Pathology of Graft vs. Host Disease

A Case Based Teaching Guide

Cecilia C. S. Yeung
Howard M. Shulman
Editors

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Editors
Cecilia C. S. Yeung
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

Howard M. Shulman
Clinical Research Division
Fred Hutchinson Cancer Research Center
Seattle, WA
USA

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

Pathology Section
Seattle Cancer Care Alliance
Seattle, WA
USA

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Foreword: Why Have a Histopathology Primer on Graft-vs-Host Disease (GVHD)?

In 45 years, HSCT has emerged from a last-ditch experimental effort to cure hematologic malignancies into an established treatment with hundreds of transplant centers throughout the world. Despite the numerous technological advances leading to successful outcomes, GVHD with its associated immunodeficiency and infectious vulnerability remains the leading cause of non-relapse mortality.

The advances in the HSCT procedure, along with changes in management of GVHD, have produced an additional set of considerations related to the interpretation of biopsies for diagnosing GVHD and evaluating response to anti-GVHD treatment. These considerations include distinguishing GVHD from pre-transplant conditioning chemo-irradiation toxicities, from coexistent infection, or from adverse post-transplant drug toxicity. There are a number of unresolved or controversial issues: when skin or gut biopsy are indicated, the best endoscopic location for diagnosing GVHD, the minimal diagnostic threshold for a likely or certain diagnosis of GVHD, what histologic activity scoring or grading systems are most useful in guiding clinical decisions that reflect the diagnosis, prognosis, or response to treatment? What "nonclassical" histological alterations are now considered to be part of the spectrum of manifestations of chronic GVHD?

Often these issues and assessment of GVHD are encountered by clinicians and/or pathologists without the expertise or limited exposure to HSCT. Unlike specialty journals and meetings devoted to HSCT, except for the European Germanic GVHD consortium group and the once-per-decade NIH consensus panels, there is a paucity or absence of pathology meetings devoted to sharing information on GVHD. The relevant literature is dispersed among a variety of publications including HSCT specialty journals, general surgical pathology, hematology-related journals, and HSCT textbooks. However, these publications may reflect the institutional practices from a single institution, and textbooks may not include recent developments or expansion of controversial issues.

This book is a primer directed at pathologists and oncologists who confront questions about the surgical pathology related to GVHD that are not necessarily addressed or controversial. We attempt to consolidate the current understanding, along with differing viewpoints from other institutions supplemented by the long years of experience by the authors from the large HSCT program at the Fred Hutchinson Cancer Research Center, where for over 40 years, over 10,000 transplants have been performed. The book format will be short case vignettes. They

cover the gamut of both typical and complex cases of acute and chronic GVHD, and pertinent infectious complications. The vignettes include a clinical case history, associated histologic images, and discussion of relevant questions related to interpretation. The two introductory overview chapters will cover the principles and caveats as related to the pathology of GVHD and a clinical overview of GVHD. The case discussions reflect both the published literature and wisdom from the FHCRC Hematopoietic Cell Transplantation team, the Seattle Cancer Care Alliance Pathology Department, and the University of Washington departments of surgical pathology. For more in-depth details on the clinical diagnosis and treatment of GVHD, please refer to the textbook *Thomas' Hematopoietic Cell Transplantation: Stem Cell Transplantation, 5th Edition*.

We would like to acknowledge the excellent skills and dedication of the staff in the Seattle Cancer Care Alliance pathology laboratory, which enabled the clear educational histology seen in these teaching cases. We would also like to give special acknowledgments and deep gratitude to Petri Muhlhauser, who developed the shared cloud computing used in the writing of this textbook and the image archival system; David Woolston, who managed book files and images, proofing and editing, in addition to communications with authors and editors; and Debbie Anderson, who helped digitize many of the rare archival cases.

Howard M. Shulman, MD
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

Contents

Contributors

Sahl Ali, BA Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Abbie Ruth Bauer, MD Division of Nephrology, Seattle Children's Hospital, Seattle, WA, USA

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

Oliver H. Chang, MD Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Department of Anatomic Pathology/Dermatopathology, VA Puget Sound Health Care System, Seattle, WA, USA

Thanh T. Dinh University of Washington, Seattle, WA, USA

Seattle Institute for Biomedical and Clinical Research, Seattle, WA, USA

Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Swedish Hospital, Seattle, WA, USA

Laura S. Finn, MD Department of Laboratories, Seattle Children's Hospital, Seattle, WA, USA

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

Sangeeta R. Hingorani, MD, MPH Division of Nephrology, Seattle Children's Hospital, Seattle, WA, USA

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

Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

David M. Hockenbery, MD Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Division of Gastroenterology, University of Washington School of Medicine, Seattle, WA, USA

Teresa S. Hyun, MD, PhD 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

H. Joachim Deeg, MD Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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

Department of Medicine, Carl Carus University, Dresden, Germany

Miklos Kohary and Natalia Zimonyi Kohary Endowed Chair, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Shiva Khoobyari, MD Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Keith R. Loeb, MD, PhD 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

David Myerson, MD, PhD 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

Marie E. Perrone, MD Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Adam James Robin, MD Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Howard M. Shulman, MD 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

David W. Woolston Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Cecilia C. S. Yeung, MD 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

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

Howard M. Shulman

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

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

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

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

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

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

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

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

(continued)

Table 1.1 (continued)

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

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

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

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

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

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

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

Skin

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

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

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

Liver

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

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

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

Gastrointestinal Tract

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

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

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

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

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

Interpretation of Biopsies

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

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

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

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

Teaching Points

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

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Evolutions in the Clinical Management of GVHD

2

Cecilia C. S. Yeung and H. Joachim Deeg

Introduction

More than 40 years have passed since the first classic clinical and pathologic descriptions of acute graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (HSCT) [1]. In that era, only a small proportion of patients survived long term. Most patients died within a few weeks or months from transplant-related complications including multi-organ acute GVHD (aGVHD), infection, interstitial pneumonia, or relapse. A few long-lived survivors of allogeneic HSCT developed a polymorphic syndrome, different from aGVHD, and resembling several autoimmune diseases that became known as chronic GVHD (cGVHD). Over the ensuing decades, the management of patients post-HSCT has improved significantly with refined strategies and algorithms based on GVHD risk stratification. These strategies have enabled us to tailor immunosuppressive regimens, to use lower drug doses or shorter treatment duration for patients with low-risk disease, and to implement earlier more intensive therapy for high-risk patients.

C. C. S. Yeung (✉)
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: cyeung@fredhutch.org

H. Joachim Deeg
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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

Department of Medicine, Carl Carus University, Dresden, Germany

Miklos Kohary and Natalia Zimonyi Kohary Endowed Chair, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
e-mail: jdeeg@fredhutch.org

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Academic sources to address the broad range of clinical and pathologic issues related to the evaluation, diagnosis, and management of acute and chronic GVHD include two journals devoted exclusively to HSCT (*Biology of Blood and Marrow Transplantation* (BBMT) and *Bone Marrow Transplantation* (BMT)) and two NIH consensus conferences. These efforts have comprehensively and reproducibly characterized various subjects concerning GVHD etiology, progression, clinical and histopathological presentation, differential diagnosis, and treatment. However, transplant physicians recognize the challenges of inter-institutional variability in the diagnosis and grading of GVHD, and, thus, recent efforts have implemented internationally standardized guidelines for managing transplant patients. The current definitions and criteria for acute and chronic GVHD were developed by consensus of expert panels [2–5] (Table 2.1). Prior to the second NIH consensus meeting, a survey of expert clinicians delineated areas of agreement and controversy regarding what clinical and histologic features were diagnostic, distinctive, or not acceptable as evidence of cGVHD [15].

Over 10,000 allogeneic and autologous HSCT were carried out in 2016 alone for a variety of hematologic malignancies, marrow failure, inherited syndromes,

Table 2.1 Adapted table based on the 2014 Recommended cGVHD-specific core measures for assessing responses in cGVHD trials [5]

Measure	Organ system	Clinician assessed	Patient reported
Signs and symptoms	Integument	NIH skin score (0–3) [6]	Skin itching (0–10)
	Ocular	NIH eye score[a] (0–3) [3, 7]	Chief eye complaint (0–10)
	Oropharyngeal	Modified oral mucositis scale (0–12) [8, 9]	Mouth sensitivity (0–10)
	Hepatobiliary	Total bilirubin (mg/dL), ALT (U/L)	
	Pulmonary	FEV-1 (liters, % predicted)	Lee symptom scale 6 (0–100) [10]
		NIH lung symptom score (0–3) [11]	
	Musculoskeletal	NIH joint score (0–3) [12]	
		Photographic range of motion (4–25)	
	Gastrointestinal (GI)	Esophagus, upper GI, lower GI response (0–3) [5]	
Global rating		None-mild-moderate-severe (0–3) [10]	None-mild-moderate-severe (0–3) [10]
		0–10 severity scale (0–10) [13]	0–10 severity scale (0–10) [13]
		7-point change scale (−3 to +3) [14]	7-point change scale (−3 to +3) [14]

ALT alanine transaminase; *FEV-1* forced expiratory volume, first second; *NIH* National Institutes of Health

[a]Components include both signs and symptoms

immunologic disorders, and assorted cancers. The increasing use of HSCT to treat multiple disorders is possible because of numerous technological advances and biological insights. Included among such advancements are less toxic conditioning regimens (reduced intensity conditioning), the use of allogeneic donor stem cells derived from peripheral blood or umbilical cord blood, more effective anti-GVHD immunosuppressive regimens for both prophylaxis and treatment, and a wider availability of donors (both related and unrelated), with more precise immunogenetic donor/recipient matching for histocompatibility antigens (HLA) and refined methods of identifying infectious agents. Furthermore, the availability and prophylactic application of new antiviral, antibacterial, and antifungal agents has markedly reduced the incidence of life-threatening infections. However, the expanded use of unrelated individuals or HLA-haploidentical family members and other partially matched individuals as stem cell donors, in addition to the inclusion of older patients as allogeneic recipients, has been associated with an increase in the incidence of acute and chronic GVHD.

