9 Persistent Gastrointestinal GVHD: The Application and Utility of Histologic Grading Schemes

Howard M. Shulman, David W. Woolston, and David Myerson

Clinical History

This 59-year-old man with myelodysplastic syndrome underwent an HLA-B mismatched transplant. On day 10, he developed signs of hyperacute GVHD characterized by skin rash, diarrhea, abdominal pain, and abnormal liver function tests. Over the next 7 months, he experienced abdominal pain, diarrhea, and four major episodes of gastrointestinal (GI) bleeding. The 7 endoscopies done between days 18 and 201 displayed dyssynchronous mucosal damage. None of the biopsies had immunohistologic evidence of CMV or adenovirus. Some regions appeared normal or had only minimal apoptotic activity (Figs. 9.1 and 9.2). Other biopsies (Figs. 9.3 and 9.4) displayed a mixed inflammatory infiltrate in the lamina propria including neutrophils and eosinophils [1, 2]. The crypts displayed a combination of regenerative changes as well as more florid apoptotic activity with apoptotic crypt abscess formation and micro-ulceration. Biopsies from the day 59 endoscopy showed the

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

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

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

D. W. Woolston
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
e-mail: dwoolsto@fredhutch.org

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colon had many apoptotic bodies and a few small crypt abscesses (Figs. 9.3 and 9.4). In contrast, the stomach and intestine had only rare apoptosis and signs of past damage with decreased glands, villus atrophy, and regeneration. In the day 96 biopsies, the stomach showed gastric vascular ectasia (GAVE) (Figs. 9.5 and 9.6) and focally enhanced gastritis (FEG) (Fig. 9.7) [3]. The jejunal mucosa had atrophy and regeneration with scattered hemosiderin, and the colonic biopsy had only rare apoptosis. Within the same day 166 biopsies, the ileum and stomach had no apoptotic changes. In contrast, in the colon there were many apoptotic crypt cells adjacent to focal crypt regeneration (Fig. 9.8). The day 201 biopsies (Figs. 9.9, 9.10 and 9.11) showed apoptotic activity in the colon, and the ileum had loss of villi and an absence of Paneth cells [4] (in contrast to Fig. 9.2). The implication of an absence of Paneth cells is discussed further in Chap. 10. Despite treatment with numerous immunosuppressive agents—budesonide and beclomethasone, oral high-dose steroids, infliximab, mesenchymal stem cells, lithium (Li^+), tacrolimus, and MMF—the patient's gut GVHD persisted. Over several months he had multiple continuing infections with bacterial, viral, and fungal organisms and succumbed at 7 months of a parainfluenza pneumonia.

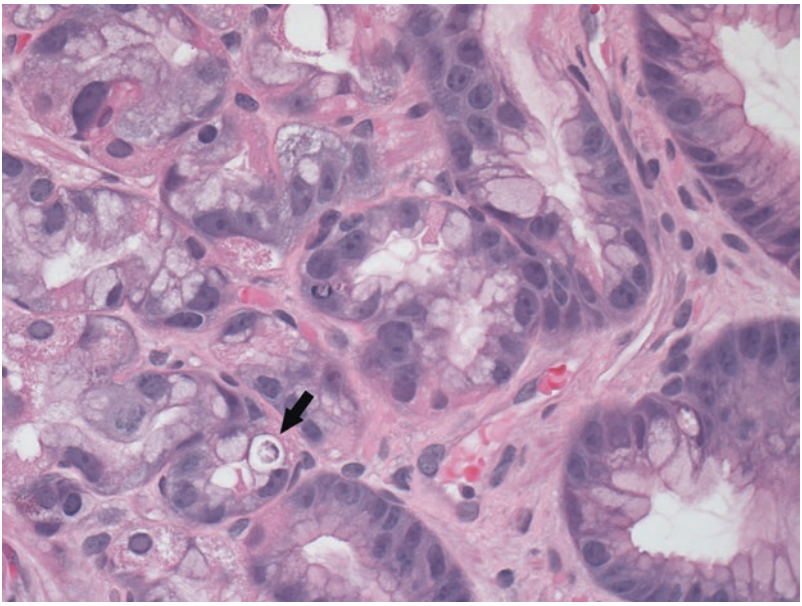

Fig. 9.1 Gastric antral biopsy from day 25. Adjacent to the normal glands are focal apoptotic bodies (one is indicated by the arrow), average 6 per tissue piece, Lerner-Sale grade I, Myerson grade 3

