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# Histopathologic Diagnosis of Chronic Graft-versus-Host Disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report

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## ABSTRACT

**This consensus document provides an update for pathologists and clinicians about the interpretation of biopsy results and use of this information in the management of hematopoietic cell transplantation patients. Optimal sampling and tissue preparation are discussed. Minimal criteria for the diagnosis of graft-versus-host disease (GVHD) are proposed, together with specific requirements for the diagnosis of chronic GVHD. Four final diagnostic categories (no GVHD, possible GVHD, consistent with GVHD, and definite GVHD) reflect the integration of histopathology with clinical, laboratory, and radiographic information. Finally, the Working Group developed a set of worksheets to facilitate communication of clinical information to the interpreting pathologist and to aid in clinicopathologic correlation studies. Forms are available at http://www.asbmt.org/cGvHD_Guidelines. The recommendations of the Working Group represent a consensus opinion supplemented by evaluation of available peer-reviewed literature. Consensus recommendations and suggested data-capture forms should be validated in prospective clinicopathologic studies.**


## KEY WORDS

Chronic graft-versus-host disease • Allogeneic hematopoietic cell transplantation • Consensus diagnosis • Pathology

*The opinions expressed here are those of the authors and do not represent the official position of the National Institutes of Health or the US Government.*

## INTRODUCTION

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 1970s [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 [2-7].

Even after 25 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 not possible to distinguish persistent, recurrent, or late acute from chronic GVHD by histologic examination. 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. In retrospective studies, the degree of inflammation or the extent of epithelial damage or apoptosis has not predicted response to treatment in the gastrointestinal tract [3], skin [4], or liver [7,8].

Several factors can influence or cause difficulty in histologic interpretation:

- Potent immunosuppressive treatment blunts the inflammatory response, one of the key indicators of activity.
- Infections and drug reactions can mimic chronic GVHD.
- Histologic characteristics change over time. Residual destruction of epithelia or glandular structures and irreversible fibrosis pose problems in separating old damage from ongoing or new activity.
- Sampling and technical factors can cause a false-negative histologic assessment of GVHD.
- The timing of biopsies and their relationship to treatment are highly variable.
- The utility of serial biopsies in judging the response to treatment has not been determined.

To conduct clinical trials of chronic GVHD, uniformly applied and interpreted criteria for pathologic diagnosis are needed. 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 to allow corollary histopathologic studies.

## PURPOSE

The purpose of this article is to provide an update for pathologists and clinicians about the interpretation of biopsy results and use of this information in the management of hematopoietic cell transplant (HCT) patients. Specifically, the Working Group sought to (1) define minimal diagnostic criteria for active GVHD in several organ systems, (2) define those features that suggest a specific diagnosis of chronic GVHD, (3) create 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, (6) define criteria for an adequate histologic sample in various organs, and (7) develop comprehensive standardized research histologic data forms for reporting histologic changes. 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 according to the results of prospective validation studies.

## SUMMARY OF RECOMMENDATIONS

- Specific recommendations for sample acquisition, assessment of adequacy of serial sections, and interpretation are found in the text and Appendix 1.
- Minimal diagnostic criteria for active GVHD and features suggesting a specific diagnosis of chronic GVHD are recommended.
- All pathology reports should report both histologic features and a final diagnosis. The final diagnosis integrates the histopathologic results and the clinical context and is summarized in 1 of 4 categories: no GVHD, possible GVHD, consistent with GVHD (equivalent to "favor," "suggestive of," or "probable"), or definite, unequivocal GVHD.
- Clinical information forms are provided for each organ site. These forms are intended to improve the flow of relevant clinical information between clinicians and pathologists. With institutional review board approval, these forms can also be used for research data collection in chronic GVHD protocols.

## RATIONALE FOR OBTAINING BIOPSY SAMPLES

Chronic GVHD has a prevalence of ≥50% in long-term survivors after HCT. As detailed in the diagnosis and scoring article [9], biopsies are recommended to confirm active chronic GVHD in situations in which 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, histopathologic analysis should be viewed as essential for establishing activity, especially if there are any atypical clinical features, confounding infections, or potential

drug toxicity. Failure to obtain biopsies can result in erroneous treatment. Jacobsohn et al. [10] 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.

Although biopsy can be of enormous 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, because asymptomatic patients with positive screening biopsy results are not considered to have chronic GVHD.

## LIMITATIONS OF DIAGNOSING GVHD BY HISTOPATHOLOGIC EXAMINATION

Whereas histologic features are purely descriptive, histologic interpretation requires additional consideration of the clinical context and the use of Bayesian logic [11]. Histopathology represents a snapshot in time of a complex and dynamic biologic process that reflects the duration of activity, use of immunosuppressive therapy, the possibility of more than 1 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. The negative predictive value of a biopsy, however, is less than the positive predictive value [12]. As criteria for the minimal diagnostic threshold become more stringent, the sensitivity of the biopsy to detect GVHD will decrease. As a result, histologic examination may not always be the gold standard for the diagnosis of GVHD. Pathologists who are reluctant to diagnose GVHD in biopsy samples without florid abnormalities should understand that the decision to treat GVHD is based not according to the histologic gold standard of a positive biopsy sample, but according to the overall clinical assessment (see the gastrointestinal section, below). In research studies, patients can be stratified for analysis according to the presence or absence of histologic changes.

Several factors can result in a false-negative histologic assessment of GVHD. Biopsies performed immediately after the onset of symptoms and signs of presumptive GVHD may be falsely negative. 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 cytologic features. Glass slides containing only a limited number of serial sections may be insufficient for the detection of focal minimal changes. GVHD may be of mild intensity or may be partially or fully suppressed by immunosuppression. In such cases, it is difficult to demonstrate that precise minimal diagnostic criteria are uniformly applied. An ongoing study of acute GVHD of the skin between dermatopathologists at the University of Washington and pathologists Fred Hutchinson bears this out (D. Myerson, Fred Hutchinson Cancer Research Center, Seattle, WA, personal communication, 2005). Conversely, 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 AND CHRONIC 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. When confronted with diagnostic ambiguity, pathologists respond by integrating the degree of the histologic changes with available clinical details to assign some degree of certainty. The qualifying phrases reflect both institutional and idiosyncratic preferences: "possible," "probable," "consistent with," "suggestive" or "favor." These are often coupled with additional terms reflecting the extent of the changes: ie, "slight," "minimal," "mild," "focal," or "marked," which convey a sense of whether the changes are borderline or dramatic. Thus, criteria that suggest a diagnosis of GVHD or, more specifically, chronic GVHD are guidelines that may change with better knowledge of the clinical data and after the clinicopathologic results are known. Examples of the inexact minimal criteria for GVHD in a biopsy sample include the number of apoptotic bodies required in a skin, oral mucosal, or gastric biopsy sample to diagnose GVHD; the number of hematoxylin and eosin (H&E) serial sections necessary before concluding that epithelial apoptosis is absent; the need for apoptosis when lymphocytic exocytosis is present in a skin or mucosal biopsy sample taken immediately after the onset of symptoms; the amount and location of inflammation in the minor salivary glands required for the diagnosis; and the extent or degree of dysmorphic interlobular bile duct changes that qualify portal inflammation as GVHD rather than nonspecific reactive changes or alterations ascribed to hepatitis C virus (HCV) or a hepatotoxic drug.