Many of the original descriptions of GVHD were based on observations in patients with undertreated or refractory aGVHD. Subsequently, the histologic interpretation of biopsy tissue was affected by numerous modifications in the HSCT procedure. In the initial era of HSCT, certain cytotoxic changes in the skin and gut, presumably related to high-dose pre-transplant conditioning with chemo-radiotherapy, were found to mimic GVHD and persist for up to 3 weeks [16]. A reliable histologic diagnosis of GVHD was understandably challenging. However, many modern conditioning regimens using reduced intensity conditioning lessen or eliminate confounding cytotoxic changes; thus, censoring interpretation of any biopsy taken during this early period may no longer be necessary. Differing degrees of HLA incompatibility between stem cell donors (related or unrelated) and patients can also lead to earlier onset of aGVHD. In the setting of such a patient with high risk for the development of early and severe GVHD, the first day post-transplant that a skin biopsy may be considered informative relies on clinical judgment. However, several confounding differentials can mimic GVHD in its early stages, such as preexisting conditions, reactions to drug toxicity, engraftment syndrome, or infection. Different sources of hematopoietic stem cells, e.g. marrow versus peripheral blood or cord blood and a variety of new immunosuppressive (IS) agents, all may affect the manifestations of early acute, chronic, and late-onset acute GVHD.

How to Use This Book

The classic target organs of aGVHD are the skin, gastrointestinal tract, and liver. The clinical approaches to deciding when pathological interpretation would be most helpful and from which site a biopsy should be obtained are outlined in the remainder of this chapter. Details of the pathologic features and the associated differentials are discussed in the ensuing chapters.

AGVHD presents most frequently in the gastrointestinal (GI) tract, followed by the skin and then by the liver. Some 30–50% of patients experience

symptoms or exhibit histopathological changes in multiple organs. Historically, cGVHD occurred in 30–70% of patients as a polymorphous multi-organ syndrome with features similar to various autoimmune disorders (Chaps. 6, 7, 12, 17, 18, 19, and 20). Results of ongoing investigations incorporating antithymocyte globulin (ATG) in conditioning regimens and administering cyclophosphamide after donor cell infusion suggest that the current incidence of cGVHD is closer to 35%. Among the most prominent manifestations is the pleiotropic biphasic skin involvement with both a lichenoid inflammatory and a later fibrotic sclerodermatous phase. Other histologic manifestations of cGVHD include a generalized sicca syndrome with oral, lacrimal, and diffuse mucosal involvement (Chap. 17), bronchiolitis obliterans syndrome (Chap. 18), immune mediated cytopenias, ductopenic cholestatic liver disease, polymyositis, and various kidney disorders [17]. Some patients with cGVHD manifest an overlap with aGVHD in the skin and gut, so distinction between acute and chronic GVHD can be difficult around day 100 post-transplant. Furthermore, neither the liver nor the gut exhibits histologic changes specific for acute or chronic GVHD. The findings of esophageal webs and muscularis mucosae fibrosis are an exception to this exclusionary rule (Chap. 12). A multivariate analysis comparing the risk factors for acute and chronic GVHD identified differences in the mechanisms of development of acute and chronic GVHD. A recent review of the immunopathogenetic relationship between acute and chronic GVHD suggests that reconstitution of the immune repertoire following stem cell infusion plays a critical role in GVHD development (Chap. 20) [18, 19]. The current NIH indications for an open lung biopsy to rule out the bronchiolitis obliterans syndrome are provided in Chap. 18. Recent studies show that cGVHD patients have antibodies which cross-react with surface membrane antigens on the tissues of infected organs [20].

Skin

Erythematous maculopapular rashes from cutaneous aGVHD in the early post-transplant period are related to allogeneic lymphocytic attack and cytokine release [21–24]. The differential diagnosis of early skin rashes includes conditioning-associated cytotoxicity drug reactions (especially those caused by antibiotics), reaction to blood products, and viral infection (Chap. 4). The histology of early skin GVHD, even in the hyperacute presentation, is not pathognomonic even when keratinocyte apoptosis occurs. Thus, there is a lack of consensus regarding the necessity of obtaining a skin biopsy for suspected aGVHD in the early post-transplant period. In a hypothetical analysis study, the decision of whether a skin biopsy was necessary to confirm suspected aGVHD was influenced by the estimated prevalence of GVHD and the value of potential outcomes, e.g. the need to treat potentially aggressive GVHD immediately [25]. In a study aimed at determining the best time point for biopsy and workup of cutaneous GVHD, 88% of European pathologists, dermatologists, and transplant physicians believed a skin biopsy was necessary when *chronic* GVHD was suspected. However, only 62% believed a skin biopsy was needed when

aGVHD was suspected and no other organ showed features of aGVHD [26]. The results of this study, especially the lack of consensus regarding the necessity of a biopsy in aGVHD, are not entirely surprising. Because the need for performing a biopsy is a prevailing issue [27], it has prompted the development of established guidelines for diagnosis. A large, international multicenter panel of experts has developed guidelines for the standardization of the clinical and histological data used for diagnosing and staging of aGVHD with the goal of improving uniformity and reproducibility of the diagnosis of GVHD in clinical trials [4].

Chronic GVHD in Skin and Genitalia

Both the severity and prevalence of cGVHD have increased in the past decade due to increased use of mobilized peripheral blood stem cells for transplantation, improved survival in the post-transplant period, and increased rate of transplantation in older patients [28–30]. The current NIH consensus recommendations, which are followed by most clinicians (82%), do *not* recommend skin biopsies for patients with suspected genital/vulvar cGVHD unless there are no other diagnostic features as defined in NIH 2014 [31]. However, a study from a large tertiary treatment center for cGVHD showed that in 7% of referred patients, GVHD was not confirmed when biopsied [32]. Assessment of morphic and sclerodermatous cGVHD typically relies on visual and physical evaluation as a biopsy of sclerotic skin may not be able to distinguish active changes from static preexisting changes [33].

Liver

Liver dysfunction is common after transplantation and occurs with varied severity due to a wide range of etiologies. At the onset of liver dysfunction, the following variables must be considered to deduce differentials of liver dysfunction: time and type of recent treatments, any preexisting conditions, specific parameters of the transplant regimen, and the constellation of laboratory tests.

The incidence of liver GVHD has decreased over the last few decades from a reported incidence of around 70% in the 1970s to less than 20% during this past decade [34, 35]. Liver GVHD can present as multisystem GVHD, with an acute hepatitic onset (see Chap. 16) requiring treatment, or it can present as a slowly progressive cholestatic disorder with elevated serum liver enzyme levels and jaundice, sometimes without other manifestations of GVHD (Chap. 14).

Aside from GVHD, sources of liver dysfunction can be categorized into those that occur early (generally before full engraftment) (Chap. 13), those which occur in the immediate post-transplant period, and those that occur late (beyond day 100) after transplantation (Chap. 16). Sources of early liver dysfunction include veno-occlusive disease/(sinusoidal obstruction syndrome, (VOD/SOS), infections, drug toxicity, sepsis, and congestive hepatopathy from cardiac decompensation [36] (Chaps. 13 and 14). Late liver dysfunction may have similar etiologies as early

dysfunction, such as infection with viral or fungal organisms, drug toxicity, and preexisting conditions (e.g., nonalcoholic steatohepatitis). All of these are potential comorbidities that can complicate GVHD cases (Chaps. 15 and 16).

The decision to obtain a liver biopsy is based on the urgency to identify the likely cause of elevated liver function tests that are not clearly identifiable by the clinical context. This applies especially to the identification of causes such as infections or a malignant process. Thin-gauge needles should be avoided for biopsies since they distort the tissue architecture and complicate interpretation of the biliary structures—the cardinal target of liver GVHD. A transvenous approach with a needle or forceps biopsy should include measurement of the manometric intrahepatic pressure gradient to aid in the diagnosis of VOD/SOS. Workup of any liver biopsy, if suggested by the clinical history, should consider markers of viral infection, and potentially hepatotoxic drugs, the timing of administration of IS, the pattern and level of elevated liver function tests, information on GVHD in other organs, and any previous liver biopsies. Of note, though CMV commonly involves the liver when there is a systemic infection in the gut or lungs, it is not a cause of marked liver dysfunction in the early or later periods [37, 38].

Gastrointestinal (GI)

GVHD of the GI tract is common with incidence rates of over 50% [39, 40] to as low as 15% in a recent study restricted to reduced intensity conditioning transplants [41]. Gut GVHD will typically present after day 20 post-transplant. It is clinically categorized either as a milder upper tract syndrome with primarily gastric symptoms of anorexia, nausea, and vomiting or as a more severe lower gut syndrome with abdominal pain, diarrhea, and hematochezia. Other ancillary laboratory studies that may help in narrowing the differential diagnosis, if the patient's GVHD involves the lower or upper gut, include diarrhea volume, increased protein in diarrhea fluid (protein-losing enteropathy), and declining serum albumin levels [40, 42]. Due to the patchy nature of the mucosal changes in GI GVHD, histopathology (which is based on tiny, usually millimeter, and fragments) should be assessed simultaneously with the gross appearance of the gut by endoscopy to render a more accurate diagnosis. Histology should be considered complementary to the clinical picture and macroscopic endoscopy findings. Discrepancies between clinical signs, endoscopic findings, and histology are not uncommon and should be reviewed together with the clinical teams [43–45].