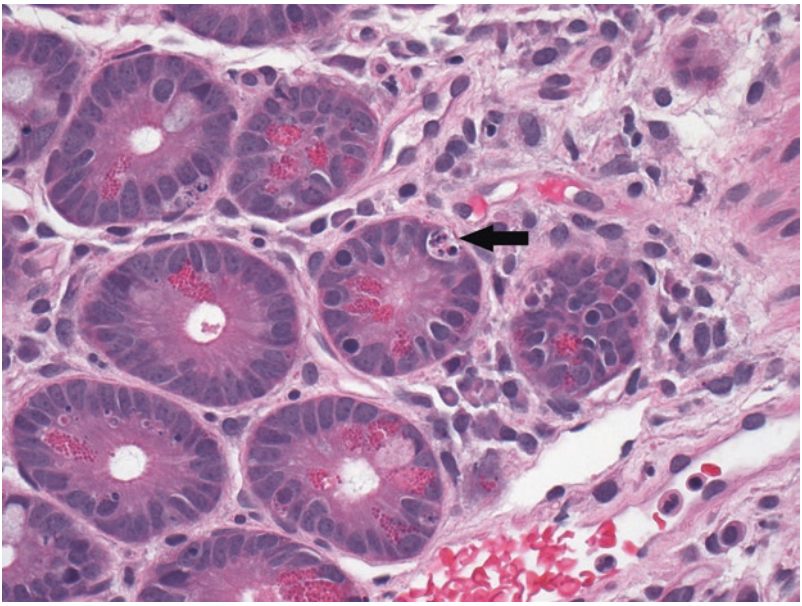

Fig. 9.2 Duodenal biopsy from day 34 demonstrating crypts have many Paneth cells in contrast to Fig. 9.10 below. The arrow denotes an apoptotic body

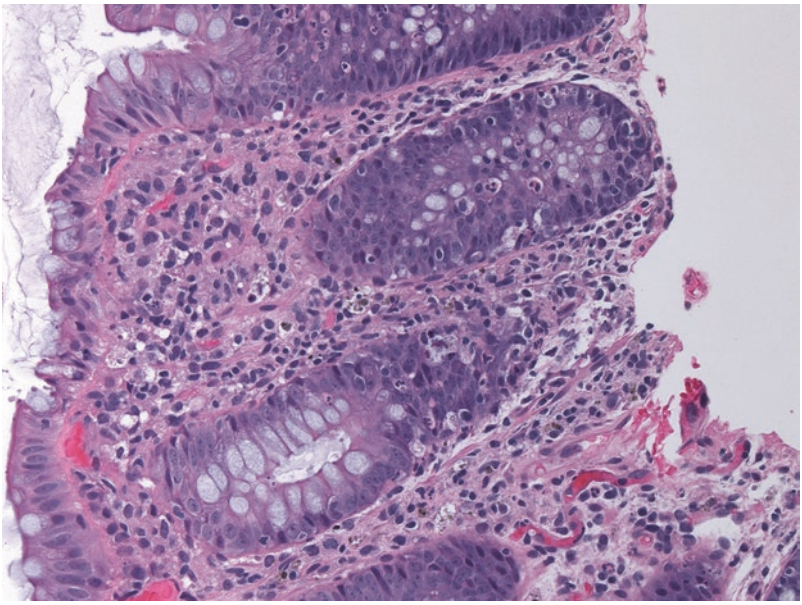

Fig. 9.3 Colon biopsy from day 59 has a marked mixed inflammatory infiltrate in the lamina propria with lymphocytes and many scattered neutrophils infiltrating crypts, neutrophilic cryptitis. The distinction of apoptotic activity from the inflammatory cells is challenging. Because many neutrophils are present, the possibility of concurrent CMV or MMF toxicity was considered, and immunostains for viruses were negative

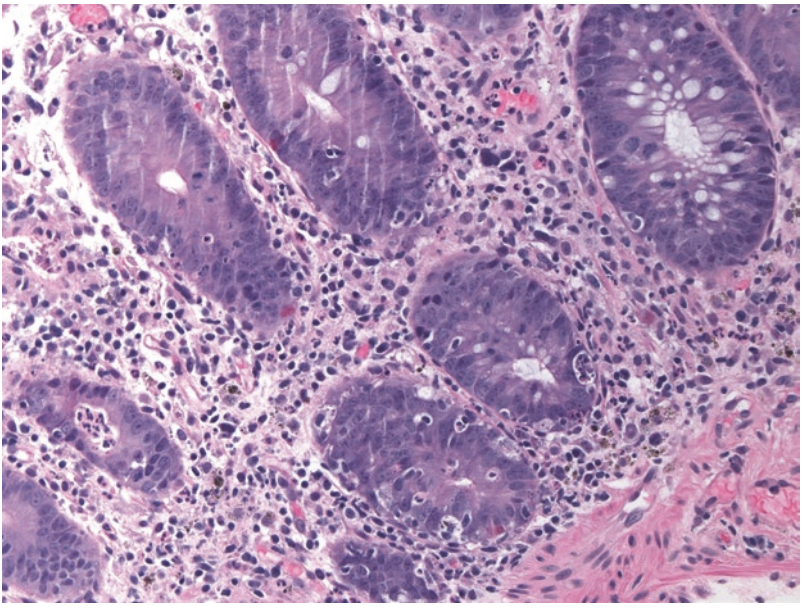

Fig. 9.4 A second view of the same colon biopsy as in Fig. 9.3 shows similar inflammatory infiltrate in the lamina propria, including an early neutrophilic crypt abscess, though less overall apoptotic activity. There is neither apoptotic crypt microabcess as defined by Star et al. [29] nor crypt destruction as defined by Kreft et al. (apoptotic involvement of ≥1/3 of the crypt circumference) [13] (see Chap. 8). This view of the biopsy would be graded as Lerner-Sale grade I and Myerson grade 4

