepancies between upper or lower endoscopic findings are well documented as are differences between endoscopic and histologic diagnoses [46, 49-53]. Although different institutional studies have suggested particular sites in the GI tract as having the highest endoscopic yield, there is lack of consensus that one particular site is better than others. The highest diagnostic yield occurs when visibly injured or erythematous regions of both the upper and lower gut are sampled[52]. Therefore, for clinical practice, the diagnosis of GI GVHD requires integration of clinical, endoscopic and histologic findings and exclusion of competing causes of injury. Importantly, when a biopsy is negative for evidence of GVHD, the probability of involvement by GVHD would still remain high in patients with typical signs and symptoms, characteristic gross endoscopic findings (i.e. mucosal edema) and when infectious or medication related etiologies are excluded. Similarly, for the purpose of designing clinical trials and studies, a combination of factors, not one indication alone, should be taken into account when making a diagnosis of GVHD.

Defining a threshold for the minimal histologic changes needed for a diagnosis of GVHD in endoscopic biopsies remains controversial and dependent on institutional standards and biopsy practice.
Attempts to refine the threshold of apoptosis involve a tradeoff between sensitivity and specificity. Using a cut-off similar to that used for acute cellular allograft rejection in small bowel transplants (≥6/10 consecutive crypts)[54, 55] was felt by the consensus group to be too high. Others have demonstrated that using the previously recommended >1-2 apoptotic bodies per biopsy piece (on average) increases sensitivity with some loss of specificity[56, 57]. Because GI GVHD may be unevenly distributed in the segments of the GI as well as within a segment of the GI, false negative conclusions could result from a limited number of biopsies 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. A clinical study quantitating regulatory T cells in blood and gastric mucosal biopsies failed to demonstrate any correlation with clinical or histological severity of GVHD[58].

It should be noted that enterocyte apoptosis is not limited to GVHD. Infectious associations with apoptosis (CMV, cryptosporidia) were discussed in the earlier document (ref 1st paper). If the histologic picture has mixed acute and chronic inflammation or enlarged cytologically atypical non-diagnostic cells and if viral cultures or shell vial assay have not been obtained, IHC for CMV should be performed. Adenovirus may be cultured from stool but may mimic the injury in GVHD, is difficult to identify on routine stains and often requires IHC to detect.

Since the last consensus document, there have been numerous publications describing mycophenolate mofetil (MMF) GI toxicity. MMF induced injury can mimic GVHD, inflammatory bowel disease, ischemia and granulomatous processes. In the upper GI tract, one may see parietal cell ballooning, chronic active gastritis, active esophagitis and celiac-like features in the duodenum[57, 59-63]. 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[60]. Contrary to prior reports [64], the presence of increased number of eosinophils within the lamina propria and epithelium should favor a diagnosis of MMF induced injury over GVHD[65, 66].

Comment [DEK16]: From DC: Is this our final recommendation?

Comment [AK17]: What could be said of the cases with increased apoptotic bodies, below the threshold. They may represent some sub clinical and innocuous GvH reaction? There may be mentioned, that crypt destruction and mucosa denudation (in the right context) are trustworthy signs of (higher grade) GvHD.

Comment [DEK18]: From DC: The use of apoptotic stains (e.g., caspase 3) is neither recommended nor proven to be more useful than good-quality H&E serial sections.??

Comment [AK19]: There may be added, that those infection may trigger and coexist with (and do not preclude) GvHD.
Additionally, loss of neuroendocrine cell nests was seen in MMF related injury as opposed to GVHD, where these neuroendocrine cells are spared[66, 67]. 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 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)[68].

The cord colitis syndrome (CCS), was a newly described entity in 2011 by Herrera et al. restricted to Boston Dana Farber cord blood recipients with late onset secretory diarrhea, an absence or GVHD or detectable infections, chronic active colitis, including some cases with multicentric granulomas, and responsiveness to antibiotics[69]. They used a unique non-standardized definition of GVHD that excluded GVHD in colonic biopsies if there were low or rare apoptosis and/or any features of chronicity as described above. Subsequently, two much larger studies including controls who received cord blood and non-cord blood stem cells found that neither the histological features of chronicity (crypt distortion, paneth cell metaplasia, chronic active colitis) nor granulomas were confined to or increased in cord blood recipients[70, 71]. In Milano’s study all of the non-cord blood allogeneic recipients with late onset secretory diarrhea and symptoms ascribed to CCS responded to anti-GVHD treatment[70]. The presence of granulomas with neutrophilia was more common in the Japanese study which included many cases with both GVHD and CMV[71]. These studies serve as a reminder that GVHD and infection are not mutually exclusive diagnoses.