While histologic features of GVHD overlap with other diagnostic entities (e.g. engraftment syndrome, drug reactions, other autoimmune diseases, and infections), they can inform the clinician in several ways: First, according to the 2015 WHO consensus criteria, a pathologist can inform the clinician of a likely GVHD diagnosis and the need to initiate or continue treatment. Quantifying the apoptotic activity or particular location of the gut histology can aid in stratifying risk and advise the need for treatment (Chap. 10). Histology can be an effective parameter for assessing efficacy of treatment when serial biopsies can be obtained. A post-treatment biopsy

can be used to gauge repair and response to therapy and rule out infections such as *C. difficile* enteritis (Chap. 14). Treatment decisions, which are based on the severity of the symptoms, range from observation and follow-up evaluation, to systemic steroids, to alternative second-line therapy options such as anti-thymocyte globulin (ATG), ruxolitinib, mesenchymal stem cells, phototherapy, lithium and alpha-1-antitrypsin, or ibrutinib (an inhibitor of Bruton tyrosine kinase) [46].

Histologic features associated with poor prognosis include GVHD in the lower gut [47], the lack of re-epithelialization after a fortnight of systemic steroid administration, and the loss of intestinal Paneth cells [48]. Late-onset acute GVHD superimposed on cGVHD also has a poor prognosis [49]. Other factors associated with increased mortality include steroid-resistant disease, older age (>18 years), increased serum bilirubin, and GI bleeding [44].

Specific biomarkers may facilitate early identification of high-risk patients. Serum proteins such as TIM3, sTNFR1, ST2, IL-6, and Reg3a have been positively correlated with more severe GVHD [48, 50–54]. Markers of vascular injury and ensuing endothelial activation, such as loss of thrombomodulin, increased blood levels of angiopoietin-2 [55, 56], and other alterations of circulating angiogenic factors have been associated with risk of GVHD or GVHD responsiveness to steroid treatment [4, 52, 57, 58].

Additional Reading

This chapter is meant to be a brief introduction and a clinical overview of GVHD. More extensive and detailed information is provided in the following references [59–61]. For a comprehensive review of the immunobiology and recent treatments for acute and chronic GVHD, please refer to the review articles by Zeizer and Blazar [62, 63]. Good review articles on new therapeutic options for cGVHD are also available [46, 64].

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Early GVHD with Follicular Rash

3

Cecilia C. S. Yeung, Thanh T. Dinh, and Howard M. Shulman

Clinical History

Our patient was a 52-year-old man with high-risk chronic lymphocytic leukemia, (Rai stage III–IV disease, refractory to chemotherapy) with recent transformation in peripheral blood. He received the HSCT from an HLA-matched sibling donor. On day 22, he developed a punctate red rash on both arms and back which progressed to clinical grade III (Figs. 3.1 and 3.2). He received treatment with prednisone. Microscopic photographs of the punctate rash were taken (Figs. 3.3, 3.4, and 3.5). The rash was resolved after treatment with prednisone. No other long-term complications of HSCT were noticed for this patient.

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

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

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

T. T. Dinh
University of Washington, Seattle, WA, USA

Seattle Institute for Biomedical and Clinical Research, Seattle, WA, USA

Swedish Hospital, Seattle, WA, USA
e-mail: thanhd2@uw.edu

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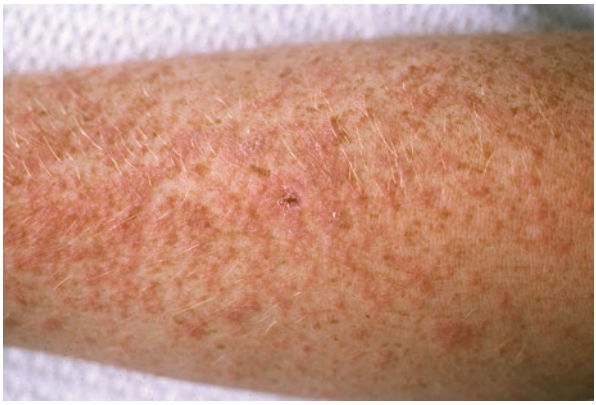

Fig. 3.1 This gross photo of the patient's arm on day 22 has a diffuse rash with small red punctuate lesions

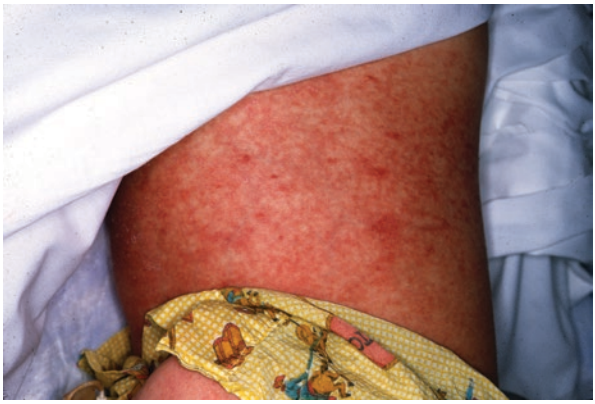

Fig. 3.2 This is a gross photo of a separate patient presenting with an early confluent erythematous macular rash over his torso on day 15, which was diagnosed on skin biopsy as early GVHD. The illustrated rash resembles clinical grade III that our patient developed

Diagnosis

Early onset of acute graft-versus-host disease (aGVHD) of the skin with involvement of the follicular unit

Key Pathology Features

- Variable keratinocyte apoptosis affecting the epidermis, follicular unit, and acrosyringium.

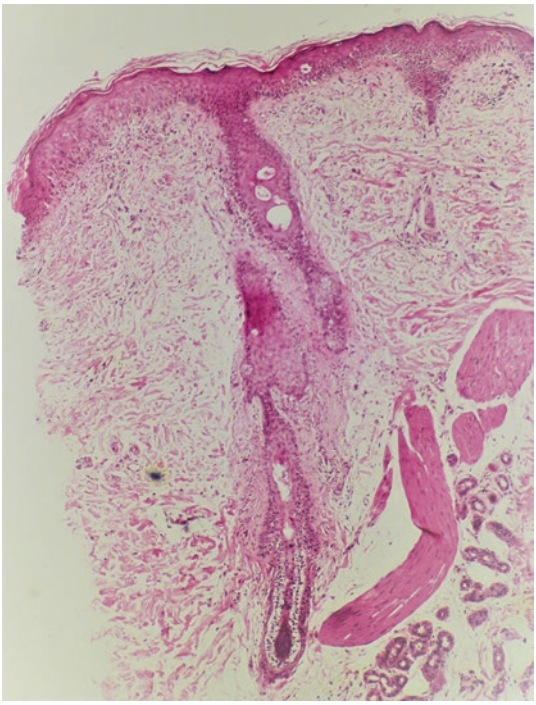

Fig. 3.3 This lower-power image of the entire hair follicle demonstrates an inflammatory reaction surrounding the follicular adventitial dermis

- Regions rich in progenitor cells are preferentially involved, basal keratinocytes, tips of rete ridges, and follicular bulb and bulge.
- Basilar vacuolization, lymphocytic satellitosis of epidermal basilar layer, melanin incontinence in the superficial dermis, and RBC extravasation may be seen.
- Biopsy performed soon after onset of rash may have only nonspecific basilar vacuolization and mild inflammation.
- Heavy lymphocytic inflammation with spongiosis and little or no keratinocyte apoptosis is most consistent with spongiotic dermatitis of non-GVHD origin.
- There is no clear histologic distinction between aGVHD that arises in the first several months or as a late-onset occurrence.

Differential Discussion

Post-transplant skin rashes are very common, especially in the first ~100 days. The histologic spectrum of early GVHD reflects several factors, the degree of allogeneic disparity between the graft donor and host recipient, cytotoxic conditioning, type and length of any prior exposure to IS prophylaxis or treatment, and duration of the rash before biopsy. The use of screening skin biopsies for aGVHD in the early post-transplant period in an asymptomatic patient is not a standard practice. Even if histopathologic criteria for GVHD are present, the biopsy still may not be accepted as GVHD [1]. A biopsy obtained in the early post-HSCT period may demonstrate

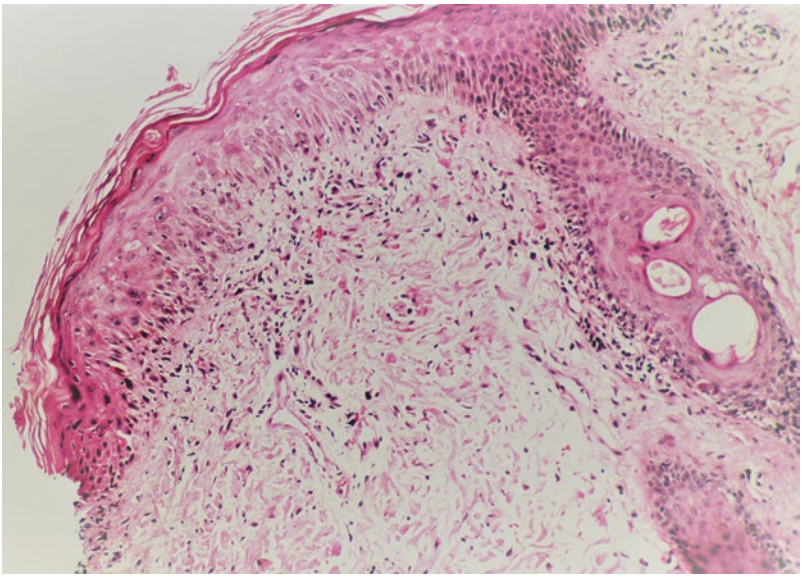

Fig. 3.4 This higher-power image of the same hair follicle and epidermis as in Fig. 3.3 demonstrates an inflammatory reaction comprised dominantly of lymphocytes. There are many apoptotic cells along the basilar portions of the epidermis and hair follicle