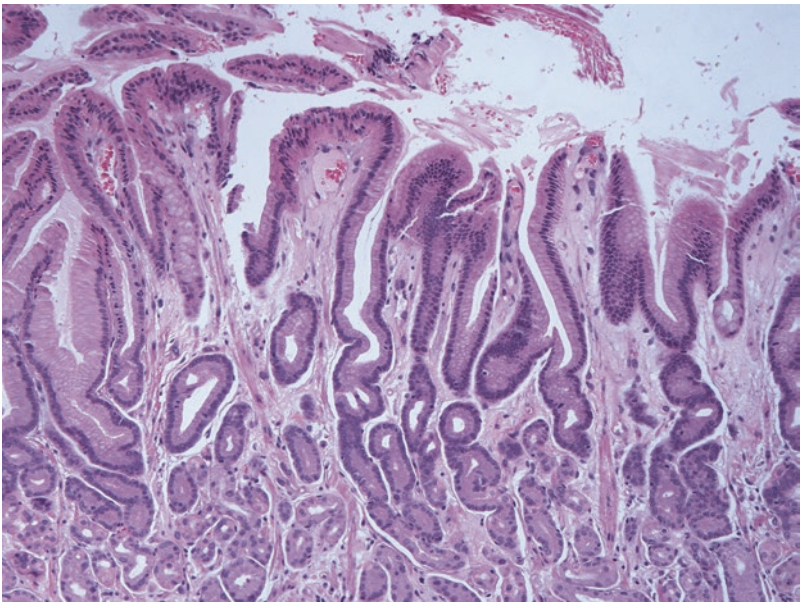

Fig. 9.5 GAVE: Low power of the gastric antrum shows edematous gastric fronds containing dilated capillaries and smooth muscle bundles which extend perpendicular to the muscularis mucosa toward the villus surface

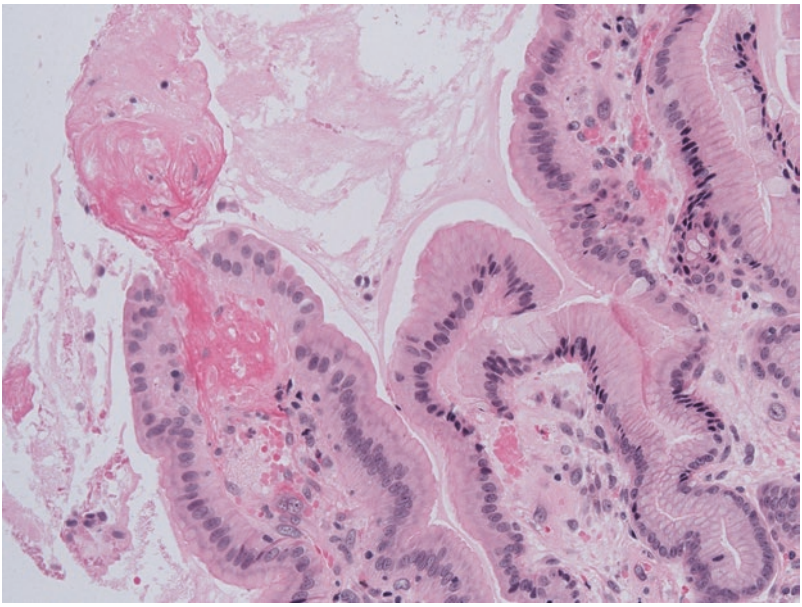

Fig. 9.6 GAVE: Higher power view of another biopsy shows a ruptured dilated capillary with extravasation of red blood cells onto the surface

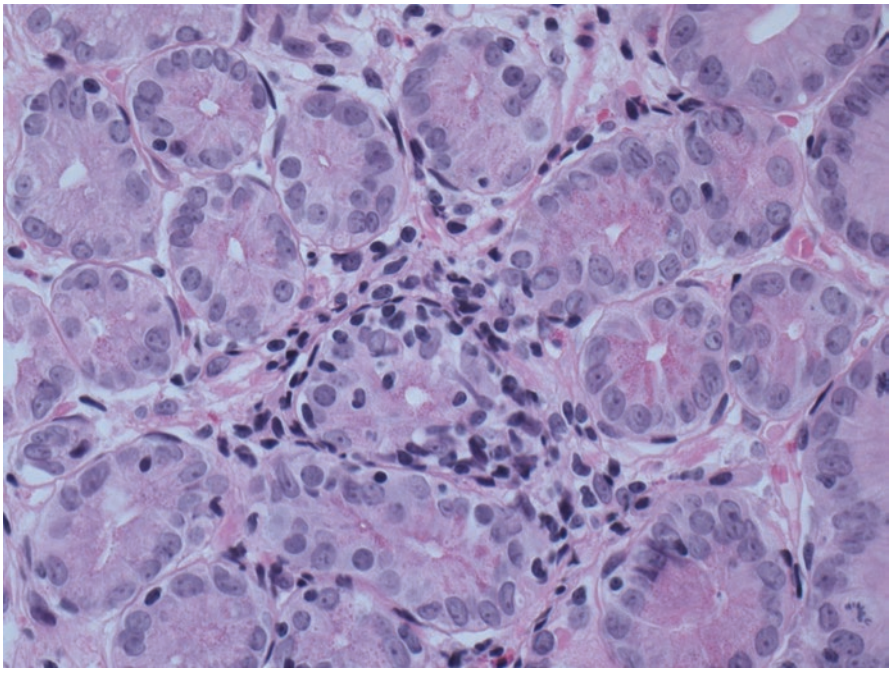

Fig. 9.7 This figure illustrates focally enhanced gastritis (FEG). A cross-sectioned gastric gland is encircled and infiltrated by a lymphoid infiltrate without accompanying apoptotic change. FEG has an increased association with GVHD, but is not a diagnostic indicator for GVHD [3]