The pathology committee did not develop or recommend using any previously developed grading schemata. Existing schemata are a composite of activity, gen-

**Table 1.** *Histologic Criteria for GVHD by Organ System*

| Organ or System | Minimal Criteria for Active GVHD* | Specific Criteria for Chronic GVHD† |
|---|---|---|
| Skin, any stage | Apoptoses in epidermal basal layer or lower malphigian layer or outer root sheath of hair follicle or acrosyringium ± lichenoid inflammation ± vacuolar change ± lymphocytic satellitosis | |
| Skin lichen planus–like | | Combination of epidermal orthorkeratosis, hypergranulosis, and acanthosis with lichenoid changes ± syringitis of eccrine units ± panniculitis |
| Skin sclerotic | | Collagenous deposition with thickening throughout the papillary dermis, or pan-dermal collagenosis ± panniculitis |
| Skin morpheic | | Clinically focal or localized lesion predominated by sclerosis in the lower reticular dermis or along the dermal-hypodermal border ± epidermal and appendigeal involvement |
| Skin fasciitis | | Fibrous thickening of fascial septa with adjacent inflammation ± panniculitis |
| Liver | Global assessment of dysmorphic or destroyed small bile ducts ± cholestasis, lobular and/or portal inflammation | Ductopenia, portal fibrosis, and chronic cholestasis reflect chronicity but are not specific for chronic GVHD |
| Gastrointestinal | Variable apoptotic criteria (≥1/piece) in crypts | Destruction of glands, ulceration, or submucosal fibrosis refelects long-standing disease but are not specific for chronic GVHD |
| Oral mucosa and conjunctiva | Lymphocytic infiltration of mucosa with variable apoptosis‡ | |
| Minor salivary or lacrimal gland | | Infiltration and damaged intralobular ducts, fibroplasia in periductal stroma, and inflammation with destruction of acinar tissue§ |
| Lung | | Obliterative bronchiolitis: dense eosinophilic scarring beneath the respiratory epithelium, resulting in complete fibrous obliteration or some degree of luminal narrowing‖ |

*Conditions that result in lesser degrees of change include immunosuppressive treatment, biopsy very soon after the 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 after 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 chemoirradiation or prior inflammation. The distinction between acute and chronic GVHD requires the addition of distinctive oral manifestations [9].

§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, indicates chronic GVHD activity.

‖Obliterative bronchiolitis [48] should be distinguished from bronchiolitis obliterans–organizing pneumonia [49], which is also associated with GVHD but has a different clinicopathologic presentation and a more favorable outcome.

erally assessed by inflammation or apoptosis, and accumulated damage or destruction, which reflects duration (stage). Some schemata were developed without testing data against useful clinical end points, and all are strongly affected by the degree of immunosuppressive treatment for GVHD. The sections below summarize consensus opinions.

## Liver

The 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 consideration of confounding causes of liver disease from infection or drug injury. Characteristic bile duct changes may be absent or may affect only a minority of portal spaces if the liver biopsy is performed soon after the onset of liver dysfunction related to GVHD [13]. The duration of active liver GVHD, the anti-inflammatory effects of immunosuppression, and the anticholestatic influence of ursodeoxycholic acid affect these alterations.

There is no clear dichotomy between acute and chronic GVHD in the liver. Long-term persistence of GVHD increases the amount of portal fibrosis [13]. Patients with acute hepatitis 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 [8,14]. Liver biopsy samples vary greatly in the number of portal spaces that can be evaluated. The greater the number, the more accurate the overall assessment. The minimum diagnostic threshold

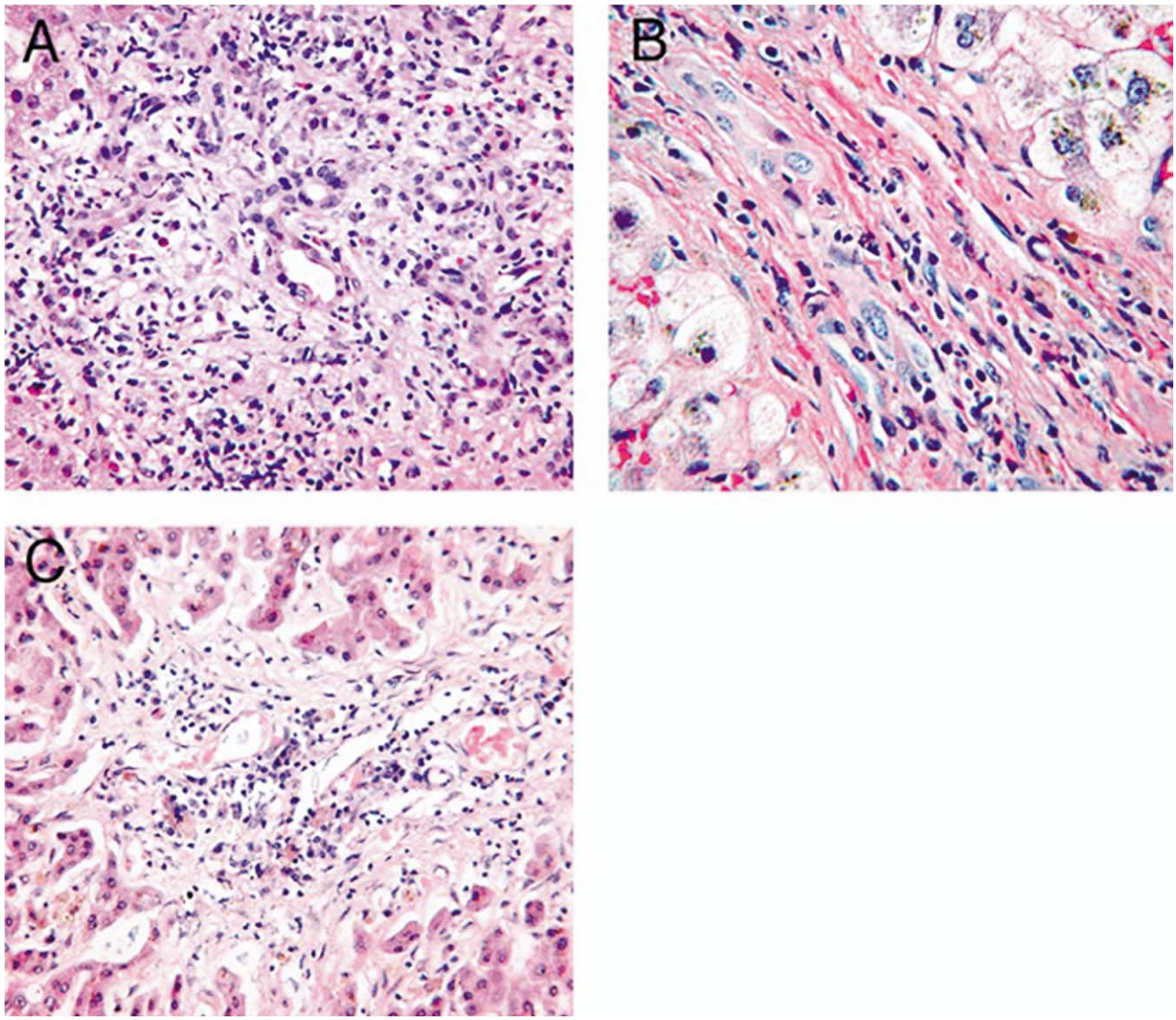



**Figure 1.** Hepatic GVHD. A, Late onset of acute GVHD, day 123. The expanded portal space contains a mixed infiltrate of lymphocytes and scattered eosinophils. The interlobular bile duct shows destructive changes of GVHD with infiltration by lymphocytes, segmental loss of nuclei, cytoplasmic vacuolization,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

relies on a global assessment of characteristic withered interlobular bile ducts with nuclear and cytoplasmic alterations, with or without lymphocytic ductitis (Figure 1). Although these changes may not be present in every portal space, they should be representative of the overall picture. Unlike some other epithelia, apoptotic bile duct changes are infrequent.