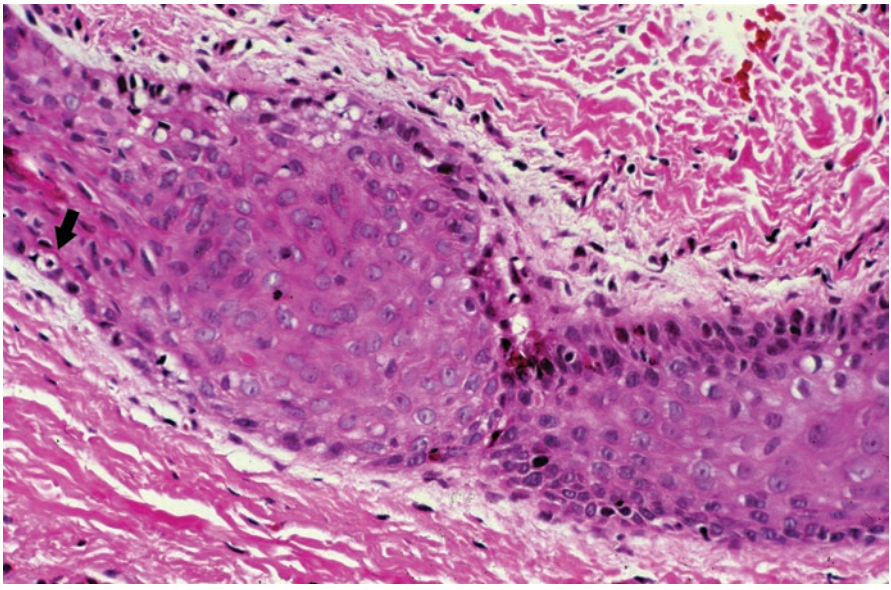

Fig. 3.5 This is a high-power microscopic image of the follicular bulge region (the widened region of the hair follicle where the arrector pili attaches) and a site of progenitor cells demonstrating characteristic GVHD features including inflammation and apoptosis (arrow) along the outer root sheath of the bulge region

nonspecific histologic features and/or overlap with other histologically similar entities. It once was a common practice at our institution to obtain serial biopsies to verify the diagnosis of aGVHD especially in the early post-transplant period. Examination of the serial biopsy would either confirm persisting GVHD or otherwise present further nondiagnostic changes [2]. However, clinicopathologic criteria developed from a 2015 consensus panel have replaced the examination of serial biopsies [3].

Pathobiology

The initial investigations into the pathobiology of GVHD focused predominately on T cell-mediated injury of target epithelia while largely ignoring the contributions of the endothelium [4] in T cell trafficking as well as a major target in solid organ rejection. In 1985, Sale et al. noted squamous epithelial basal cells in the epidermal rete tips were targeted preferentially in aGVHD [5], and Cotsarelis et al. showed that similar cells reside in the bulge region of the hair follicle [6]. Additional studies proved these basal squamous stem cells expressed cytokeratin 15, enabling immunohistochemistry labeling of squamous epithelial progenitors [7]. Subsequent murine studies by Zhan et al. showed that cytokeratin 15-positive progenitors when exposed to cytokines change their apoptotic vulnerability from antiapoptotic to proapoptotic phenotype, thereby becoming preferential epithelial targets in GVHD [8, 9]. Of relevance, some drugs, such as lovastatin, may interfere with the expression of GVHD by blocking T cell adhesion, proliferation, and cytokine production [10]. Pulses of anti-GVHD prophylactic methotrexate given before the skin is biopsied will suppress the lymphocytic inflammatory component [11].

Early GVHD Histologic Features Classic histologic features of GVHD include superficial interface dermatitis with vacuolar change mostly occurring in the basilar layer, sometimes accompanied with lymphocyte satellitosis or a lichenoid pattern of lymphocytic inflammation [1, 12, 13] (Fig. 3.6). The lymphocytic infiltrates are often sparsely scattered within the papillary dermis and around superficial venules. Lymphocyte satellitosis describes intraepithelial lymphocytes which surround apoptotic keratinocytes in the basilar layer and rete ridges. However, this is not a pathgnomonic diagnostic feature of aGVHD because drug reactions can show similar features [14] (Fig. 3.7). Cardinal histologic features that provide stronger support for the diagnosis of GVHD in the skin include apoptosis in the epidermal basilar and lower spinosum layers. A comparative study of aGVHD after T cell depletion vs non-GVHD skin rashes found features more suggestive of aGVHD were diffuse basal vacuolization, extensive keratinocytes apoptosis involving the entire epidermis, and mild rather than dense inflammatory infiltrates [15]. The hallmark of GVHD-induced cell death, apoptosis, is a shrunken hypereosinophilic keratinocyte with a pyknotic nucleus [16] (Figs. 3.3 and 3.5).

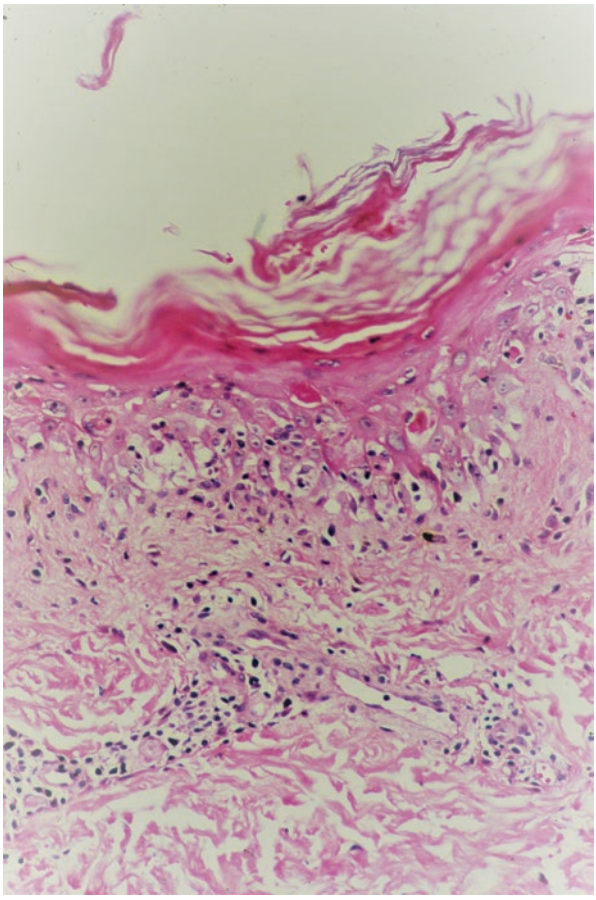

Fig. 3.6 This is the image of severe aGVHD with marked destruction of the cells in the stratum spinosum with reticular degeneration of the basal layer, extensive apoptosis, and lymphocytic inflammation

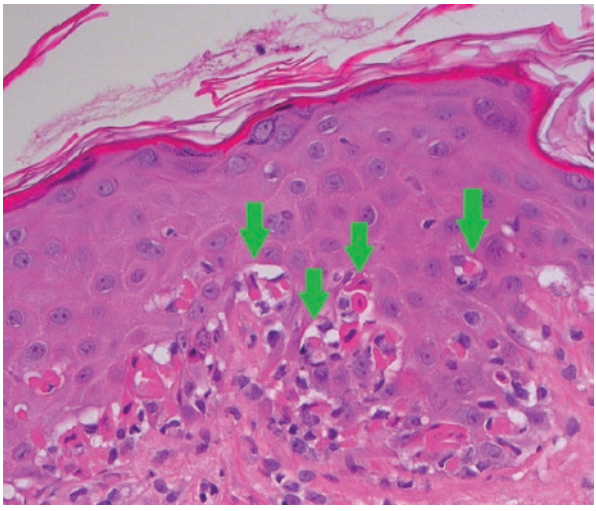

Fig. 3.7 This is a high-power image of a skin involved by aGVHD of severe histologic activity. The green arrows are pointing to a confluence of apoptotic keratinocytes which are surrounded by lymphocytes

Differential Diagnosis

Early post-transplant skin rashes may occur from toxicity to conditioning chemo-irradiation, reactions to drugs or antibiotics, transfusion reaction, infections, engraftment syndrome, or GVHD [17–23]. Presentations of dermatoses such as atopic dermatitis (eczema) can also bare strikingly similar histology to GVHD [24]. Histopathological changes seen in GVHD are often nonspecific. A GVHD diagnosis is aided by the gross appearance of the rash, the clinical context such as timing of engraftment, the number of days post-transplant, and the concurrent treatments, e.g. antibiotics and/or immunosuppressive agents [11]. Kohler et al. studied 16 histologic parameters in 179 skin biopsies (i.e., dyskeratotic keratinocytes, basal vacuolization, satellitosis, and necrotic cells in appendages) in an attempt to discern statistically distinct histological features of GVHD: but no single feature was expressed with greater statistical significance, failing to suggest a reliable single predictor or combination of predictors [25]. An early study by Sale et al. had also recommended avoiding the histologic diagnosis of skin GVHD before day 20 post-transplant because of the similar findings in autografted recipients who received intense myeloablative regimens [2]. The conclusions from these studies cannot be corroborated nor extrapolated to all HSCT centers because of differences in institutional practices, e.g. conditioning regimens, basis for study patient selection, and the uncertainty of how GVHD was clinically defined. Today, with the widespread use of mismatched unrelated allogeneic donors and the use of reduced intensity conditioning regimens, it is no longer tenable to avoid doing skin biopsies until day 20. This conundrum challenges accurate interpretation of skin biopsies in the early post-transplant period. There are different opinions on if and when skin biopsy is needed for diagnosis [26]. In Firoz's classic study on decision analysis for deciding when to do a skin biopsy, clinical estimation of the prevalence of GVHD was less influential than the possibility of not treating early severe GVHD [27].