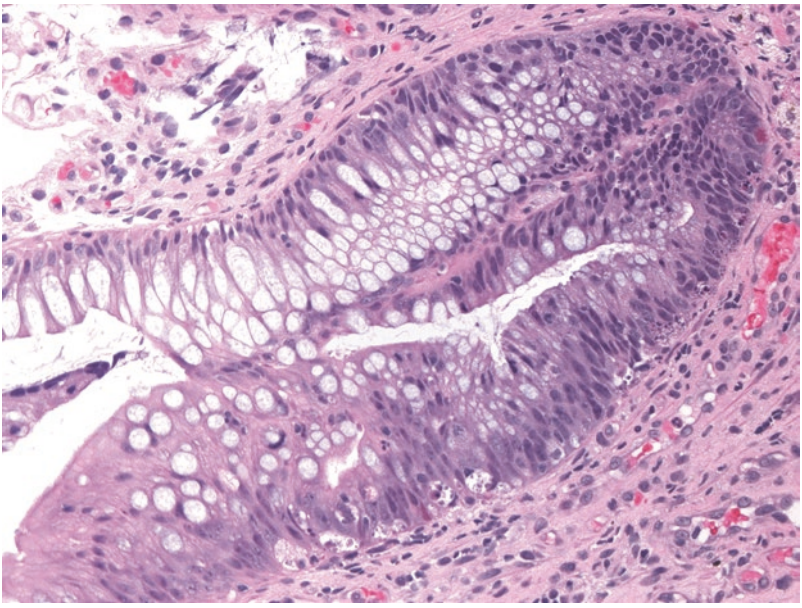

Fig. 9.8 Colonic biopsy from day 166 demonstrates enlarged, irregular regenerative crypts with confluent apoptosis consistent with prior crypt destruction. Lerner-Sale II, Myerson grade 5

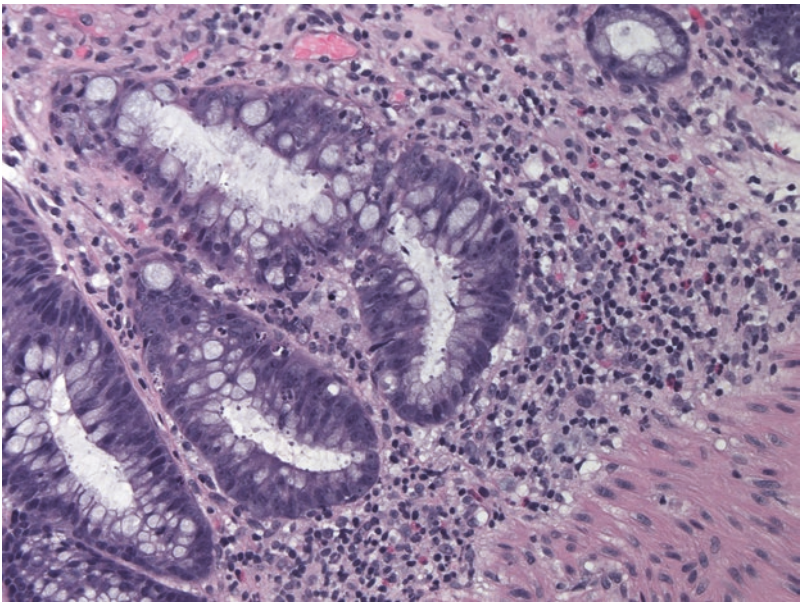

Fig. 9.9 Active colonic GVHD on day 201. The marked inflammatory content in the lamina propria includes a number of eosinophils and neutrophils which are contributory to the cascade of injury caused by GVHD [1, 2]