Lymphocytic infiltration, nuclear and cytoplasmic alterations, and proliferative ductular reactions along the margins of portal spaces, previously described as bile ductule proliferation (see Web link; liver worksheet), are similar to those in affected interlobular bile ducts. The ductular reaction seems to represent both a reparative effort and a secondary target of GVHD. Data are insufficient to determine whether quantitative immunohistologic assessment of bile duct loss by staining for cytokeratins 7 or 19 or replicative senescence by P21 staining provides additional information above and beyond the usual histologic evaluation [15].

Refractoriness or a delay in starting treatment is associated with greater loss of bile ducts and a longer time to recovery [8]. Refractory GVHD in the liver usually produces a picture of chronic cholestasis with ductopenia and, rarely, a picture of bile ductular prolif-

eration (reaction) with or without bridging fibrosis [6,8,16]. In young pediatric patients with chronic liver disorders, the developing hepatobiliary tract is especially vulnerable to injury and prone to fibrosis [17].

Although rare cases of cirrhosis have been attributed to chronic GVHD [6,16,18], most were reported before the identification of HCV infection, which occurred in up to 32% of patients who received HCT before the advent of blood-product screening for HCV [19]. Longitudinal studies indicate that nearly all cases of cirrhosis that develop after HCT are caused by chronic HCV [20]. The long-term cumulative incidence of cirrhosis from HCV acquired after HCT is 24% at 20 years [21]. The usual manifestation of HCV is a mild self-limited increase of serum aminotransferases during tapering of immunosuppression [19]. If no other signs of GVHD are present, a liver biopsy may be necessary. Although HCV causes inflammation and reactive bile duct changes [6,13,22], the withered degenerative bile duct changes of GVHD are qualitatively different from those caused by HCV (Figure 1A and B).

Assessment of the response to therapy requires integration of the clinical and pathologic data, especially with liver biopsies, where improvement in liver tests and histology may take 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 1 year [8].

### Gastrointestinal Tract

Upper esophageal webs are a diagnostic clinical feature of chronic GVHD [9]. In contrast, no histologic changes in the gastrointestinal tract are specific for chronic GVHD. Features that reflect the duration of the disease, fibrosis within the lamina propria, crypt loss, lymphocytic-plasmacytic inflammation, colonic Paneth cell metaplasia, or submucosal or serosal fibrosis are sequelae of refractory acute or late acute GVHD [2,3,7,23] (Figure 2).

Consensus as to whether clinical, endoscopic, or histologic data define the gold standard for the diagnosis of gastrointestinal GVHD has not been reached. Large discrepancies can occur between gross endoscopic and histologic findings [24]. Endoscopy can visualize the full extent of the changes, whereas biopsy is subject to the vagaries of sampling and histologic preparation. When a gut biopsy sample fails to show features of GVHD, the probability of GVHD remains high in patients at risk who have typical signs and symptoms and gross endoscopic appearance (mucosal edema) or ultrasound evidence of mucosal edema, provided that all studies for infection are negative. After an extended discussion of this issue between clinicians and pathologists, it was recommended that patients with only clinical evidence of gut GVHD could be eligible for clinical trials, but that

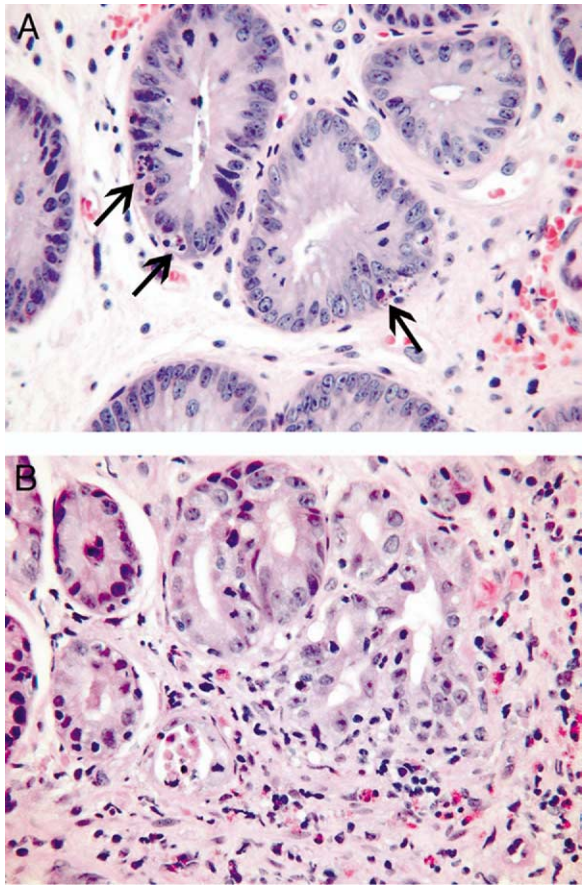
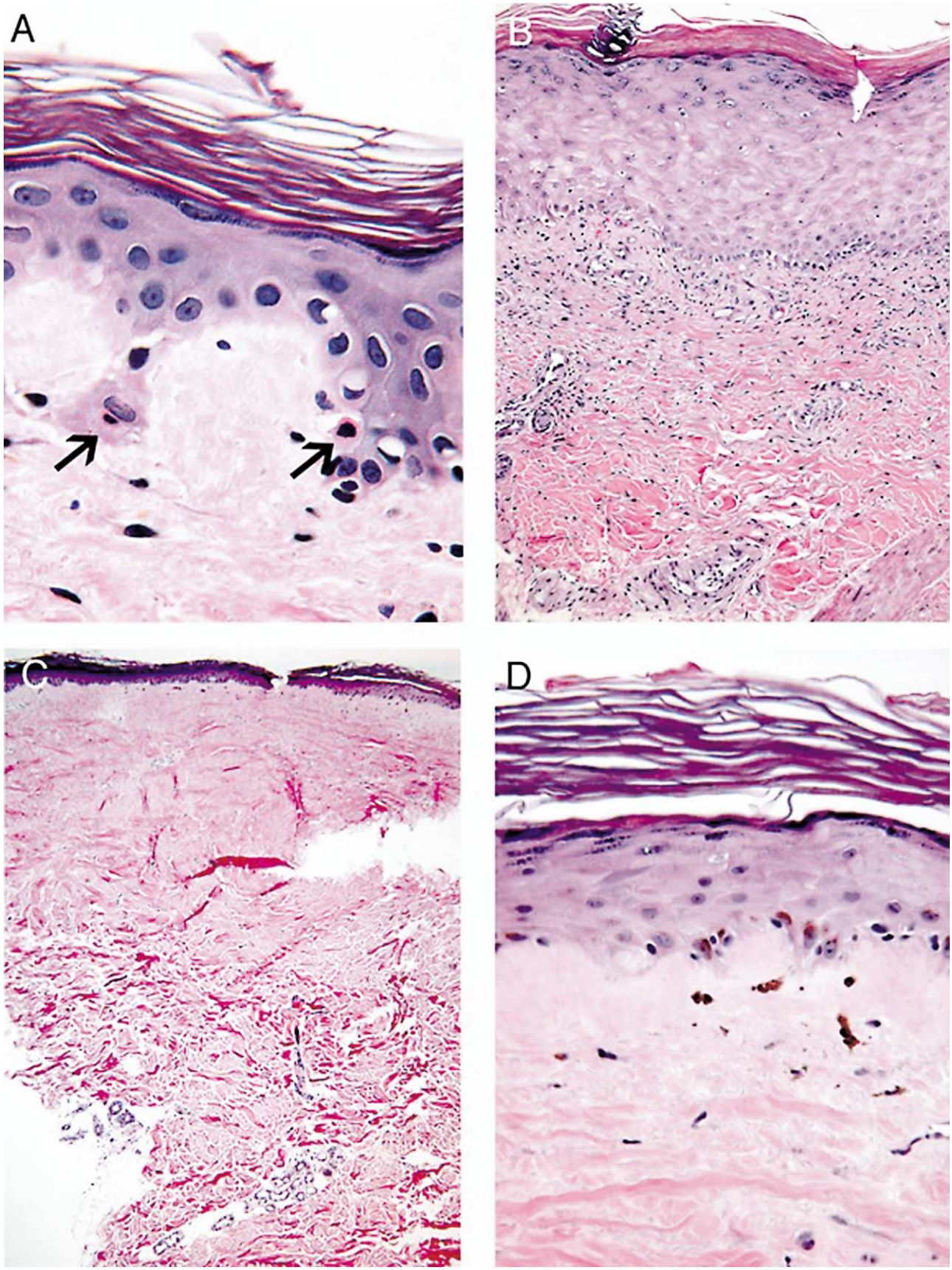