Distinguishing between aGVHD and drug hypersensitivity reactions (DHR) can be especially challenging because aGVHD and DHR may not be distinguishable based on histologic features [14, 20, 28, 29]. In addition, the presence of eosinophils neither proves a drug reaction nor excludes GVHD [28]. Further complicating the problem are cases where components of both GVHD and DHR are likely. In these cases, having good communication between the pathobiologist and the clinical team over specific initiation of new drugs and onset of rashes as well as a trial of withdrawing the suspected drug may be needed to make a definitive diagnosis. Knowledge of additional clinical features can help narrow the differential, including facial involvement, presence of diarrhea, or hyperbilirubinemia, which are more likely due to GVHD [29]. Atopic dermatitis cannot be reliably distinguished from GVHD, as they have similar microscopic features; the diagnosis of atopic dermatitis relies primarily on the clinical physical exam and history [24]. Keratosis pilaris is a common condition with small, bumpy, hard follicular papules and pustules on the posterolateral upper arms, cheeks, anterior thighs, or buttocks, which are generally flesh colored but on occasion present as erythematous papules. In these latter instances, keratosis pilaris cannot be easily distinguished from GVHD in gross

appearance (Fig. 3.8). Engraftment syndrome may be more prevalent with certain sources of stem cells and conditioning regiments, e.g. T cell depletion with cord blood transplant, particularly if post-HSCT prophylaxis is not utilized.

In the early transplant era, rigorous multi-agent chemo-irradiation conditioning therapy could produce severe epidermal changes that resembled dysplasia, defined by Li et al. as severe keratinocyte dysplasia (SKD) [30]. SKD histological changes include enlarged, aberrantly shaped nuclei, enlarged keratinocytes, prominent nucleoli, possible multi-nucleation, loss of polarity, and mitotic figures in the epidermis which can be seen following conditioning with busulfan and have been reported to persist for months [30] (Fig. 3.9). SKD may be very difficult to distinguish from enlarged dyskeratotic cells found in some precancerous dysplasia. SKD has been reported in up to 92% of HSCT recipients who received a busulfan-conditioning regimen [31] and can occur concurrently with GVHD.

Rashes mimicking GVHD infrequently occur secondary to opportunistic infectious agents such as viruses, bacteria, and/or fungi in an immunocompromised host. Scabies is a contagious skin infestation by the mite called *Sarcoptes scabiei* that causes an intensely pruritic erythematous rash with macules/papules with a predilection for skinfolds and creases [32]. aGVHD has a predilection for palms and soles of the feet and presents in the acute phase with erythematous macules (Fig. 3.2). However, GVHD rashes are typically described as tender, whereas scabies are typically severely itchy (Fig. 3.10).

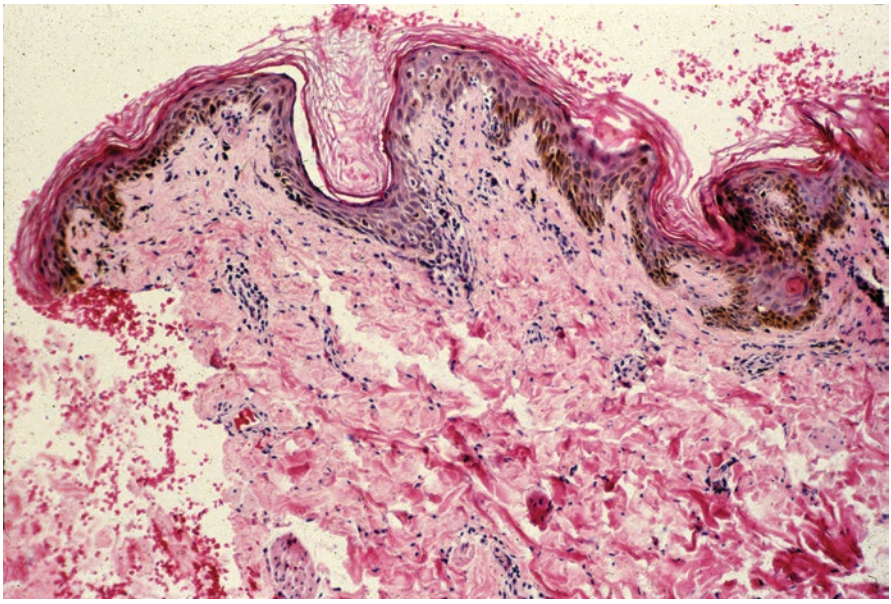

Fig. 3.8 This is a low-power image demonstrating keratinous plugging and lymphocytosis, which may resemble keratosis pilaris at low power

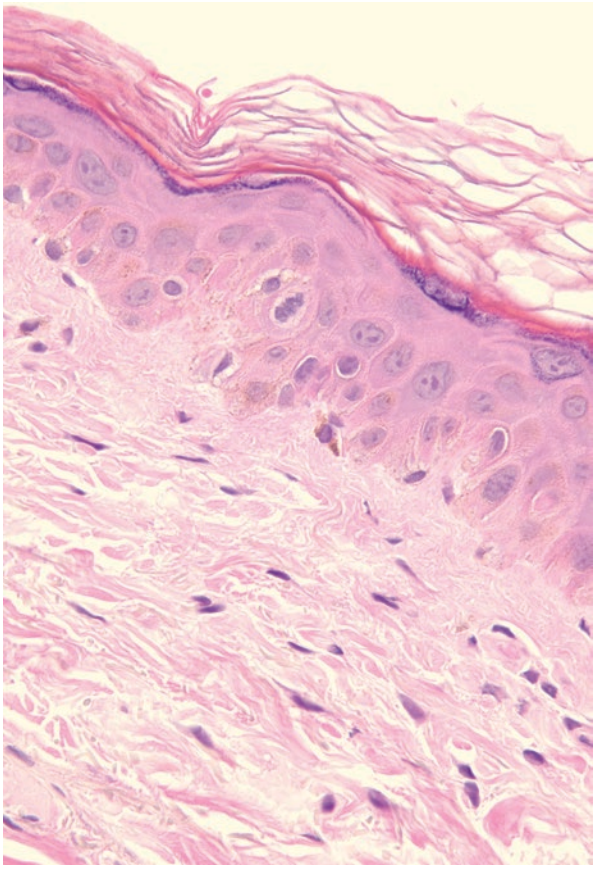

Fig. 3.9 This high-powered microscopic image of an H&E-stained skin shows an epidermis demonstrating severe keratinocyte dysplasia from a patient with AML, who is s/p HSCT day 40. Note the enlarged irregular keratinocytes with prominent nucleoli and the rare mitotic figure

CMV vasculitis is a rare serious complication in immunocompromised patients with high rates of morbidity and mortality [33, 34]. CMV vasculitis involving the cutaneous vessels has a characteristic skin rash with small erythematous papules. The microscopic changes show characteristic enlarged endothelial cells with prominent eosinophilic nuclear inclusions (Fig. 3.11). If the patient is taking prophylactic antiviral medication, these pathognomonic features of CMV can be suppressed, and additional immunohistochemistry will have to be employed to confirm CMV infection.

Uncommon cutaneous infections by both bacterial and fungal organisms have also been described secondary to severe disseminated systemic infectious such as with *Staphylococcus aureus* or various fungal organisms including *Aspergillus*, *Fusarium*, and zygomycetes (Fig. 3.12).

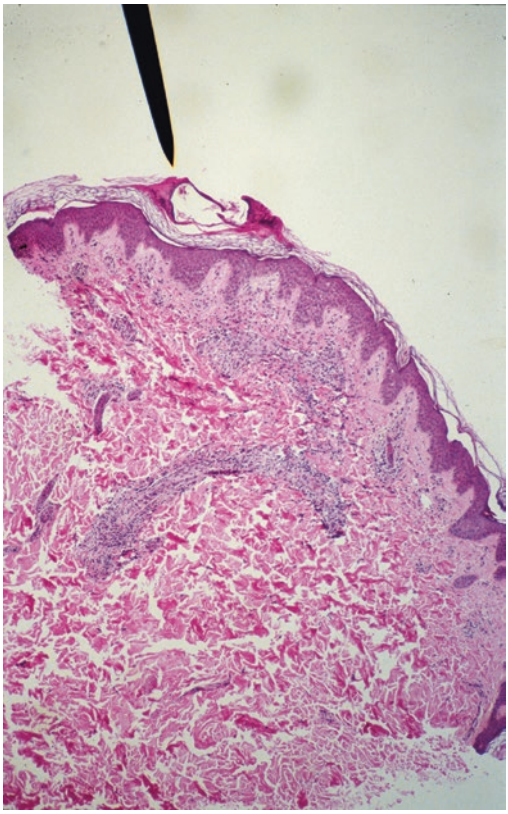

Fig. 3.10 This low-powered microscopic image of H&E-stained skin shows an epidermis with perivascular chronic inflammation associated with a mite, whose cross section is embedded into the stratum corneum (arrow) and clinically confirmed as scabies infection