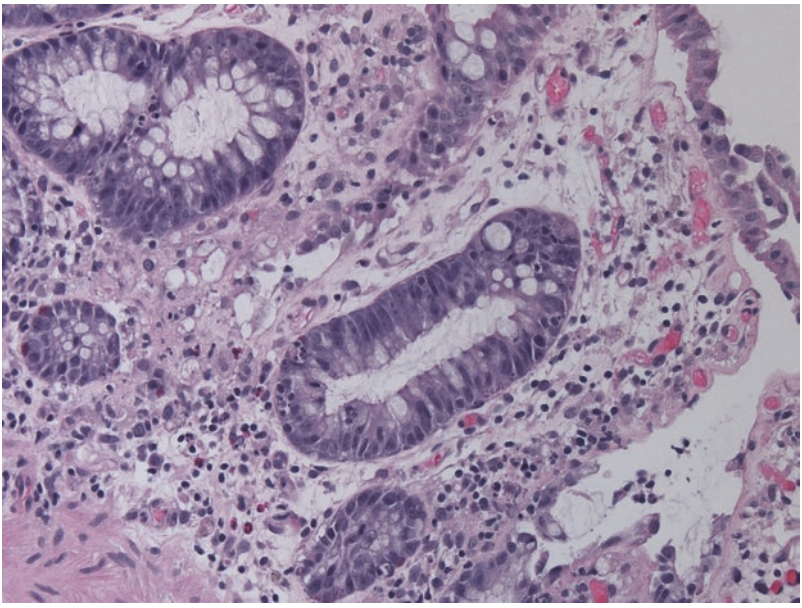

Fig. 9.10 Damaged ileal crypts in the day 201 biopsy have nuclear hyperchromatism, loss of villus architecture, and flattened surface epithelium without goblet cells. Crypts lack Paneth cells (in contrast to Fig. 9.2), a feature associated with a poor outcome [4]. The dropout of crypts is Lerner-Sale grade III, Myerson grade 5

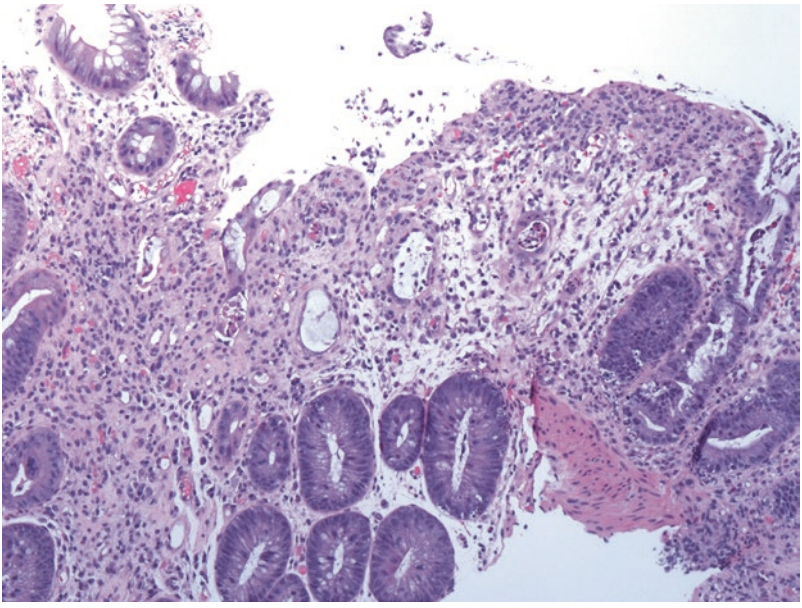

Fig. 9.11 Colonic mucosa on day 201 displays spectrum of severe mucosal damage, from exploding crypts lined by flattened crypt epithelium whose lumina contain apoptotic debris, to crypt destruction with mucosal ulceration. Lerner-Sale grades II–IV, Myerson grade 5

Diagnosis

Persistent refractory GVHD most pronounced in the lower gut

Differential Discussion

"Defining with certainty the futility of further therapy is impossible in patients with severe gut GVHD, but studies indicate that there are no long-term survivors. It is more difficult to gauge the outcome of less severe gut GVHD patients who require interminable therapy" [5].

This patient had persistent gut GVHD that was only partially responsive to the full gamut of IS interventions. His numerous endoscopic biopsies demonstrated patchy distribution and variation in the degree of damage within different portions of the gut. The stereotypic symptoms of GI GVHD reflect the portions involved. When affecting primarily the upper gut, symptoms include nausea, vomiting, and anorexia, while those from the lower gut, including the intestines and colon, symptoms are cramping, secretory diarrhea, abdominal pain, and bleeding. Depending on these symptoms, various publications have opined whether biopsy limited to the colorectal region is sufficient versus sampling of both the upper and lower gut. A recent study with extensive concurrent sampling of the upper and lower gut found that the duodenum, ileum and right colon were the most informative sites for the diagnosis of GVHD [6]. Nonetheless, there are still disagreements about the standard practice for sampling. Different HSCT centers employ different conditioning, post-transplant IS prophylaxis regimens, and clinical algorithms which dictate when or where to perform concurrent upper and lower endoscopic biopsies or just rectal biopsies [5, 7]. Imaging and autopsy studies demonstrate that GVHD has uneven involvement throughout the GI tract and that symptoms may not necessarily indicate the portion of the GI tract involved. For example, in a prospective study of the etiology of diarrhea with concurrent upper and lower biopsies, the biopsies from the stomach yielded the most positive diagnoses of GVHD [8]. In summary, the more biopsies that are taken from different regions, the greater the likelihood of finding diagnostic features of GVHD. The index case also emphasizes a reason for repeating a biopsy after treatment failure to rule out coexisting infectious agents.

Prompt diagnosis and initiation of therapy are essential to gaining control of GVHD before treatment-resistant mucosal sloughing develops. However, previous histologic studies based on the Lerner-Sale modified grading scheme described below [9, 10] had shown it to be ineffective at predicting survival or steroid resistance at the early stages of GVHD, whereas endoscopic visualization and clinical data were superior in predicting subsequent clinical course [5, 11, 12]. The histologic grading schemes have categorized the spectrum of gut damage from minimal pathology, i.e., only a few isolated exploding crypts cells found after observing several serial sections versus easily detected widespread changes (Fig. 9.12).