In the previous consensus document[15], the standard use of histologic grading was not recommended because the existing grading schemes (Lerner[39] and the Sale modification[2]) combine diagnostic criteria with the extent and chronologic stage of disease. Although severe changes (Lerner grade 4) are associated with poor survival[72], 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 systems in routine practice.
If an institution chooses to use a grading system, the site with the greatest damage or highest histologic grade should be documented because of the inherent variability in injury. Additional morphologic features that have been shown to correlate with disease severity and/or non-relapse mortality include the loss of Paneth cells within the small intestines and crypt loss within the colon[73, 74]. Nonetheless, since neither of these two features are present at the onset of gut GVHD or before there is extensive crypt damage, initial gut biopsies are not useful predictors of subsequent clinical course. Because of the drawbacks of the existing histological grading systems, the committee was very interested in investigating grading schemes based on the degree of apoptotic activity independent of the stage of crypt or mucosal destruction. An ideal grading system should be reproducible and simple to apply, define minimal criteria for diagnosis of GVHD and grade severity in a way that can guide clinical decision-making.

3) Skin: Since the previous consensus document the reader is directed to two comprehensive richly illustrated reviews of the gross and histologic manifestation of cutaneous cGVHD[8, 75]. The minimal histologic criteria for GVHD require apoptosis within the basilar or lower spinosum layers of the epidermis (Figure 3A).[2, 5, 39] In cases of minor alteration, the focus should be on the interpretation of vacuolar changes and apoptotic keratinocytes, including in the adnexal epithelia[76]. The archetypical features of both acute and chronic GVHD are superficial interface dermatitis with either a lichenoid pattern with lymphocytic inflammation with or without lymphocyte satellitosis or with predominately vacuolar change in the basilar layer.[4, 5] As a note of caution, since no single histologic feature is pathognomonic of GVHD, the major overall inflammatory reaction pattern should be factored into the final interpretation.[77] For example, an 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 dermatitis but encompasses a broad differential and likely excludes the diagnosis of GVHD. The inconstant finding of lymphocyte satellitosis (lymphocytes...
surrounding an apoptotic keratinocyte in the epidermis or appendages) provides evidence that the dermatitis may be caused by GVHD. However characteristic, this feature is not entirely specific and can occur in drug reactions. Of note Nishiwaki et al., noted that many cells of 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 lessened survival[78].

The diagnosis and management of sclerotic cGVHD are discussed in related consensus documents. The genesis of the dermal sclerosis and its relationship to the dermal microvasculature was studied by Biedermann et al.[79]. They reported a correlation of dermal sclerosis in chronic GVHD with elevated blood vWF levels and reduced capillary density identified by lectin IHC staining for Ulex europeus lectin. Biedermann concluded that reduced vascularity was responsible for the fibrosis and further postulated that the presence of T cells in the perivascular zone suggested that the endothelium was the target. Of note circulating vWF multimers are a non-specific acute phase reactant in the inflammatory process. The Ulex antigen is a fucose glycosylation moiety of a blood group antigen expression of which is subject to competing fucosylation in the presence of inflammation. In contrast, Fleming et al. used the specific endothelial markers CD31 and VE-cadherin, focusing on vessel density rather than vessel size. They did not find a correlation between the dermal fibrosis in chronic GVHD and a reduction in dermal capillary density [80]. They further showed that the microvasculature in chronic GVHD did not have the archetypical abnormal endothelial phenotype of capillaries in systemic sclerosis of reduced VE cadherin and vWF. A related study using capillaroscopy on nailfold capillaries confirmed the findings of Fleming study that only PSS but not CGVHD patients had both morphologically abnormal capillaries and reduced density. In the NIH Clinical Trials and Biology document, the role of PDGF-r in the genesis of dermal sclerosis is further discussed.

The histologic manifestations of chronic cutaneous GVHD evolve over time, are modified by treatment, and to some extent overlap with those of acute GVHD. Severe keratinocyte dysplasia related to conditioning with busulfan can persist for many months post transplant[81]. The histologic
counterparts to the proposed diagnostic clinical definitions of cutaneous chronic GVHD include several different histologic patterns (Figure 3B). The lichen-planus type eruptions (initially classified as early generalized extensive chronic GVHD[1, 4] referred to a specific constellation with epidermal thickening by acanthosis (hyperplasia) with orthokeratosis (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 dermal adnexae. This constellation, especially when accompanied by lymphoplasmacytic inflammation around the eccrine coils, is highly specific for chronic GVHD, but at the expense of reduced sensitivity. Since the skin lesions of chronic GVHD are not synchronous, the presence or absence of chronic GVHD features in a biopsy can be influenced by sampling or partial thickness. In practice, members of the dermatopathology subcommittee regarded a skin biopsy with the combination of epidermal compact orthokeratosis, hypergranulosis and acanthosis with blunted or saw-toothed rete ridges as features that favor or are consistent with chronic lichenoid GVHD. Rarely, a lesser degree of this combination of features can occur in skin biopsies from patients with severe clinical acute GVHD. Skin biopsies from non-sun-exposed normal appearing skin, such as iliac crest, taken between day 80 -100 often contain rare isolated keratinocyte apoptosis with little or no accompanying inflammatory content[82]. These findings were regarded as GVHD in earlier era when protocol driven day 100 screening biopsies were performed to predict the development of cGVHD[83]. Presently, the final interpretation of such findings, whether considered non specific or consistent with minimal (subclinical) GVHD is dependent 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 (Figure 3C and D).[1, 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 and/or subcutaneous septa. Depending on the clinical presentation sclerotic GVHD can manifest with localized morphea-like
features or diffuse sclerosis and lichen sclerosus-like features. Histopathologically, localized morphea-like features or diffuse sclerosis are largely confined to the reticular dermis and/or subcutaneous septa with little or no epidermal involvement. In lichen sclerosus-like GVHD sclerosis is confined to the papillary dermis often with residual interface changes in terms of melanophages in the papillary dermis and sparse lymphocytic infiltrate. Another variant, fasciitis shows fibrous thickening only of the fascia with adjacent inflammation without any epidermal or dermal involvement (Figure 4).[84]