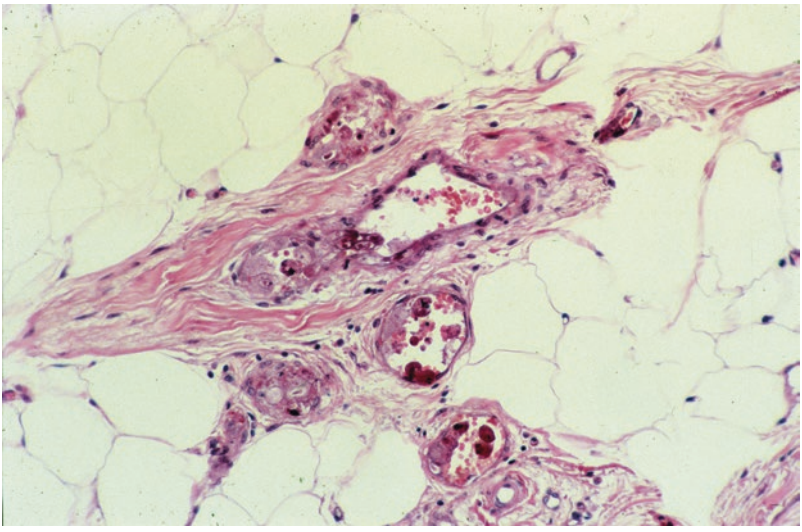

Fig. 3.11 This image is a high-power photo of the deep dermal vessels from a patient with disseminated CMV vasculitis; note the atypical endothelial lining cells with enlarged nuclei

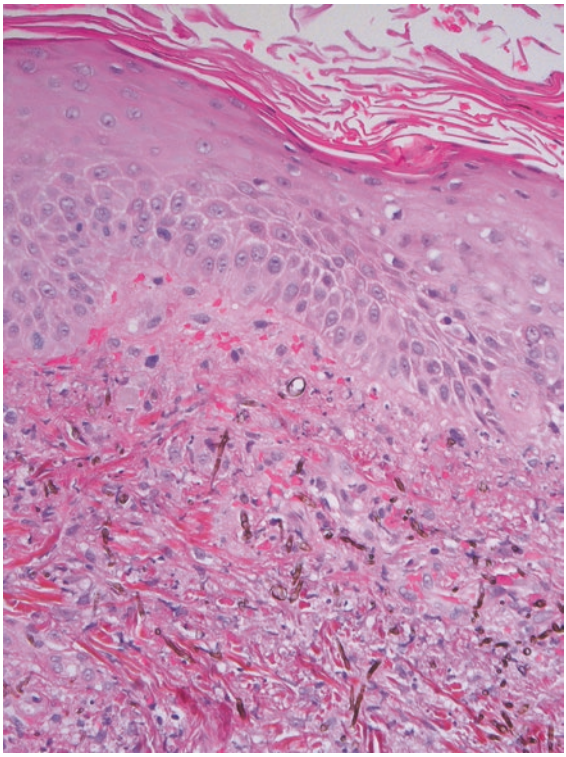

Fig. 3.12 This microscopic image of a skin biopsy stained with methenamine silver demonstrates silver positive fungal organisms in a patient with disseminated scopulariopsis infection

Teaching Points

1. The necessity of obtaining a skin biopsy for aGVHD is shaped by several factors: the associated clinical findings supporting a diagnosis of GVHD, as well as context including the donor-host allogeneic disparities, and avoiding delay for treatment of potential hyperacute aGVHD.
2. The interpretation of aGVHD is the sum of the clinical assessment plus the histologic findings which generally follow the international consensus guidelines [3]. Likewise, the presence of eosinophils neither proves a drug reaction nor excludes GVHD [28].
3. Early GVHD has features of superficial interface dermatitis with vacuolar change and keratinocyte apoptotic in the basilar layer and lymphocytic inflammation, sometimes with lymphocyte satellitosis.
4. Early post-transplant skin rashes are common and may occur from toxicity to conditioning chemo-irradiation, drug or antibiotic reactions, transfusion reaction to blood products, infections, engraftment syndrome, or GVHD.
5. The initial sites of the GVHD attack are in progenitor cell regions, the follicular hair bulb and the bulge region, and the tips of rete ridges.

Questions

1. Is it possible to ascribe different degrees of damage depending on the allogeneic incompatibility?
2. Which of the following are conditions that can mimic GVHD?
   A. Drug hypersensitivity reaction
   B. Atopic dermatitis
   C. Infection
   D. Engraftment syndrome
   E. All of the above
3. A patient develops a markedly itchy diffuse rash over the trunk back and upper arm 37 days post-transplant. A skin biopsy demonstrates lymphocytic infiltration and spongiotic change in the epidermis without apoptosis (Fig. 3.13). The patient described severe clinical excoriation (itchiness), particularly along skin creases. Which of the following diagnoses explains these symptoms?
   F. Early acute GVHD
   G. Follicular GVHD

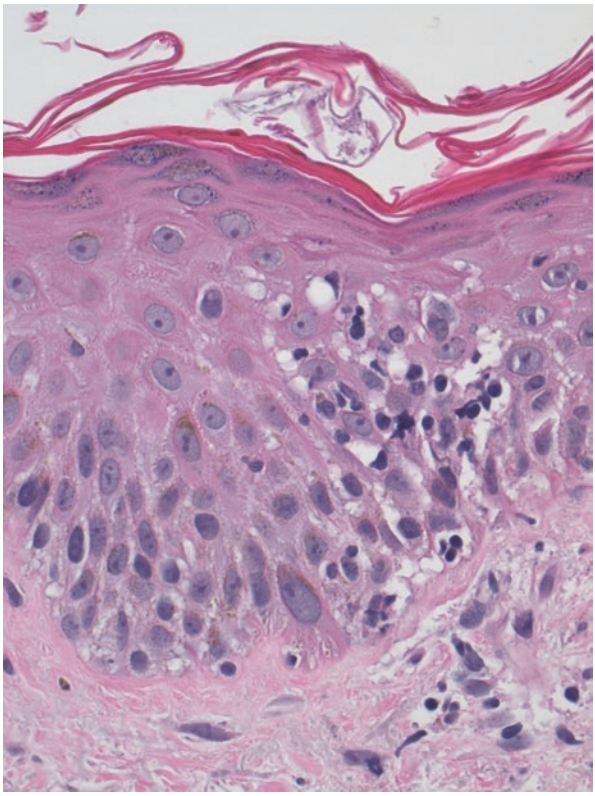

Fig. 3.13 A skin biopsy demonstrating lymphocytic infiltration and spongiotic change in the epidermis without apoptosis. Note the subcorneal structures

H. Scabies infection
I. Drug eruption
J. Contact dermatitis

Answers

1. Answer: No; however the date of onset, the tempo, and prognosis may be influenced.
2. Answer: E
3. Answer: C

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The Basic Sequence of Injury in Acute Skin GVHD

4

Teresa S. Hyun, Shiva Khoobyari, and Oliver H. Chang

Clinical History

A 65-year-old man underwent allogeneic-related matched peripheral blood stem cell transplant for high-grade myelodysplastic syndrome 35 days ago. His conditioning regimen included fludarabine and melphalan; he received cyclosporine for GVHD prophylaxis. He developed a spreading, mildly pruritic, diffuse erythematous skin rash involving the scalp, trunk, and bilateral lower extremities (Fig. 4.1). A biopsy of the skin rash was taken (Figs. 4.1 and 4.2). He was treated with prednisone and showed improvement of the rash within 1 week.

Diagnosis

aGVHD of the skin

T. S. Hyun (✉)
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: thyun@fredhutch.org

S. Khoobyari
Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA
e-mail: skhoob@uw.edu

O. H. Chang
Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Department of Anatomic Pathology/Dermatopathology, VA Puget Sound Health Care System, Seattle, WA, USA
e-mail: ochang@uw.edu

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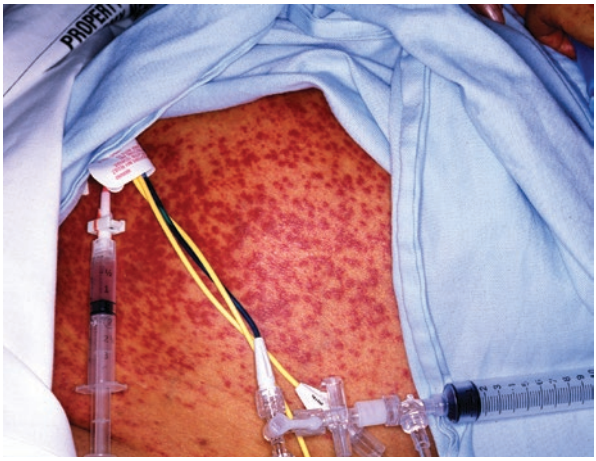

Fig. 4.1 Hyper-aGVHD on day 12 presents with an intense maculopapular rash superimposed onto an erythematous background, similar in appearance to that of our patient

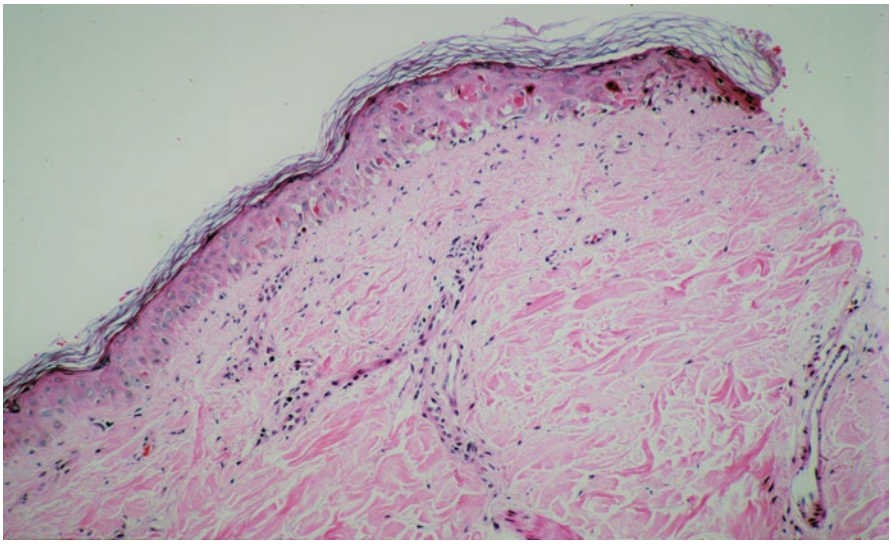

Fig. 4.2 Histology of Fig. 4.1 demonstrates aGVHD with numerous apoptotic keratinocytes, lymphocytic exocytosis, and vacuolar changes in the epidermis

Key Pathology Features

- Apoptotic keratinocytes in the basal epidermis, outer root sheath and hair bulge of follicular unit, or acrosyringium of sweat ducts.
- Basal epidermal vacuolar change.
- Interface dermatitis and perivascular lymphocytic infiltrate.
- Lymphocytes may surround apoptotic keratinocytes (lymphocyte satellitosis).