Tables 9.1 and 9.2 outline two different grading schemes of GVHD alterations in the gut, with separate approaches and goals. The initial grading scheme, published

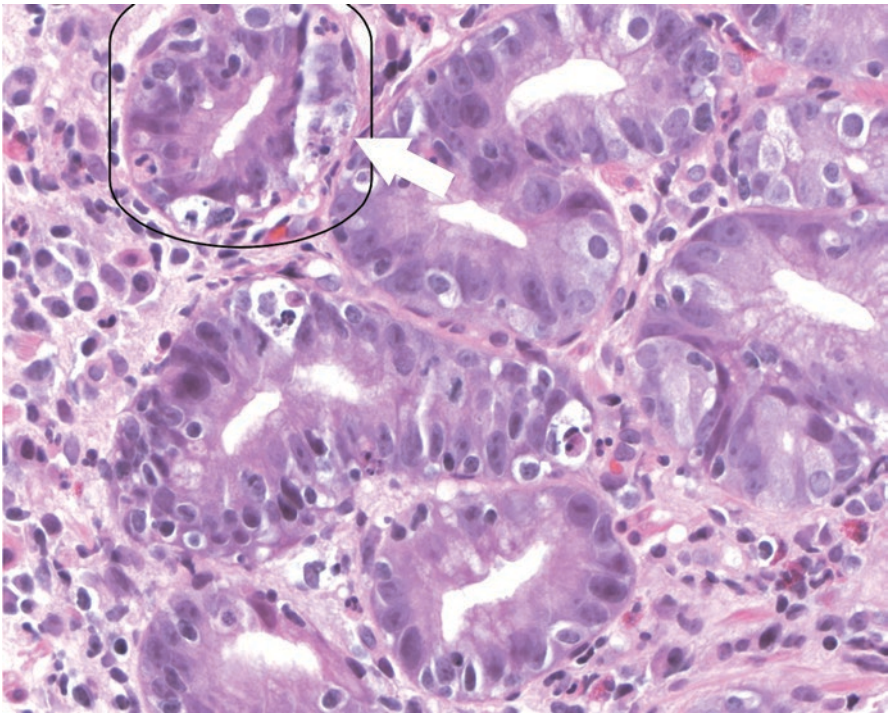

Fig. 9.12 Severe GVHD in colon day 86 with numerous apoptotic bodies. The top, leftmost crypt (circled) shows apoptotic crypt destruction involving >1/3 of the wall (white arrow). Though this field would be classified as Lerner-Sale grade II, this understates the severity of activity, which would be Myerson grade 5

Table 9.1 Modified Lerner-Sale grading system [9, 10]

Lerner-Sale grade	Description
Grade I	Apoptosis of individual cells in basal and lateral crypts, known as exploding crypt cells, sometimes with lymphocytic infiltrate
Grade II	Crypt abscess/crypt destruction, involving ≥1/3 of glandular circumference. Dilated crypt is outlined by flattened, mucin-less epithelium with lymphocytic infiltration containing apoptotic debris. Scattered lymphocytes, neutrophils, eosinophils present in the interstitium, crypt walls, and abscesses. Typically widespread damage
Grade III	Dropout of one or more crypts. Regions of crypt-devoid mucosa with some focal ulceration. Typically widespread damage
Grade IV	Extensive mucosal denudation; lamina propria completely devoid of crypts and epithelium Granulation tissue and intermittent hemorrhaging regions may be present

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Table 9.2 Myerson activity grading scale [14]

Activity grade	Diagnostic nomenclature	Apoptotic cells per section	Lerner-Sale grade
0	No diagnostic alteration/nonspecific changes/ nonspecific inflammation	<0.07	0
1	GVHD of minimal histologic activity	≥0.07 to <0.25	I
2	GVHD of mild histologic activity	≥0.25 to <4	I
3	GVHD of moderate histologic activity	≥4 to <25	I
4	GVHD of severe histologic activity	≥25	I
5	GVHD of severe histologic activity, with destruction	≥25	II–IV

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by Lerner et al. [9] in 1974, was derived mostly from severe late-stage GVHD. The 1979 modification to this scheme by Sale et al. included enterocyte (crypt cell) apoptosis within Lerner grade I [10]. This modification of Lerner (henceforth "Lerner-Sale") grade I encompasses a broad spectrum of apoptosis distribution, from only rare apoptosis to many apoptoses without crypt destruction as defined in Chap. 8 and a 2015 publication by Kreft et al. (especially Table 3 therein) [13]. The number of apoptotic enterocytes reflects several factors including the duration of active GVHD, if IS treatment was begun before biopsy, and differing degrees of allogenicity. The changes in the stomach are also generally of lower grade. More advanced stages, typically found in the intestines and colon, include crypt destruction in Lerner-Sale grade II (Fig. 9.12), dropout of crypts in Lerner-Sale grade III, and mucosal denudation or ulceration in Lerner-Sale grade IV (Figs. 9.11 and 9.13) [12]. Since the early HSCT era, changes in transplant practice and the effects of prolonged IS have altered the severity and onset of GVHD, resulting in a willingness to diagnose GVHD based on low numbers of apoptotic crypt cells. This has been compounded by debate over the minimal diagnostic numbers of apoptotic crypt cells.