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. The indication of active GVHD is residual apoptotic changes in the epidermis or appendages. During treatment, the histologic significance of persistent epidermal vacuolar degeneration requires additional correlative study, as does the assessment of activity in patients who have received psoralen and UVA irradiation 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 histologic regression of dermal sclerosis occurs a year or two following autologous transplant for scleroderma( PSS). Nash et. al. developed a scheme for grading the reduction in dermal sclerosis[85]. A similar though more complicated scheme for grading the regression of sclerosis was designed by Verrecchia et al.[86].

4) Oropharynx, vulva and eye: Based on the studies of oral labial biopsies taken 80-100 days after HCT, patients without any signs or biopsy evidence of GVHD may have chronic inflammation without apoptotic changes in the mucosa and minor salivary glands. These changes were attributed to chemotherapy or irradiation in the conditioning regimen[87]. The minimal histologic criteria for oral chronic GVHD have remained unchanged: localized or generalized epithelial changes (lichenoid interface inflammation, exocytosis and apoptosis) similar to those described in cutaneous GVHD or presence of intralobular, periductal lymphocytes with or without plasma cells and exocytosis of lymphocytes (without neutrophils) into intralobular ducts and acini. Periductal fibrosis (not 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.\[88\] Horn et al. developed a histologic grading system for chronic GVHD of minor salivary glands based on the degree of lymphocytic infiltration and destruction of glandular acini.\[89\] Soares et al. found the most specific histologic features of oral cGVHD were minor salivary gland periductal inflammation with exocytosis which correlated with extensive cGVHD and global survival. While the reduction in glandular acinar area was greatest in those with cGVHD some reductions also occurred in patients without GVHD\[90\]. In children, oncocytic ductal metaplasia may be an additional feature favoring GVHD. Similar findings are commonly seen in oral biopsies of adults over 40 and are considered evidence of previous ductal damage. Moderate to intense periductal and periacinar fibroblastic stroma is evidence of previous inflammation or chronic GVHD activity, whereas dense fibrous tissue with destruction of acinal tissue and duct ectasia may be only a marker for previous damage.

Persistent salivary dysfunction after treatment of chronic GVHD is related to continuing lymphocytic inflammation with absence of recovery or destruction of minor salivary secretory units.\[91\] Patients with oral cGVHD demonstrate a strong relationship of xerostomia to xerophthalmia and reduced salivary flow rates. All patients with salivary dysfunction had histologic damage to the minor salivary glands with mononuclear infiltration and/or fibrosis/atrophy involvement of the oral mucosa did not correlate with salivary dysfunction. In the future, quantitative proteomic analysis of saliva may be added as a biomarker to identify active oral cGVHD especially in newly diagnosed patients within 12 months of HSCT.\[93\] Quantitative proteomic analysis of saliva from patients with and without oral chronic GVHD demonstrated altered expression in the cGVHD compared to the non GVHD group. Glandular atrophy, fibrosis and inflammatory infiltrate were all associated with salivary gland function in a cohort of patients with oral cGVHD\[92\].
The diagnosis and staging document and other reviews[8] provide a detailed description of the oral changes of cGVHD. Nonetheless, clinicians and pathologist should be aware that premalignant dysplasias and oral cancers, a leading cause of secondary malignancies after allogeneic transplantation, often present with a lichenoid appearance.[94, 95]