**Figure 2.** Gastrointestinal GVHD. A, Persistent GVHD in the colon, day 87. The colonic biopsy specimen has numerous contiguous apoptotic changes (arrows; original magnification, ×250). B, Late acute GVHD in the stomach, day 133. The biopsy sample shows pronounced lymphocytic and prominent eosinophilic infiltration with destruction of gastric antral glands and formation of crypt abscesses (original magnification, ×250).

**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 (original magnification, ×400). B, Lichen planus–like chronic GVHD, day 426. The thickened epidermis displays orthokeratosis, hypergranulosis, 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 (original magnification, ×100). C, Progression of GVHD from panel A into a sclerotic stage, day 382. A zone of dense, relatively avascular homogenized collagen has replaced the papillary and upper reticular dermis (original magnification, ×63). D, High-power view shows a hyperkeratotic epidermis with flattening of the rete ridges, vacuolar changes, and lymphocytic infiltration along the basal layer, with disruption of the epidermal melanin unit and with coarse clumps of melanin in the epidermis and incontinent melanin pigment in the sclerotic papillary dermis (original magnification, ×160).


A
B
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D

the analysis should be stratified according to presence or absence of histopathologic findings of GVHD.

The histologic threshold for a minimal diagnosis of GVHD in endoscopic biopsy samples was subject to some differences of opinion regarding any apoptosis versus apoptosis in each piece. The histologic diagnostic threshold ranged from rare isolated apoptotic enterocytes to extensive lymphocytic infiltration with glandular or crypt destruction and crypt abscesses filled with apoptotic debris (apoptotic crypt abscess; Figure 2A). Eosinophils contribute to the injury, with variable scant to heavy infiltration and destruction of glands with crypt abscess formation (Figure 2B) [25]. Focally enhanced gastritis, characterized by small collections of lymphocytes, histiocytes with or without neutrophils surrounding small groups of foveolae or gastric glands, is found in GVHD and inflammatory bowel disease [26]. There was some disagreement about whether focally enhanced gastritis should be regarded as "consistent with GVHD" in the setting of HCT.

In the absence of confounding features (drug reaction or infections), several variations of minimum criteria thresholds requiring apoptotic enterocytes (excluding those on the surface) were suggested for a diagnosis of "consistent with GVHD": at least 1 apoptotic body per biopsy piece (University of Michigan), the total number of apoptotic bodies at least to equal the number of pieces (Fred Hutchinson Cancer Research Center), or scattered apoptotic bodies in more than 1 crypt (M.D. Anderson). None of these variations specifies the number of serial sections that should be cut and examined. Although this issue has not been formally studied, clearly false-negative conclusions may result from examination of too few serial sections. Because GVHD may have a patchy distribution with variable evidence of apoptosis, at least 8, and up to 20, serial sections should be analyzed to avoid missing infrequent apoptotic changes or cytomegalovirus (CMV) viral inclusions. The use of apoptotic stains (eg, caspase 3) is neither recommended nor proven to be more useful than good-quality H&E serial sections.

The minimal diagnostic criteria are also subject to false-positive interpretation, because apoptosis in the gut epithelium is not limited to GVHD. Cryptosporidia and heavy CMV infection of the gut mucosa are well-known causes of apoptosis [3,27]. Immunohistologic examination for CMV may be needed in selected cases to distinguish CMV from GVHD. Extensive apoptosis in the absence of identifiable CMV antigens by immunohistology is most likely to represent GVHD. Focal colonic ulcerations with marked apoptosis and acute and chronic inflammation coupled with normal mucosal biopsy specimens from sites distant from the lesions should raise the possibility of colitis due to mycophenolate mofetil exposure [28]. Apoptosis has also been reported with the use of proton pump inhibitors used to treat gastric disorders [29]. Small numbers of apoptotic changes in esophageal mucosal biopsy specimens can arise from a variety of chronic inflammatory conditions. Unless there is a combination of lichenoid interface changes with apoptosis along the basilar portion of the mucosa, the diagnosis of esophageal GVHD should be made with some qualification (see "Standardized reporting of GVHD in the 'Final Diagnosis'").

With the exception of diffuse ulceration or ulcerated stenotic segments verified by endoscopic or radiographic evaluations [30], it is unclear whether there is any additional prognostic value in the Lerner et al. [31] histologic grading scheme for acute GVHD above and beyond clinical parameters. Some centers use the histologic grading scheme routinely, whereas others do not. Any proposed grading or staging scheme for gut GVHD should be a clinicopathologic composite score that reflects the overall extent of symptoms and damage seen by endoscopy and imaging studies, together with the degree of histologic mucosal destruction or fibrosis.

### Skin

The minimal histologic criteria for active GVHD require apoptosis within the basilar or lower spinosum layers of the epidermis (Figure 3A) [2,5,31]. The archetypical features of both acute and chronic GVHD are superficial interface dermatitis either (1) with a lichenoid pattern with lymphocytic inflammation with or without lymphocyte satellitosis or (2) with predominately vacuolar change in the basilar layer [4,5]. As a note of caution, because no single histologic feature is pathognomonic of GVHD, the major overall inflammatory reaction pattern should be factored into the final interpretation [32]. Thus exuberant superficial spongiotic dermatitis with marked spongiosis (intraepidermal edema) and lymphocytic infiltration into the epidermis with only a rare apoptotic keratinocyte is much more likely to represent an allergic reaction than GVHD. The inconstant finding of lymphocyte satellitosis (lymphocytes surrounding an apoptotic keratinocyte in the epidermis or appendages) provides evidence that the dermatitis is caused by GVHD.