The Myerson apoptosis activity index (Table 9.2) is based on the degree of apoptotic activity independent of the degree of crypt or mucosa destruction [14]. The intent of the scheme's study was to inform and clarify early diagnosis as a guide for therapeutic intervention, particularly within the wide apoptotic spectrum within Lerner-Sale grade I changes. Employing the definitions of apoptosis from Kreft et al. [13], the study proposed an activity scale of 0–5 using the arithmetic average of total apoptotic cells per total tissue section, regardless of whether these sections were present in one or more paraffin blocks. The Myerson apoptotic activity grade 5 encompasses all changes within Lerner-Sale grades II–IV. Both schemes would require some IS intervention. The validation for this approach found that within Lerner-Sale grade I, the higher the activity index, i.e. the more apoptotic enterocytes, the greater the likelihood of therapeutic intervention. Applying the Myerson grading scheme addresses the issue of minimal diagnostic criteria by prioritizing sensitivity over specificity of an apoptosis etiology. Low numbers of apoptotic cells

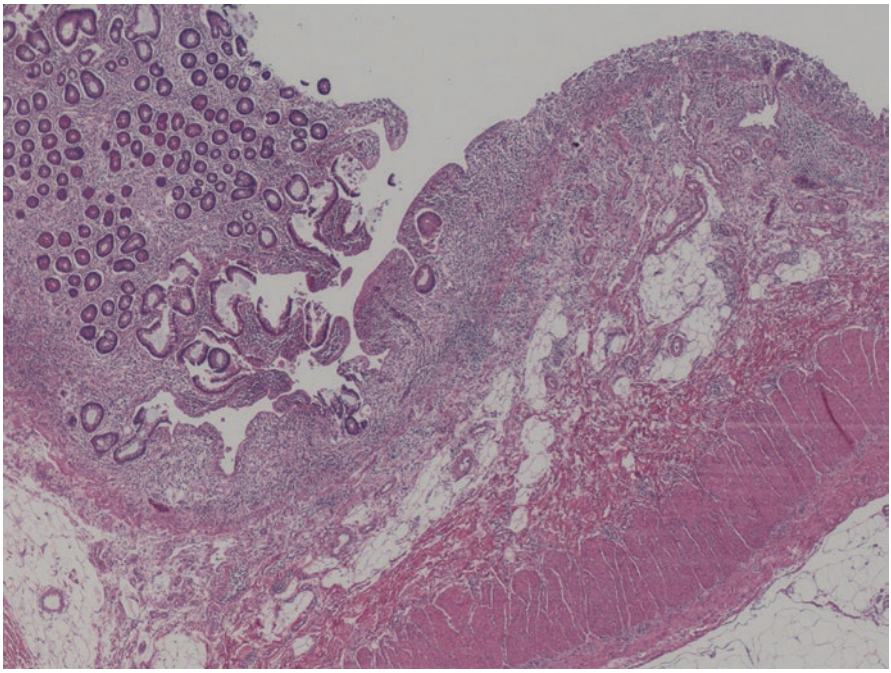

Fig. 9.13 Low-power view of the intestine demonstrates the patchy distribution of GVHD. Ulcerated segments complete mucosal destruction of crypts adjacent to areas with architectural disarray and small irregular crypts

are not dismissed but have a lower likelihood that they will influence a therapeutic intervention.

There is general agreement among pathologists and clinicians that histologic features portending a poor outcome include persistent high-grade Lerner-Sale II–IV lesions, the late onset of acute GVHD, and/or recurring or persistent gut GVHD, especially following a trial of corticosteroids [11, 12, 15–18]. However, a large recent study amends this dogma, indicating that a gut biopsy has additional prognostic implications if its source comes from the lower gut. Im et al. found that biopsy from the lower GI tract with Lerner-Sale grade I (Myerson grades 2–4) and ≤ clinical grade 2 had a significantly higher non-relapse mortality (NRM), hazard ratio 2.7×, than a comparable clinical grade I upper gut biopsy [19]. Regardless of which grading scheme is applied, it should be remembered that changes of GVHD may not be uniform in different regions. This concept is demonstrated by this chapter's index case. Radiologic imaging (Chap. 10) visualizes regions of the intestine inaccessible by endoscopy. The Myerson scheme does not classify chronic changes or provide stages of GVHD progression, but it does suggest that the final report might include an additional comment describing the types and extent of severe mucosal damage, e.g. "Myerson grade 5 with widespread or focal denudation, loss of intestinal Paneth cells" [4]. Additional descriptive changes of chronicity which