The same criteria described above for oral and esophageal mucosa are used for histologic assessment of chronic GVHD in vulvar, [96] conjunctival and lacrimal biopsies. Histopathological findings of ocular GVHD have been described in conjunctiva and in the lacrimal gland. [34, 41-44] The alterations in lacrimal gland acinar tissue resemble those in minor salivary glands with prominent infiltration of mononuclear cells around medium size ducts, loss of acinar lobules replaced by fibrosis. While lacrimal gland biopsy is relatively invasive and may result in decreased capacity of its function, conjunctival biopsy may be obtained without much risk to the patient. Histological evaluation of conjunctiva may aid in the diagnosis and management of ocular GVHD in symptomatic patients with conjunctival disease.[41.45-46] While the biopsy is not performed routinely, it may be particularly helpful in cases where ocular GVHD is in question in symptomatic patients who have normal or unchanged Schirmer’s test with or without GVHD of other organs. Conjunctival specimen may also be tested using special stains for viral involvement when indicated. Routine survey biopsy, however, is thought to serve little benefit for early detection of ocular GVHD.[41,44] Conjunctival histological features for GVHD include lymphocyte exocytosis, satellitosis, vacuolization of the basal epithelium, and epithelial cell necrosis, similar to changes that are observed in other organs.[41-45] Other features are relatively nonspecific, including epithelial attenuation, goblet cell depletion, which are not sufficient for the diagnosis of ocular GVHD.[43] Corneal and conjunctival pseudomembranous histological findings are clinical manifestations generally associated with acute pattern of ocular GVHD.[45-47]

5) Lungs: The pathologic finding of constrictive bronchiolitis obliterans (CBO) is considered to be a diagnostic feature of pulmonary chronic GVHD. CBO resembles chronic lung allograft rejection,[97] systemic pulmonary Castleman’s disease[98], post-infectious scarring and toxic fume
exposure[99, 100]. The bronchioles show intraluminal connective tissue and chronic inflammation that develops into dense fibrotic scarring of the bronchioles, resulting in luminal narrowing (Figure 6). 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.[97]

In the previous consensus document[15], a lung biopsy was not deemed necessary to diagnose pulmonary chronic GVHD in the presence of other distinctive organ features and a constellation of suggestive pulmonary function tests and chest CT scans[16]. However, two recent studies challenge this assumption. The study by Holbro et al. based on 33 open lung biopsies for suspected CBO reported discrepancies between the histologic findings and the NIH consensus criteria[101]. Half of the fourteen biopsies with histologic CBO did not meet the consensus criteria for CBO. In addition, of the 9 biopsies with lymphocytic bronchiolitis (LB) three met the NIH consensus for CBO. Though CBO and LB had similar pulmonary function tests and clinical manifestations, the patients with LB fared considerably better with treatment and had improved survival. LB may represent an earlier stage in the final common pathway towards the development of CBO of several different disorders as suggested in a recent review.[102] Many of the discrepant cases with clinical CBO had infection without histologic CBO. Gazourian et al also found a variety of pulmonary histopathologies in the autopsy lungs of 35 patients who lived at least one year, 80% of who had cGVHD. Airway disease was present in 33 patients and clinically unrecognized interstitial fibrosis and pulmonary venoocclusive disease were seen in 8 and 12 patients respectively. Thus the clinical diagnosis of BO/CBO may be confounded by a variety of pathologies and the criteria for deciding if and when an open lung biopsy is needed for diagnosis is not entirely settled. Since the new proposed diagnostic and staging clinical criteria for BOS no longer includes DLCO, it is unknown if the assumed increased clinical sensitivity coupled with improved CT imaging studies will lead to the inclusion of both lymphocytic bronchiolitis and COP.
Cryptogenic organizing pneumonia (formerly termed idiopathic bronchiolitis obliterans organizing pneumonia, BOOP) is associated with both acute and chronic GVHD. COP is clinicopathologic syndrome 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.[103] COP should be distinguished from CBO because COP has a different clinicopathologic presentation and a more favorable outcome.

6) Kidney Since the initial consensus document[15] an accumulating body of evidence has linked cGVHD with several kidney disorders including nephrotic syndrome(NS) with or without renal insufficiency,[104] membranous nephropathy[104-106], transplant associated microangiopathy TAM and chronic kidney disease (CKD). For a more comprehensive review the reader should refer to (Reference needed).

Acute and chronic kidney disease is common following hematopoietic stem cell transplant (HSCT) with acute renal failure occurring in 30-50% of patients and CKD occurring in up to 66% of patients. Patients with NS usually present with proteinuria, edema, and hypoalbuminemia. The majority of these case reports demonstrated membranous nephropathy with subepithelial deposits on biopsy; it is postulated that these deposits are antigen–antibody complexes representing GVHD in the kidney.

However, cases of minimal-change disease (MCD), which is thought to be a T-cell-mediated process, have been described and one case in a cord blood transplant patient without GVHD was thought to be associated with recipient T-cell activation leading to both graft failure and MCD. Case reports of membranous nephropathy (MN) after HCT are much more frequent than MCD 61% versus 22%, respectively. The majority of reported patients with MN had a history of acute and chronic GVHD. Both MCD and membranous nephropathy occur later after transplant at 8 and 14 months, respectively, and tend to occur within 1–5 months of the development of GVHD and/or the tapering of immunosuppression for chronic GVHD. Additional reported kidney diseases associated with chronic GVHD and/or tapering of immunosuppression include of diffuse proliferative glomerulonephritis,
antinuclear cytoplasmic antibody-related glomerulonephritis, focal segmental glomerulosclerosis, and IgA nephropathy.