The histologic manifestations of chronic cutaneous GVHD evolve over time, are modified by treatment, and to some extent overlap with those of acute GVHD. 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]) refer to a specific constellation with epidermal thickening by acanthosis (hyperplasia) with orthokeratosis (stratum corneum) and parakeratosis, hypergranulosis, a bandlike infiltrate along the dermal-epidermal junction, extensive apoptosis and vacuolization of basilar keratinocytes, sawtoothed (short blunted) rete ridges, and inflammation around the dermal adnexa. This constellation, especially when accompanied by lymphoplasmacytic inflammation

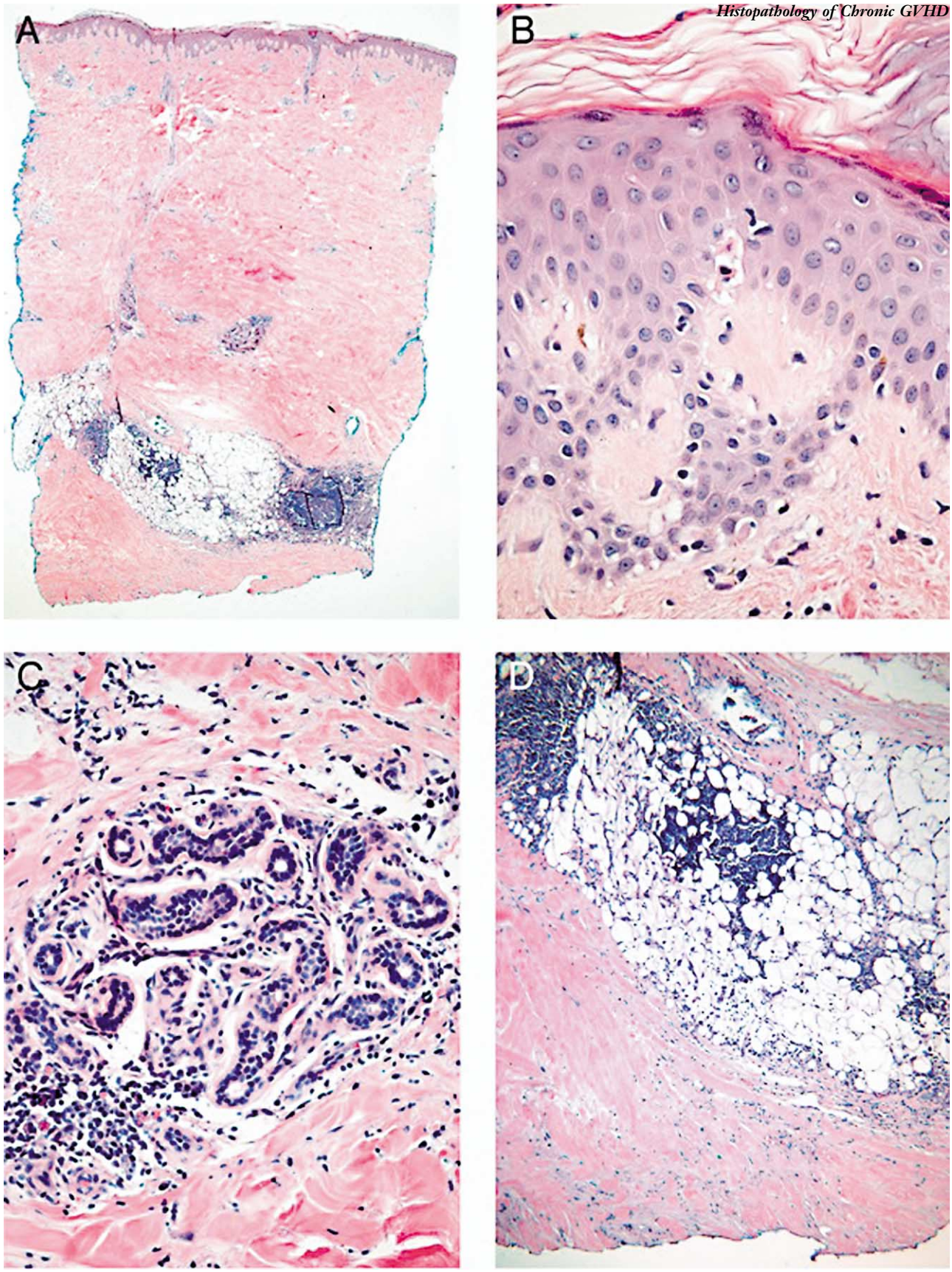


**Figure 4.** Morpheic GVHD lesion, day 607. A, Low power shows a thickened dermis with sclerotic widening of the lower reticular dermis and fascia (original magnification, ×20). B, The epidermis shows activity of GVHD with scattered apoptotic bodies. Note that the papillary dermis is not sclerotic, in contrast to the deep dermis and fascia in panel D (original magnification, ×400). C, Syringitis: eccrine coils are infiltrated by lymphocytes with a loss of adjacent fat tissue replaced by fibrous tissue (original magnification, ×200). D, Interface of the reticular dermis and the fascia shows fasciitis with fibrous thickening of the septa, lymphocytic panniculitis, and formation of lymphoid follicles (original magnification, ×63).