Differential Discussion

Skin biopsy obtained for histologic evaluation of aGVHD is often initiated when a patient develops a maculopapular rash following HSCT. The differential diagnosis in the case described above includes drug eruption from medications such as antibiotics, toxicity from chemotherapy and transfusion reactions. The presence of five or more apoptotic keratinocytes in association with apoptotic changes in adnexal structures within an inflammatory background of predominantly lymphocytes can help distinguish aGVHD [1]. Although the presence of eosinophils in the dermis in a non-transplant setting is commonly assumed to suggest a drug reaction, some eosinophils do occur with acute and cGVHD and should not exclude this diagnosis [2]. In the early posttransplant period, engraftment syndrome can occur in the period of granulocyte recovery and characteristically includes fever, skin rash, pulmonary edema, and organ dysfunction [3]. Of note, institutions which have used cord blood for the source of donor stem cells with T cell depletion without giving posttransplant methotrexate have reported engraftment syndrome; other institutions using similar transplant protocols but giving posttransplant methotrexate did not [4]. An inflammatory pattern of interface dermatitis with only rare apoptotic keratinocytes raises a broad differential and includes allergic contact dermatitis. Viral exanthem can also present with a similar rash.

There are multiple factors that need to be considered in the interpretation of skin biopsies used to diagnose GVHD. These include the timing of the biopsy, treatment schedules, concomitant use of immunosuppression (IS), and distribution of the rash, as well as the appearance of the skin at the site of biopsy. The timing of the skin biopsy and the severity of GVHD will influence the differential diagnosis. As there are no absolutely specific histopathologic findings in acute skin GVHD, biopsies must be appropriately correlated with posttransplantation timeline and clinical history. If the histologic findings are equivocal, empiric treatment may be given in suspected cases.

Grading of GVHD in the Skin

The histologic grading system for skin aGVHD was first proposed by Lerner et al. in 1974. It assigned the sequential histologic stages with grades as well as defining the minimal histologic criteria for skin aGVHD. In grade I GVHD, a superficial perivascular dermatitis with vacuolization of the epidermal basal region is seen, nonspecific features. Grade II GVHD encompasses an interface dermatitis with occasional dyskeratotic or apoptotic keratinocytes in the basilar or lower spinosum layers and may display lymphocytic satellitosis. Grade III GVHD is represented by extensive apoptotic keratinocytes with reticular degeneration, destruction of the basal layer, and supra-basilar bulla formation. Grade IV GVHD shows full-thickness destruction and ulceration of the epidermis. Grade II changes were proposed as minimal diagnostic criteria for distinguishing GVHD [5]. Current practice recognizes that histologic features are not always definitive for a

specific diagnosis with the NIH consensus pathology recommending using the terms “not GVHD,” “possible GVHD,” and “likely GVHD” as categories of final diagnoses [6].

Differential Diagnosis of Grade I/II GVHD

In practical terms, most skin biopsies taken from post-HSCT patients with aGVHD typically have grade II changes with infrequent apoptotic keratinocytes and only mild inflammatory infiltrates; therefore, review of multiple serial sections is beneficial. The differential diagnosis for low-grade GVHD of the skin includes drug reaction, eruption of lymphocyte recovery, infection, and other preexisting skin conditions (Chap. 3) [7]. Features that favor a diagnosis of GVHD are apoptotic keratinocytes along the outer root sheath of hair follicles and in the basilar epidermis and rete ridges. Eccrine units with architectural disarray and apoptosis are also supportive of GVHD.

Adverse drug reaction is a frequent consideration in HSCT patients, particularly when a rash presents with a distribution or pattern that is somewhat atypical for GVHD. Histologically, spongiosis with a prominent perivascular lymphocytic infiltrate and rare or no apoptotic keratinocytes suggests drug eruption rather than GVHD. Nonspecific features include edema and vascular dilation. Dermal eosinophils can be present in both entities and should not be considered specific to drug reactions. The causative medication may not always be identified due to polypharmacy use in HSCT patients, but antibiotics are a frequent cause.

Eruption of lymphocyte recovery is classically characterized as a maculopapular rash occurring 14–21 days after cytotoxic therapy, which coincides with recovery of peripheral blood lymphocytes. Skin biopsy at this time shows a scant perivascular lymphocytic infiltrate and dilation of vessels with rare or no apoptotic keratinocytes. Sweet’s syndrome, also known as acute febrile neutrophilic dermatosis, is another rash for diagnostic consideration, though the histology of neutrophilic dermatosis is quite different from that of GVHD.

In the subset of HSCT patients who have received busulfan as part of their conditioning regimen, biopsies can show severe keratinocyte dysplasia (SKD) in up to 92% of patients [8]. SKD is characterized by enlarged keratinocytes with bizarrely shaped or enlarged nuclei, prominent nucleoli, mitotic figures, and/or loss of polarity, which can resemble dysplasia of a precancerous epidermal lesion [9] (see Fig. 3.9 in Chap. 3). SKD and GVHD can coexist in the same biopsy, but there is no association between the two entities.

Clinical Characteristics

Histologic findings from skin biopsies do not always correlate well with the severity of rash seen clinically. Normal-appearing skin in a post HSCT patient can

show histologic alterations of aGVHD with scattered apoptotic keratinocytes without significant inflammation (Fig. 4.3). aGVHD typically presents as a maculopapular rash, which typically occurs within the first 100 days after HCT and affects 30–50% of transplant patients. With progression, the rash can become confluent with epidermal exfoliation (Figs. 4.4 and 4.5) or bulbous lesions. Histologically, apoptotic keratinocytes become more numerous, and basal vacuolization and interface dermatitis become more diffuse (Fig. 4.6). Severe cases can eventually resemble toxic epidermal necrolysis (TEN) with full-thickness involvement, subepidermal clefting, and complete dermal-epidermal separation and can be fatal (Figs. 4.7 and 4.8).

Recurrent or new cases with classic features of aGVHD occurring at >100 days can follow withdrawal of IS and are now termed "late acute" GVHD based on NIH criteria [10, 11]. The cumulative incidence of late aGVHD is 10% with a nonrelapse mortality of 23%, indicating the need for continued consideration of aGVHD in later posttransplant evaluation [12].

"Classic cGVHD" refers to cGVHD manifestations occurring without diagnostic features of aGVHD: an "overlap syndrome" is defined by concurrent features

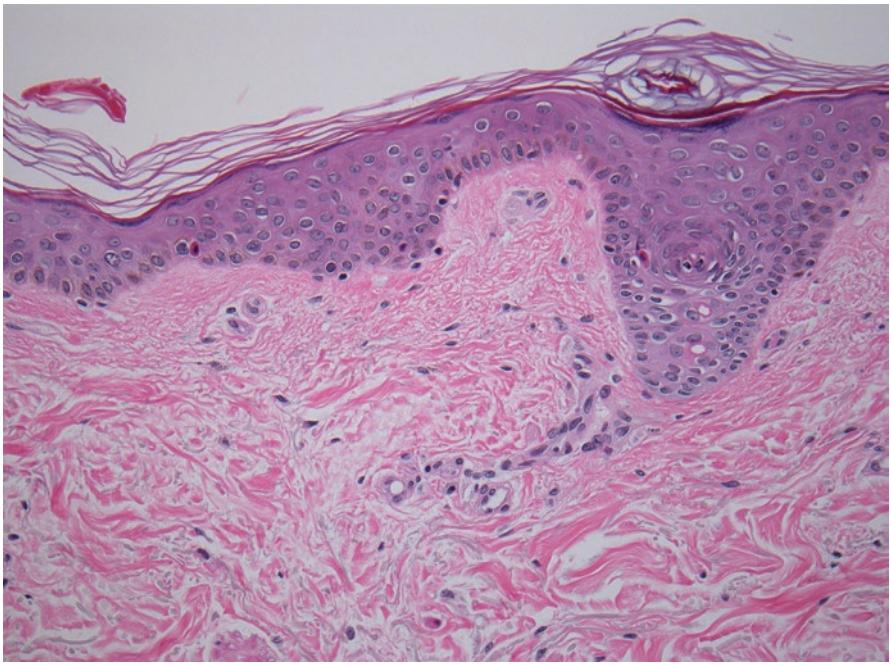

Fig. 4.3 Normal-appearing skin demonstrating GVHD of mild histologic activity with occasional scattered apoptotic keratinocytes and minimal inflammation