The genesis of TA-TMA with thrombosis within the glomerular capillary tufts and adjacent small arterioles may reflect direct injury to endothelial cells by several different etiologies. Clinical and histologic studies have supported both acute and chronic GVHD as potential risk factors. The risk of TA in autopsy kidneys was increased four-fold in patients with acute GVHD in recipient/donor gender mismatch pairs. Plasma markers of endothelial injury and coagulation activation are elevated in patients with acute GVHD after HCT suggesting secondary perturbation to the endothelium from circulating inflammatory cytokines related to GVHD[10].

Post HSCT patients are labeled idiopathic CKD because they do not present with NS, nor satisfy the definition of a TMA nor have a documented viral infection. The incidence of idiopathic CKD varies from 13% in patients receiving high-dose conditioning to 16–66% in patients who have been treated with reduced intensity conditioning. This suggests that TBI was not associated with an increased risk of CKD. Other risk factors for CKD identified in the high-dose group include older age, CSP use, acute kidney injury, and acute and chronic GVHD. In the RIC transplant group, long-term calcineurin inhibitor use and prior autologous transplant, as well as AKI and GVHD. Among the posited explanations for the CKD post-HSCT is that the kidney is directly targeted via T-cell-mediated renal damage. In a case report of minimal-change nephrotic syndrome after HCT, large numbers of CD8 (+) donor T cells were found infiltrating the interstitium and periglomerular areas of the kidney. Alternatively, CKD may be a result of the chronic systemic inflammatory state of GVHD. A third explanation is that chronic exposure to calcineurin inhibitors, such as CSP, leads to CKD. These are not mutually exclusive hypotheses as T-cell-mediated injury in GVHD is intertwined with cytokine effects and the effects of CSP may be potentiated in the presence of a chronic inflammatory state. Renal tubulitis identical to that seen in renal allograft rejection was present in 67% of patients in an autopsy study of six autologous and 20 allogeneic HCT patients. However, a later study found only 12% of patients had tubulitis associated with more severe forms of GVHD.
7) Other sites: Several other sites of chronic GVHD are less commonly involved or biopsied. Myositis is clearly a phenomenon associated with chronic GVHD. A comprehensive description with comparison to other myositis entities has not been made. The skeletal muscle biopsy changes range from mild perimycial lymphocytic infiltrates to extensive endomycial inflammation with necrosis and regeneration of fibers. Clinical presentations and pathologic changes resembling both polymyositis and dermatomyositis have been reported.[107, 108].

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 can cause obliteratorive coronary artery changes resembling transplant atherosclerosis.

**Standardized reporting of GVHD in the “FINAL DIAGNOSIS”**

In the prior document, we propose terminology that can be used to qualify the certainty of a histologic diagnosis of GVHD from any particular site (Table 2 in ref [15]). This schema allows the diagnosis to be expressed as a continuum rather than “yes” or “no,” and separates the objective histologic findings in the microscopic description from the subjective global interpretation. This has been subjected to study for reproducibility by a German consortium[109]. 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 one 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 Diagnosis and Staging Working Group considered a biopsy read of likely GVHD sufficient to establish the diagnosis of chronic GVHD if accompanied by at least 1 distinctive feature of chronic GVHD (ref. Diagnosis and Staging Working Group Report).

No new recommendations regarding data collection and formal communication from clinicians to pathologists are made. These forms can be found in the previous document[15].
Acknowledgments

[Insert boilerplate acknowledgments for the 2014 chronic GVHD project]

NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD Steering Committee

[Insert steering committee acknowledgement here]
Table 1. Histologic Criteria for GVHD by Organ System