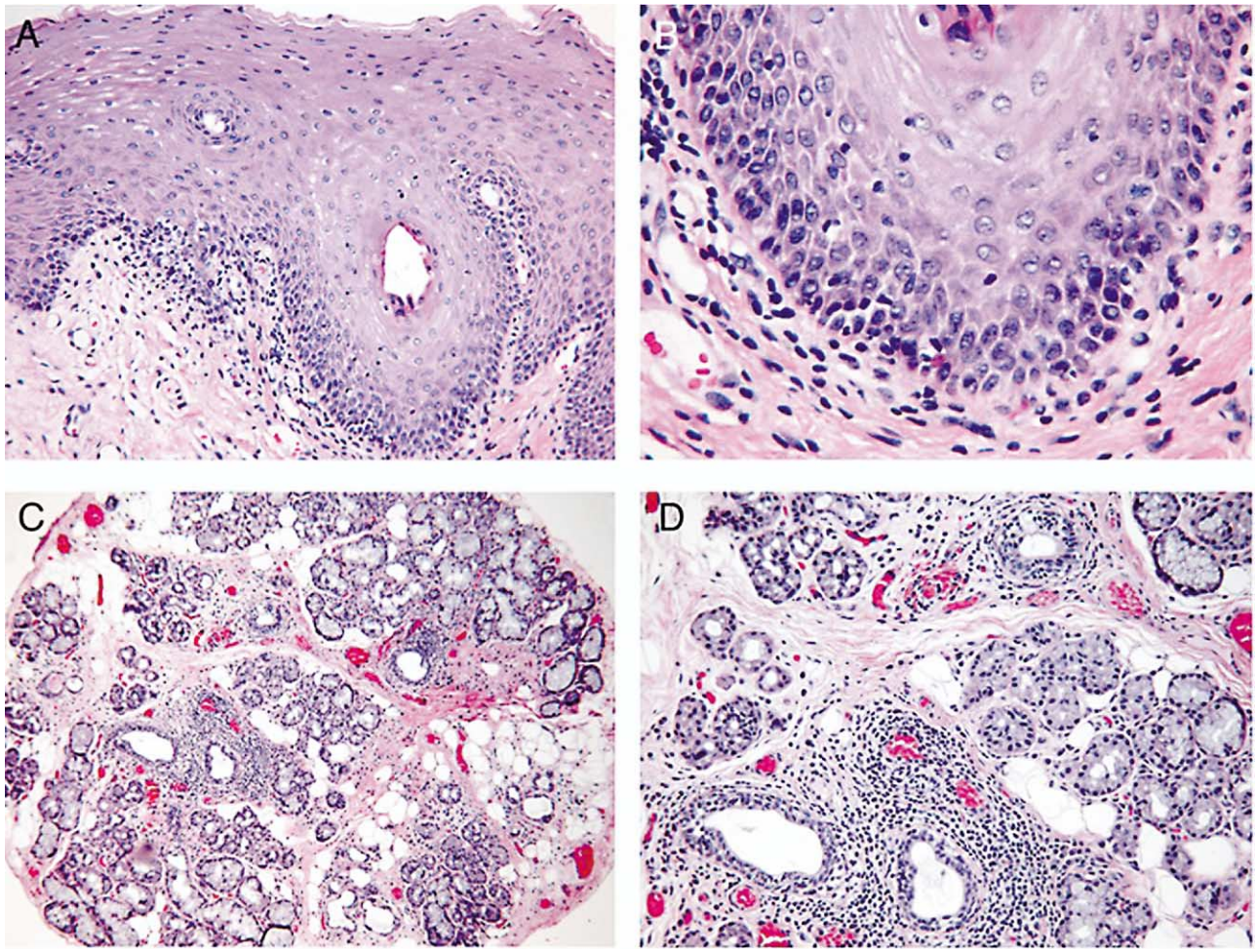


**Figure 5.** Oral GVHD. A, Oral mucosal biopsy sample, day 75. This view shows lymphocytic infiltrate along the basal layer of the mucosa (original magnification, ×160). B, High-powered view shows apoptotic changes along the tip of a rete ridge (arrow; original magnification, ×400). C, Minor salivary gland, day 364. Early lobular involvement shows focal periductal lymphocytic infiltrates with minimal loss of acinar tissue (original magnification, ×63). D, High-powered view of an intralobular duct with marked lymphocytic infiltration, cytoplasmic vacuolization, and focal destruction of the ductular epithelium (original magnification, ×160).

around the eccrine coils, is highly specific for chronic GVHD, but at the expense of reduced sensitivity. Because the skin lesions of chronic GVHD are not synchronous, the presence or absence of chronic GVHD features in a biopsy sample can be influenced by sampling or partial thickness. In practice, members of the dermatopathology subcommittee regarded the combination of epidermal compact orthokeratosis, hypergranulosis, and acanthosis as features that favor or are consistent with chronic lichenoid GVHD (Figure 3). As a note of caution, a lesser degree of this combination of features can occur occasionally in skin biopsy specimens from patients with severe clinical acute GVHD.

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 the 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. In the morpheic variant of chronic cutaneous GVHD, sclerosis may be largely confined to the reticular dermis and underlying fascia with little or no epidermal involvement. Another variant, fasciitis, may show fibrous thickening only in the fascia, with adjacent inflammation but without any epidermal or dermal involvement [33] (Figure 4).

The cutaneous pathology group addressed the changes in chronic GVHD histologic characteristics as related to immunosuppressive treatment. After treatment, skin biopsy samples display a combination of residual damage, with loss of rete ridges and dermal appendages, some increase in papillary or dermal sclerosis, and reduced to absent lichenoid inflammation. The hallmark of an incomplete response indicating ongoing GVHD activity is residual apoptotic changes in the epidermis or appendages. After treatment, the histologic significance of minor residual perivascular lymphocytic inflammation or persistent epidermal vacuolar degener-

ation requires additional 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. The diagnosis and staging committee recommended that keratinocyte apoptosis without other chronic GVHD features in day 80 to 100 screening skin biopsy samples does not indicate chronic GVHD and does not necessarily predict that a flare may follow cessation of immunosuppressive therapy.

The cutaneous subgroup (E.F.) proposed a working definition of marked apoptotic activity in the skin as >5 epidermal apoptotic bodies per section from a 4-mm punch biopsy. Previous attempts to quantify the inflammatory changes of lichen planus–like chronic GVHD did not correlate with the response to therapy, whereas continued histologic signs of activity after treatment increased the likelihood of some irreversible damage to tissues. Clinicopathologic correlation from prospectively obtained data will be used to evaluate these guidelines for assessing activity (see "Standardized reporting of GVHD in the 'Final Diagnosis'").

### Oropharynx, Vulva, and Eye

On the basis of studies of oral labial biopsy samples taken 80 to 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 [34]. Accordingly, lip biopsies to screen for chronic GVHD were rarely performed before day 80. Even so, some patients with aplasia who received only cyclophosphamide before HCT developed typical gross changes of oral chronic GVHD before day 80. A similar situation may also apply to reduced-intensity conditioning.

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 the presence of intralobular, periductal lymphocytes with or without plasma cells and exocytosis of lymphocytes (without neutrophils) into intralobular ducts and acini (Figure 5). A later variation proposed the minimal criteria as >3 mucosal apoptotic bodies and, for salivary changes, a >10% loss of acinar tissue or ductal epithelial cell necrosis [35]. Periductal fibrosis (not generalized interstitial fibrosis) is often present. Horn et al. [36] developed a histologic grading system for chronic GVHD of minor salivary glands based on the degree of lymphocytic infiltration and destruction of glandular acini. This GVHD grading schema and others like it most accurately reflect the stage of the disease.

Persistent salivary dysfunction after treatment of chronic GVHD is related to continuing lymphocytic inflammation and absent recovery of minor salivary secretory units [37]. In children, oncocytic ductal metaplasia may be an additional feature favoring GVHD (T. Morton, University of Washington School of Dentistry, Seattle, WA, personal communication, 2005). Similar findings are commonly seen in oral biopsy specimens of adults older than 40 years 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. The assessment of GVHD activity should focus on lobules that are not completely fibrotic. In these nodules, fibroplasia, acinar and periductal inflammation, and damage to ducts indicate GVHD activity. Finally, 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 [38,39].

The same criteria described previously for oral and esophageal mucosa are used for histologic assessment of chronic GVHD in vulvar [40], conjunctival, and lacrimal biopsy specimens. Histopathologic findings of ocular GVHD have been described in the 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, with loss of acinar lobules replaced by fibrosis. Whereas lacrimal gland biopsy is relatively invasive and may result in decreased functional capacity, conjunctival biopsy samples may be obtained without much risk to the patient. Histologic evaluation of the conjunctiva may aid in the diagnosis and management of ocular GVHD in symptomatic patients with conjunctival disease [41,45,46]. Although the biopsy is not performed routinely, it may be particularly helpful in cases in which ocular GVHD is in question in symptomatic patients who have normal or unchanged Schirmer tests with or without GVHD of other organs. The conjunctival specimen may also be tested by 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 histologic 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 and goblet cell depletion, which are not sufficient for the diagnosis of ocular GVHD [43]. Corneal and conjunctival pseudomembranous histologic findings are clinical manifestations generally associated with an acute pattern of ocular GVHD [45-47].