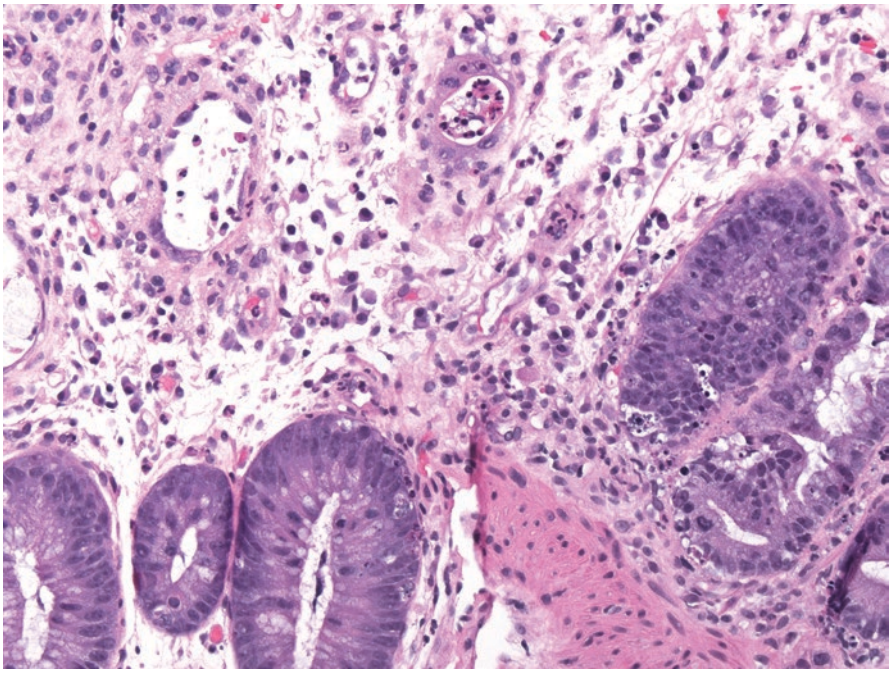

Fig. 9.14 Higher magnification of Fig. 9.11 demonstrates crypt abscesses containing, eosinophils and/or apoptotic debris. Extensive apoptosis is present in some residual crypts

may be included in the report include architectural distortion with crypt loss, formation of nubbins of irregular cystic glands, areas of atrophy alternating with partial regeneration or ulceration, basement membrane fibrosis in the lamina propria (see case #2 in Chap. 10), nuclear atypia, and loss of mucin [20, 21]. These chronic changes may persist with little apoptosis or inflammation (Fig. 9.10), which may reflect the anti-inflammatory effect of prolonged IS. Recent studies demonstrate that neutrophils contribute to the initiation of gut GVHD [22, 23]. Our index case also demonstrates that eosinophils and neutrophils are involved in the mucosal damage [1, 2] (Fig. 9.14). Estimate of risk stratification for treatment decisions is based on several parameters including biomarker data, stool volume, radiologic imaging, endoscopic visualization, histologic grade, and region of gut biopsy [24–28].

Teaching Points

1. GVHD of the gut may be approached clinically as upper gut disease, which presents with nausea and vomiting, while lower gut involvement of the intestines and colon is characterized by a secretory diarrhea and abdominal cramping.

2. Prognosis at the onset of GVHD is best predicted by a combination of clinical data, endoscopic findings, biopsy if taken from the lower gut, and biomarkers.
3. If gut symptoms persist after treatment, repeat biopsies of several regions are useful to exclude infectious causes and to help define the affected regions of the gut.
4. The modified Lerner-Sale grading scheme describes the stages of GVHD activity, which reflects its severity and duration from the accumulated mucosal damage.
5. High-grade Lerner-Sale stages of activity (Myerson grade 5) persisting after treatment are correlated with a poor outcome.
6. Useful information for prognosis and management can be derived from Lerner-Sale grade I. Biopsies from the lower gut, even of mild clinical severity, have a 2.7× higher NRM outcome than upper gut biopsies of comparable clinical severity.
7. Grades 2–4 of the Myerson grading scheme, which are based on the number of apoptotic bodies, are contained within Lerner-Sale grade I and serve as a guide for recommendation of therapeutic intervention. As the activity grade of the Myerson scheme increases, the likelihood for therapeutic intervention increases.
8. The Myerson grading scale does not factor in changes associated with duration such as crypt destruction and crypt abscess. The inclusion of these finding should be mentioned in the diagnosis.

Pathological evaluation is complimentary to the overall assessment, but is no longer considered the gold standard for gut GVHD diagnosis. A positive clinical assessment may sometimes be discordant with negative pathology. This may reflect that the early clinical manifestations of both gut and liver GVHD are produced by cytokines before the development of histologic changes, if IS treatment was begun before biopsy, or variances in endoscopic zones sampled.

Questions

1. Within Lerner grade 1, how many Myerson apoptotic activity grades are included?
2. What are the shortcomings of the Myerson activity grade scheme?
3. What is the best way to prognosticate at the time of initial gastrointestinal involvement?

Answers

1. Answer: It is possible to have Myerson activity grades 1–4 within Lerner-Sale grade I, indicating that biopsies of Lerner-Sale grade I contain a large amount of useful clinical information.

2. Answer: The Myerson activity grading index does not account for stage and destructive mucosal changes nor chronic changes such as architectural distortion or collagen deposition in the lamina propria.
3. Answer: The combination of clinical data (diarrhea volume), endoscopic findings, and biomarkers. Pathology is confirmatory of the diagnosis and predictive of greater NRM if the biopsy comes from the lower gut or if there is associated advanced lower gut GVHD (Lerner-Sale grades III–IV).

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