<table>
<thead>
<tr>
<th>Organ or system</th>
<th>Minimal criteria for acute/ active GVHD*</th>
<th>Specific criteria for Chronic GVHD†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin, in general</td>
<td>Apoptoses 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</td>
<td>Combination of epidermal orthokeratosis, hypergranulosis and acanthosis with lichenoid changes ± lichenoid inflammation and / or vacuolar changes of eccrine units</td>
</tr>
<tr>
<td>Skin lichen planus-like</td>
<td></td>
<td>Collagenous deposition with thickening and homogenization throughout reticular dermis or pandermal sclerosis ± thickening and homogenization of adjacent subcutaneous septa</td>
</tr>
<tr>
<td>Skin morpheic (localized or diffuse)</td>
<td>Predominated by sclerosis in papillary dermis with residual interface changes most often in terms of melanophages in the papillary dermis and sparse lymphocytic infiltrate</td>
<td></td>
</tr>
<tr>
<td>Skin lichen sclerosus like</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin fasciitis</td>
<td>Fibrous thickening of fascial septa with adjacent inflammation ± sclerosis of adjacent subcutis</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular and portal inflammation</td>
<td>Ductopenia, portal fibrosis, chronic cholestasis reflect chronicity but are not specific for chronic GVHD</td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>Variable apoptotic criteria (≥1/piece) in crypts</td>
<td>Destruction of glands, ulceration or submucosal fibrosis reflect severe or long-standing disease but are not specific for chronic GVHD</td>
</tr>
<tr>
<td>Oral mucosa and conjunctiva</td>
<td>Lymphocytic infiltration of mucosa with variable apoptosis‡</td>
<td></td>
</tr>
<tr>
<td>Minor salivary or lacrimal gland</td>
<td>Infiltration and damaged intralobular ducts, fibroplasia in periductal stroma, inflammation with destruction of acinar tissue§</td>
<td>Obliterative bronchiolitis: dense eosinophilic scarring beneath the respiratory epithelium, resulting in complete fibrous obliteration or some degree of luminal narrowing¶</td>
</tr>
</tbody>
</table>

*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.

Comment [z39]: In lichenoid GVHD there is no panniculitis present

Comment [DEK40]: am confused by “Skin sclerotic” and “Skin morpheic”. Are these distinctions made routinely in diagnostic texts? For one thing, “morpheic” is a strictly clinical term and while I note that a clinical element was put in the definition, it is not a term that can be used with confidence by a pathologist who often has no clue, sadly, as to the clinical picture. Also, for “Skin sclerotic” one of the histologic features is “+/- panniculitis”. Panniculitis is an absolutely classic feature of active morphea (see below) so that confuses me even more. Can these two terms not be combined?

Comment [AK41]: This may also be the signs of acute GvHD. As the previous consensus paper does not describe histological signs of GI cGVHD, is there now an agreement on this.

Comment [AK42]: This may also be the signs of acute GvHD. As the previous consensus paper does not describe histological signs of GI cGVHD, is there now an agreement on this.

Comment [AK43]: This may also be the signs of acute GvHD. As the previous consensus paper does not describe histological signs of GI cGVHD, is there now an agreement on this.

Comment [NT44]: Specify “lichenoid” to better define the actual pattern?
Once the diagnosis of chronic GVHD has been established or following immunosuppressive treatment, the histologic manifestations of active disease may meet only minimal diagnostic criteria for activity.

Inflammation of the oral mucosa and within the minor salivary glands may persist from prior chemoradiation or prior inflammation. The distinction between acute and chronic GVHD requires the addition of distinctive oral manifestations.[16]

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. Fibroplasia, with acinar and periductal inflammation and features of damage to ducts, such as vacuolar change, lymphocytic exocytosis nuclear dropout, dyspolarity or apoptosis, indicate chronic GVHD activity.

Obliterative bronchiolitis (BO)[97] should be distinguished from BOOP,[103] which is also associated with GVHD but has a different clinicopathologic presentation and a more favorable outcome.

### Table 2. Recommendation for FINAL DIAGNOSIS categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not GVHD</td>
<td>No evidence for GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Possible GVHD</td>
<td>Evidence of GVHD but other possible explanations</td>
<td>[Obvious CMV enteritis with inclusions near the apoptotic changes][11]</td>
<td>Indicate possible alternate diagnoses and reasons for suspicion</td>
</tr>
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<td></td>
<td></td>
<td>[Focal colonic ulcers with marked apoptotic cryptitis and destruction of crypts associated with use of MMF][11]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Co-infection with known active viral hepatitis][11]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>[Clinical features which suggest or favor a drug reaction][11]</td>
<td></td>
</tr>
<tr>
<td>Likely GVHD</td>
<td>Clear evidence of GVHD with mitigating factors OR</td>
<td>[Unequivocal evidence of CMV yet abundant apoptotic epithelial changes that are not associated with CMV infected cells by immunostaining][11]</td>
<td>Included old categories of “Consistent with” and “Unequivocal” GVHD</td>
</tr>
<tr>
<td></td>
<td>GVHD mostly likely diagnosis but relevant clinical information is limited OR</td>
<td>[Single or rare apoptotic epithelial changes without other features of active GVHD and no alternative explanations][11]</td>
<td></td>
</tr>
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<td></td>
<td>GVHD is validated by sequential biopsy or by absence of competing diagnosis</td>
<td>[Limited sample or minimal or focal findings][11]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>[Proximity to recent chemotherapy or radiotherapy][11]</td>
<td></td>
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</tbody>
</table>
Figure Legends