### Lungs

The pathologic finding of obliterative bronchiolitis is considered to be a diagnostic feature of pulmonary chronic GVHD and resembles chronic lung allograft rejection [48]. Lung biopsy shows unequivocal dense eosinophilic scarring of the bronchioles, resulting in some degree of luminal narrowing (Figure 6). The process begins with inflammation around the small arteries and veins and beneath the respiratory epithelial lining in small airways. Eventually, the submucosal layer is replaced by fibrous tissue, and the lumen is obliterated (also called *constrictive bronchiolitis*). Secondary changes include distal mucostasis or aggregates of foamy macrophages. Inflammation is common but variable and insufficient for diagnosis. The extent and severity of changes should be correlated with functional studies, particularly if only a single affected airway is present in the biopsy specimen. Other causes, such as infection and chronic aspiration, should be excluded [48]. Interstitial lung disease is not considered to be a direct manifestation of GVHD. In the presence of other distinctive organ features and a constellation of suggestive pulmonary function tests and chest computed tomographic scans, a lung biopsy is not necessary to diagnose pulmonary chronic GVHD [9].

Idiopathic bronchiolitis obliterans–organizing pneumonia (BOOP) is associated with both acute and chronic GVHD. BOOP is a 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 [49]. BOOP should be distinguished from obliterative bronchitis because BOOP has a different clinicopathologic presentation and a more favorable outcome.

### Other Sites

Several other sites of chronic GVHD are less commonly involved or subjected to biopsy. 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 perimysial lymphocytic infiltrates to extensive endomysial inflammation with necrosis and regeneration of fibers [1].

Biopsies may be useful in the evaluation of other rare manifestations that may be related to chronic GVHD. These syndromes include nephrotic syndrome and glomerulonephritis, inflammatory neuropathies, and synovitis. Chronic GVHD can cause obliterative coronary artery changes that resemble transplant atherosclerosis.

## STANDARDIZED REPORTING OF GVHD IN THE "FINAL DIAGNOSIS"

With this background, we propose terminology that can be used to qualify the certainty of a histologic diagnosis of GVHD from any particular site (Table 2). 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. For the diagnosis of GVHD, the 4 categories are the following: not GVHD, possible GVHD, consistent with GVHD (synonymous with probable, favor, or suggestive), and GVHD (yes, without equivocation). The pathologist should add these qualifiers, as needed, in the final diagnosis.

Of these categories, the most difficult to define are the qualified categories. A practical example involves the long-standing controversy over whether GVHD of the gut can be diagnosed in the presence of CMV. "Consistent with GVHD" in a gut biopsy sample with unequivocal evidence of CMV would be appropriate if there were abundant apoptotic epithelial changes not associated with CMV-infected cells detected by immunostains, given that the 2 diagnoses are not mutually exclusive. Alternatively, if the pathologist believes that the changes can likely be ascribed only to CMV, then the diagnosis would be "no GVHD or possible GVHD" with a comment. "Consistent with GVHD" applies in the frequent situations in which a single or rare apoptotic change can be found without other accompanying features. "Yes" is used when there is unequivocal GVHD with no further comment needed. The pathologist may choose to use "GVHD with a comment" when the changes are atypical for chronic GVHD, or

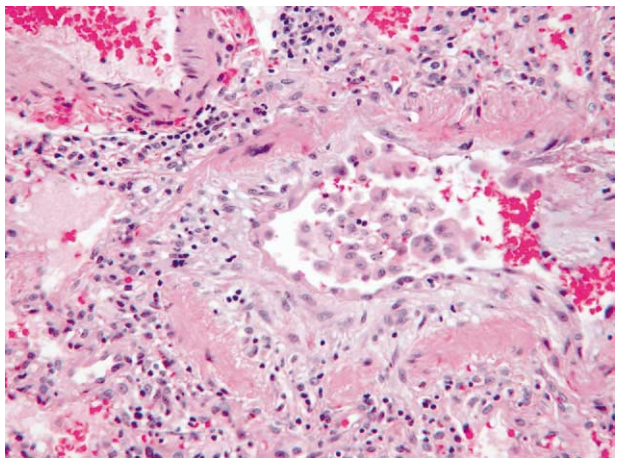

**Figure 6.** Pulmonary GVHD with obliterative bronchiolitis: lung biopsy specimen, day 194. A small airway shows constriction of the bronchiole lumen by a subepithelial expansion of fibrous tissue. A lymphocytic infiltrate surrounds the outer bronchiole smooth muscle layer (original magnification, ×250; photo courtesy of Dr. Robert Hackman, of Fred Hutchinson Cancer Research Center).

**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<br>• Focal colonic ulcers with marked apoptotic cryptitis and destruction of crypts associated with use of MMF<br>• Coinfection with known active viral hepatitis<br>• Clinical features that suggest or favor a drug reaction | Indicates possible alternate diagnoses and reasons for suspicion |
| Consistent with GVHD | Clear evidence of GVHD with mitigating factors | • Unequivocal evidence of CMV yet abundant apoptotic epithelial changes that are not associated with CMV-infected cells by immunostaining<br>• Single or rare apoptotic epithelial changes without other features of active GVHD and no alternative explanations<br>• Limited sample or minimal or focal findings<br>• Recent chemotherapy or radiotherapy | Equivalent to "probable," "favor," or "suggestive of" GVHD |
| GVHD | Unequivocal evidence of GVHD and no further comment necessary | • Inflammation may be minimal despite extensive destruction of the targeted epithelia | If extensive destruction of epithelium, ducts, or crypts or marked inflammation, may note "marked activity," but the features that define "marked" activity have not been prospectively defined and are highly dependent on the degree of secondary immunosuppressive treatment |

MMF indicates mycophenolate mofetil.

"marked" to indicate extensive destruction of epithelium, ducts, or crypts, severe apoptosis of epithelia, or severe inflammation. For example, with the skin worksheet, the guideline recommended by Johns Hopkins (Evan Farmer) for marked epidermal apoptosis is >5 per section.

## DATA COLLECTION AND COMMUNICATION BETWEEN PATHOLOGISTS AND CLINICIANS

Standardized reporting of results can also advance research related to chronic GVHD. In addition to standard information regarding the sample and processing, the Working Group recommends that all pathology reports in which GVHD is questioned include the following information: date of the transplantation or the day after transplantation or after donor lymphocyte infusion, the question that the clinician is asking or the reason for the biopsy, and other clinical information as provided by the clinician. The pathology report should mention of the adequacy of the sample. For small biopsy specimens of skin and for oral and gastrointestinal mucosa biopsy samples, this means preferably 2 slides, each with 8 to 10 serial sections. Within the microscopic description, histologic details should be provided. A final diagnosis that combines the histologic and clinicopathologic interpretation should be provided according to the categories suggested in the relevant sections above.

Standardized methods of data collection can facilitate clinical interpretation, communication between clinicians and pathologists, and prospective data collection for histopathology studies. The Working Group has

**Table 3.** *Forms Recommended for Use by Clinicians and Pathologists*

| Organ | Clinical Information Form | Research Histology Form |
|---|---|---|
| Liver | A | B |
| Gastrointestinal tract | C | D |
| Cutaneous | E | F |
| Oral | G | H |

developed clinical information forms (Table 3) and research histopathology worksheets. Readers can obtain these forms with their respective accompanying clinical information forms by accessing the American Society for Blood and Marrow Transplantation (ASBMT) Web site (http://www.asbmt.org/cGvHD_Guidelines).

## CLINICAL INFORMATION FORMS

Standardized forms that suggest relevant clinical data to accompany a biopsy have been developed. The forms suggest a format for collection of such data as the date and type of transplantation or donor lymphocyte infusion, the reason for the biopsy and the differential diagnosis, patient symptoms, and laboratory or radiology test results (http://www.asbmt.org/cGvHD_Guidelines). In general, these forms allow clear communication with the interpreting pathologist about the patient's clinical situation, potential comorbid conditions that could affect the observed histology, the clinician's differential diagnosis (especially diagnoses that require special testing, such as viral pathogens), and specific questions. The pathologist should form his or her initial histologic impression before integrating all the clinical data.

These forms serve several purposes. First, they may accompany outside consultations to ensure that the interpretation addresses the reasons for the biopsy. In addition, the forms provide demographic transplantation data and contact information for rapid communication. Second, the forms could be used to improve clinical care within institutions by ensuring that pathologists have the relevant clinical data when evaluating a biopsy specimen. Finally, with appropriate institutional review board approval, data collected on these forms could be used in histopathology research studies, in which standardized clinical and pathologic data are important.

We recognize many potential barriers to greater use of these forms. It may be difficult in a busy clinical setting, hospital, or outpatient procedure room to locate and complete these forms. To improve access, these forms may be printed directly from the Web site for use. Some institutions also require any forms used for clinical care to be approved by a forms committee appointed by the institution. However, these forms could be appended to a standard pathology requisition sheet to facilitate communication of complete information while minimizing interference with hospital documentation requirements.

A sample cover letter requesting clinical information and histologic material for outside consultations is also presented on the ASBMT Web site: http://www.asbmt.org/cGvHD_Guidelines (form 1). All outside consultants should include information indicating how to contact the attending clinician by e-mail, fax, and telephone; the surgical pathology report; and, if possible, the paraffin blocks or several unstained recuts. Properly prepared H&E-stained serial sections remain the primary source for diagnosing GVHD.

## RESEARCH HISTOPATHOLOGY WORKSHEETS

The Working Group also developed standardized data collection research worksheets for liver, gut, skin, and oral/mucosal surface biopsy specimens. These worksheets are available on the ASBMT Web site (http://www.asbmt.org/cGvHD_Guidelines). The worksheets are intended not for daily diagnostic use, but rather for guidance in the evaluation of the biopsy specimens used in clinicopathologic correlative studies when paired with the clinical information forms discussed previously. The research histopathology forms are in pilot testing to determine their suitability as communication tools. For example, study pathologists will score the histologic changes in representative biopsy samples that demonstrate the spectrum of minimal to obvious GVHD, to test their understanding of the features listed and determine the range of observations.

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## APPENDIX 1: CONSIDERATIONS FOR SAMPLE ACQUISITION AND PROCESSING

### Liver Biopsies

*Specimen.* Needle biopsies (either percutaneous or transjugular) are recommended that use the widest gauge compatible with local clinical practice and safety, with a total core length of at least 1.5 to 2 cm. There should be at least 10 portal areas available for evaluation. If GVHD is a diagnostic consideration, then 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.

*Processing and Staining.* Using the shorter 2-hour processing schedule, especially with formalin-fixed biopsy specimens, results in less shrinkage and improved histology. If a portion of the biopsy specimen is to be frozen, then this procedure should also be performed as soon as possible after the biopsy is performed. If a rapidly progressing viral infection is clinically suspected, then a portion should be sent for rapid shell vial centrifugation culture. The clinician should advise the pathologist regarding priorities for special studies.

Recommended routine and special stains include H&E, Masson trichrome, iron, periodic acid–Schiff with and without diastase, and reticulin for evaluation of liver architecture. As needed, cytokeratin 19 (or cytokeratin 7, if 19 is unavailable) may be used for examination of the biliary epithelium. Special stains should be obtained as indicated including copper or Hall's bile stain for cholestasis, ubiquitin, or P62 for evaluation of Mallory bodies in steatohepatitis, methenamine silver, and Kinyoun acid fast stains. Special immunostains for infectious agents include hepatitis B surface and core antigens, adenovirus, herpes simplex, herpes zoster, and CMV. Of note, liver involvement with CMV almost always occurs as part of a systemic infection, often coexisting with GVHD, and by itself rarely results in significant liver dysfunction or icterus [30]. Additional immunostains for striking cellular infiltrations include lymphoid and/or myeloid antibodies, and antigens for the detection of posttransplant lymphoproliferative syndrome, Epstein-Barr-encoded RNA, and latent membrane protein.

### Gastrointestinal Biopsies

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

*Processing and Staining.* Endoscopic biopsy specimens must be properly oriented and placed directly into fixative. At least 8 to 10 serial sections (recommended 16-20) stained with H&E should be obtained to detect minimal criteria changes for the diagnosis of GVHD or rare viral inclusions. Special stains for *Helicobacter* species and viral infections should be obtained as indicated.

### Skin Biopsies

*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 4-mm full-thickness punch biopsy of lesional skin is sufficient. If the patient is not acutely ill, delaying the biopsy for a day or two will allow the rash to become better developed and will avoid equivocal histologic results (spongiosis and perivascular infiltrate only). It is important to remember that cutaneous lesions are not synchronous. Concurrent biopsy specimens from several different sites may show classical features of chronic GVHD in only 1 specimen. Certain types of lesions are more difficult to interpret. If the skin is sclerotic or if fasciitis is suspected, a 6-mm punch is better suited for assessment of deep involvement of the hypodermis and the response to treatment. Morpheic lesions also require larger and deeper biopsy samples to appreciate the focal remodeling of the dermis and changes in the deep fascia. In some situations, a combination of skin, oral, and mucosal biopsies samples will be needed to assess the completeness of the response.

*Processing and Staining.* Routine processing of formalin-fixed material with at least 8 to 10 H&E-stained serial sections, and preferably 16 to 20, is necessary. Several studies have used immunostaining against T-cell subsets to discriminate GVHD from other non-GVHD inflammatory dermatitides. In most biopsy samples with GVHD, the infiltrate is sparse. Furthermore, the phenotypic markers may not indicate a cell's function. Stains for apoptosis, TUNEL, 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.

### Oral Mucosal and Lacrimal Biopsies

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

Vulvar mucosal biopsy specimens are often sheared fragments with a high background of nonspecific chronic inflammation. Proper orientation of vulvar or conjunctival mucosal biopsy samples is needed to evaluate the features of GVHD.

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

*Processing and Staining.* Routine formalin fixation and processing with serial sections are recommended.

### Open Lung Biopsy

*Specimen.* Histologic evaluation for pulmonary obliterative bronchiolitis cannot be performed with transbronchial biopsy or bronchoalveolar lavage specimens. A diagnostic biopsy of pulmonary chronic GVHD requires evaluation of peripheral lung that contains bronchioles. The lung biopsy specimen, obtained via a fiberoptic transthoracic or open thoracotomy approach, should be at least 2 cm long.

*Processing and Staining.* 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, Verehoeff van Gieson, or Movat are necessary [40].

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