Figure 1. Hepatic GVHD
A. Late onset of acute graft-versus-host disease, day 123. The expanded portal space contains a mixed infiltrate of lymphocytes and scattered eosinophils. Interlobular bile duct shows destructive changes of graft-versus-host disease with infiltration by lymphocytes, segmental loss of nuclei, cytoplasmic vacuolization and nuclear dyspolarity. Ductular proliferation at the margins of the portal space also shows some features of GVHD. 250x
B. Refractory chronic graft-versus-host disease, day 556. Interlobular bile ducts have characteristic withered appearance with dyspolarity, dropout of nuclei, nuclear anisonucleosis, infiltrating lymphocytes and eosinophilia of cytoplasm. Fibrotic portal space contains scattered lymphoid cells and periportal hepatocytes show changes of chronic cholestasis with cytoplasmic ballooning. 250x
C. Refractory untreated chronic graft-versus-host disease, day 350. Portal spaces have marked ductopenia with loss of bile ducts, a lymphoplasmacytic infiltrate and fibrosis with focal bridging (not shown). 250x

Figure 2. Gastrointestinal GVHD
A. Persistent GVHD in colon, day 87. The colonic biopsy has numerous contiguous apoptotic changes (arrows). 250x
B. Late acute GVHD in stomach, day 133. The biopsy shows pronounced lymphocytic and prominent eosinophilic infiltration with destruction of gastric antral glands with formation of crypt abscesses. 250x
Figure 3. Progression of histologic changes from acute to chronic cutaneous GVHD

A. Screening skin biopsy, day 85. A focal apoptotic body formation is present at the tips of rete ridges (arrow) with focal surrounding lymphocytic satellitosis. 400x

B. Lichen planus-like chronic GVHD, day 426. The thickened epidermis displays orthokeratosis, hyperkeratosis and acanthosis. The striking lichenoid reaction along the damaged basal layer includes a prominent lymphocytic inflammation and infiltration, apoptotic changes, loss of rete ridges and prominence of the superficial vascularity. 100x

C. Progression of GVHD from Panel A into a sclerotic stage day 382. Zone of dense, relatively avascular homogenized collagen has replaced the papillary and upper reticular dermis. 63x

D. High-power view shows a hyperkeratotic epidermis with flattening of rete ridges, vacuolar change, and lymphocytic infiltration along the basal layer with disruption of the epidermal melanin unit with coarse clumps of melanin in the epidermis and incontinent melanin pigment in the sclerotic papillary dermis. 160x

Figure 4. Morpheic GVHD lesion, day 607

A. Low-power shows a thickened dermis with sclerotic widening of the lower reticular and fascia. 20x

B. The epidermis shows activity of graft-versus-host disease with scattered apoptotic bodies. Note that the papillary dermis is not sclerotic, in contrast to the deep dermis and fascia in Panel D below. 400x

C. Syringitis. Eccrine coils are infiltrated by lymphocytes with loss of adjacent fat tissue replaced by fibrous tissue. 200x

D. Interface of the reticular dermis in the fascia shows fasciitis with fibrous thickening of the septa, lymphocytic panniculitis, and formation of lymphoid follicles. 63x
**Figure 5.** Oral Graft-versus-host disease

A. Oral mucosal biopsy, day +75. This view shows lymphocytic infiltrate along the basal layer of the mucosa. 160x

B. High-powered view shows apoptotic changes along the tip of a rete ridge (arrow). 400x

C. Minor salivary gland, day +364. Early lobular involvement shows focal periductal lymphocytic infiltrates with minimal loss of acinar tissue. 63x.

D. High-powered view of intralobular duct with marked lymphocytic infiltration cytoplasmic vacuolization and focal destruction of ductular epithelium. 160x

**Figure 6.** Pulmonary GVHD with obliterative bronchiolitis, lung biopsy, day +194. A small airway shows constriction of the bronchiole lumen by a subepithelial expansion of fibrous tissue. A lymphocytic infiltrate surrounds the outer bronchiole smooth muscle layer. 250x (photo courtesy of Dr. Robert Hackman)
Appendix 1. Considerations for sample acquisition and processing

1) Liver biopsies

   a) Specimen: Percutaneous liver biopsy, laparoscopy-guided, or transjugular liver biopsies are recommendend using the widest gauge compatible with local clinical practice and safety, with total core length of at least 1.5 to 2 cm. The technique used relies largely on the expertise available in individual centers. If GVHD is a diagnostic consideration, transvenous forceps biopsies are less desirable because of the inevitable distortion of architecture. Thin bore needle biopsies crush portal spaces and distort bile ducts: if possible they should be avoided. There should be at least 10 portal areas available for evaluation. Smaller biopsies are recommended to be interpreted with caution.

   b) Processing and staining: Using the shorter 2-hour processing schedule, especially with formalin fixed biopsies results in less shrinkage and improved histology. If a portion of the biopsy is to be frozen, this procedure should also be done as soon as possible after the biopsy is performed. If a rapidly progressing viral infection is clinically suspected, a portion should be sent for microbial detection (e.g., PCR, culture). The clinician should advise the pathologist regarding priorities for special studies.

   Recommended routine and special stains include H&E, a connective tissue stain (e.g. Masson trichrome), PAS with and without diastase, reticulin for evaluation of liver architecture, and iron. As needed, keratin 7 or 19 may be used for examination of biliary epithelium. If indicated, copper may be used for evaluation of chronic cholestatic changes, Hall’s stain for bile, hepatitis B surface and core antigen, ubiquitin or P62 for evaluation of Mallory bodies in steatohepatitis, methenamine silver, Kinyoun acid fast, stains, immunostains for Adenovirus, VZV, HSV Hepatitis B surface and core antigen, CMV, lymphoid and/or myeloid antibodies, EBER and LMP for EBV.

2) Gastrointestinal biopsies
a) Specimen: A variety of institution-dependent sampling strategies have been used: gastric antrum vs. fundus vs. duodenum. Discordance between clinical severity, endoscopic observations and biopsy findings may be observed. The most severely affected areas may not be sampled, particularly in the lower GI tract. It is important for the endoscopist to record whether the biopsy represents a localized or diffuse process.

b) Processing and staining: Endoscopic biopsies must be properly oriented and placed directly into fixative. At least 8-10 serial sections, recommended 16-20 stained with H&E should be obtained in order to detect minimal criteria changes for the diagnosis of GVHD or rare viral inclusions. Special stains for Helicobacter and viral infections should be obtained as indicated.

3) Skin biopsies

a) Specimen: The gross appearance of the lesions and the clinical context, whether for diagnosis or determining therapeutic response, dictate the type of biopsy. For most purposes, a 4mm full thickness punch biopsy of lesional skin is sufficient. If the patient is not acutely ill, delaying biopsy for a day or two will allow the rash to become better developed and avoid equivocal histologic results (spongiosis and perivascular infiltrate only). It is important to remember that cutaneous lesions are not synchronous. Concurrent biopsies from several different sites may show classical features of chronic GVHD in only one biopsy. Certain types of lesions are more difficult to interpret. If the skin is sclerotic or if fasciitis is suspected, a narrow but deep incisional wedge biopsy is better suited in order to assess deep involvement of the hypodermis and response to treatment. Morpheic lesions also require larger and deeper biopsies to appreciate the focal remodeling of the dermis and changes in the deep fascia. In some situations, combination of skin, oral, and mucosal biopsies obtained will be needed to assess completeness of response.

b) Processing and staining: Routine processing of formalin fixed material with at least 8-10 H&E stained serial sections, and preferably 16-20. Several studies have utilized immunostaining against T cell subsets to define GVHD from other non-GVHD inflammatory dermatidities. In most biopsies
with GVHD, the infiltrate is sparse. Furthermore the phenotypic markers may not indicate a cell’s function. Stains for apoptosis, tunnel and anti-caspase-3 do not label cells that have the diagnostic appearance of apoptosis. At this time, these studies are not considered appropriate for diagnostic use.

4) Oral mucosal and lacrimal biopsies

   a) Specimen: An incisional biopsy (non-ulcerated site to include underlying gland lobules) with 5-10 lobules is recommended. Mucosal and glandular disease may not be synchronous, and the disease may be at various stages of development even in lobules of gland removed at the same time in the same specimen.

   Vulvar mucosal biopsies are often sheared fragments with a high background of non-specific chronic inflammation. Properly orientation of vulvar or conjunctival mucosal biopsies is needed in order to evaluate the features of GVHD.

   For conjunctival, and lacrimal biopsies, an incisional biopsy (non-ulcerated site to include underlying gland lobules) with 5-10 lobules is recommended. Mucosal and glandular disease may not be synchronous and the disease may be at various stages even in lobules of gland removed at the same time in the same specimen. Conjunctival biopsy from inferotemporal bulbar conjunctiva is recommended. A “snip” biopsy (approximately <3mm) specimen is usually sufficient to check of apoptotic cells in the conjunctival epithelium.

   b) Processing and staining: routine formalin fixation and processing with serial sections.

5) Open lung biopsy

   a) Specimen: Histologic evaluation for pulmonary obliterative bronchiolitis cannot be done with transbronchial biopsies or bronchoalveolar lavage specimens. A diagnostic biopsy of pulmonary chronic GVHD requires evaluation of peripheral lung that contains bronchioles. The lung biopsy, obtained via fiberoptic transthoracic or open thoracotomy approach, should be at least 2 cm in length.
b) Processing and staining: In order to visualize the characteristic concentric or eccentric submucosal collagenous deposits beneath the epithelium that result in partial to complete obliteration of the bronchiole lumen, connective tissue stains for elastica, Verhoeff’s-Van Gieson or Movats are necessary.[96]

References


[70] Milano F, Shulman HM, Guthrie KA, Rifkin I, McDonald GB, Delaney C. Late onset colitis after cord blood transplant is consistent with Graft-Versus-Host Disease: Results of a blinded histopathological review. Biol Blood Marrow Transplant. 2014.