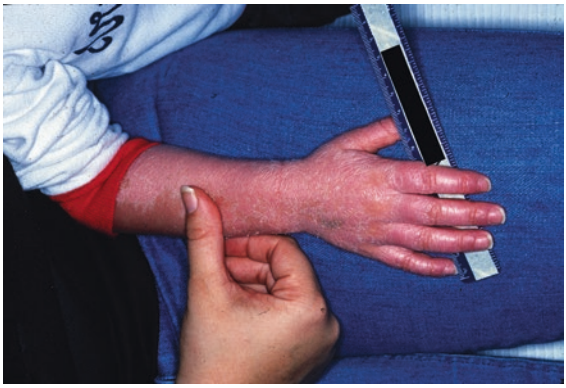

Fig. 4.4 Upper extremity of patient with diffuse rash and severe aGVHD

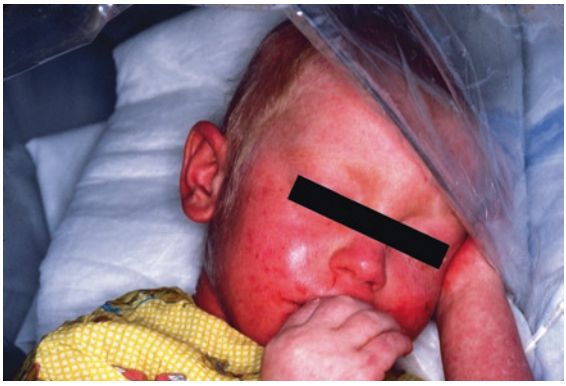

Fig. 4.5 aGVHD with diffuse edematous and erythematous rash

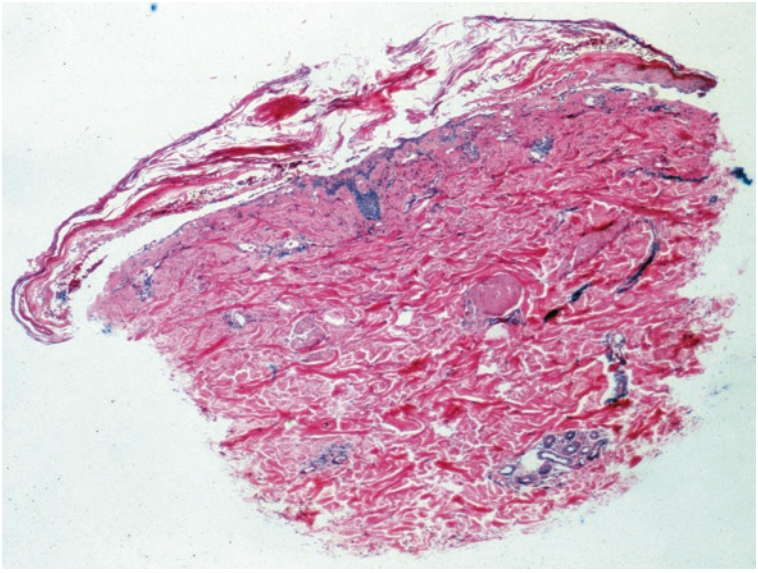

Fig. 4.6 Severe (Lerner grade IV) GVHD has necrosis and separation of the epidermis from the underlying dermis

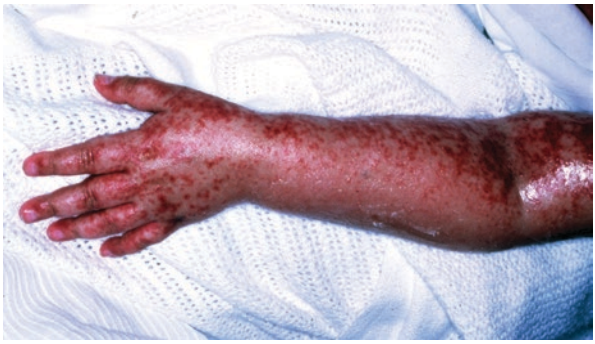

Fig. 4.7 Upper extremity of patient with TEN-like skin changes of severe aGVHD

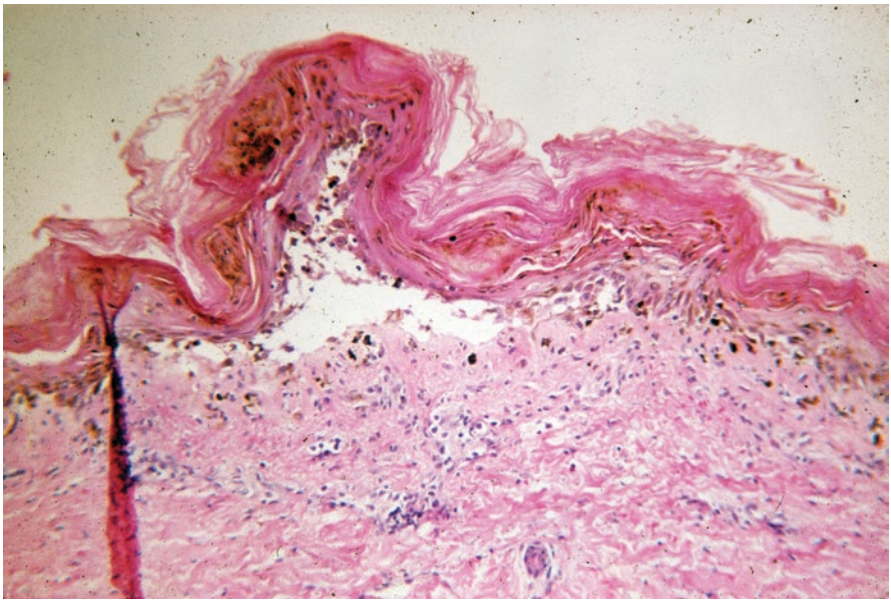

Fig. 4.8 Skin biopsy from patient with TEN-like skin changes demonstrating subepidermal clefting and full-thickness epidermal necrosis

with both chronic and aGVHD. Overlap syndrome features most often involve the skin or gastrointestinal tract and less commonly a cholestatic liver disorder. A prospective study by Pidala et al. in 2012 demonstrated the importance of recognizing the overlap syndrome as significantly higher functional impairment and subsequent adverse prognosis were observed in these patients compared to those with classic cGVHD [13].

Differential Diagnosis of Grade III/IV GVHD

Grade III/IV GVHD is characterized by extensive keratinocyte apoptosis, reticular degeneration, destruction of the basal layer, bulla formation, and eventual full-thickness epidermal destruction and ulceration. The differential diagnosis for this spectrum of histologic findings includes erythema multiforme, Stevens-Johnson syndrome (SJS), and TEN, but the specific entity cannot be distinguished based solely on histologic features and requires clinical context.

Erythema multiforme presents as acute, self-limited targetoid papules, vesicles, or plaques. Histologic evaluation shows vacuolar interface or lichenoid dermatitis, spongiosis, epidermal apoptosis of single or clustered cells often above the basal region, and clefting of the dermal-epidermal junction. Dermal eosinophils are usually absent or rare. With bullous formation, keratinocyte apoptosis becomes confluent with subepidermal clefting. Severe drug eruption manifesting as life-threatening SJS/TEN shows progression to full-thickness epidermal necrosis and blistering and may involve the oral mucosa and conjunctiva.

Chemotherapy-induced acral erythema is a form of toxic epithelial injury caused by cytoreductive conditioning. Though the acral distribution on the palms and soles is highly suggestive of conditioning toxicity, histologically it is difficult to distinguish from severe GVHD. Therefore, factoring in the type of conditioning regimen, waiting until posttransplant day 20–30 to biopsy skin has been suggested [14]. Similar histologic features of these two entities include keratinocyte atypia, vacuolar damage, dyskeratosis, apoptosis, and interface dermatitis. Current transplant protocols using reduced intensity regimens are much less likely to have such prolonged epidermal changes that overlap with aGVHD. A clinician's decision to observe a rash, treat without biopsy, or wait for biopsy before treatment is influenced by their estimated prevalence of GVHD and the desire to avoid delaying treatment of a potential case of hyperacute aGVHD [15].

Pathogenesis of aGVHD

The pathogenesis of aGVHD involves the interaction of donor T lymphocytes with host major histocompatibility complex (MHC) and/or host minor histocompatibility antigens [16]. Mismatches in MHC class or minor histocompatibility antigens are associated with increased risk of severe aGVHD. However, some degree of mismatch may contribute to the graft vs. leukemia response preventing posttransplant relapse of disease. The inflammatory effector response by donor T cells targets selective host tissues including epithelial cells of skin, intrahepatic bile ducts, and gut. Regulatory T cells (Tregs) are play a role in modulation of GVHD severity [17]. Other factors participating in GVHD include T cell costimulatory pathways, cytotoxic cytokines such as TNF-α, and other cell types including natural killer cells.

Serum biomarkers to predict the risk of GVHD and response to IS are an ongoing area of study. For example, soluble tumorigenicity-2 (ST2) has been associated with therapy resistance, and high levels were correlated with overall survival [18, 19].

Key Teaching Points

- Skin aGVHD occurs in 30–50% of HSCT patients. Factors influencing the incidence include whether the donor and recipient are the same gender, whether the donor is related or unrelated to the recipient, the age of the recipient, and prophylactic post-HSCT IS.
- Findings in early posttransplant period may overlap with the residual effects of conditioning toxicity. The posttransplant cutoff for when skin biopsy for aGVHD is evaluable is empiric but occurs much earlier with modern reduced intensity regimens.
- The clinical decision to obtain a skin biopsy to diagnosis GVHD is influenced by the constellation of clinical findings in other organs suggestive or indicative of GVHD based on the criteria of a panel of experts [20].
- The decision to biopsy an isolated rash reflects the clinical estimate of the prevalence of GVHD and the desire to avoid delaying treatment for potentially severe aGVHD.
- Histologic findings may not correlate well with clinical characteristics of the skin rash; clinically normal-appearing skin can show changes of GVHD on histologic evaluation.
- Progression of damage from mild aGVHD begins with infrequent apoptotic keratinocytes and minimal inflammation, followed by interface dermatitis with basal vacuolization and more frequent apoptotic keratinocytes.
- Severe aGVHD may progress to fulminant lesions resembling TEN.
- Presence of dermal eosinophils occurs in GVHD and, in isolation, does not favor drug reaction.
- Overlap syndrome, defined by simultaneous features of both acute and cGVHD, is significantly associated with higher morbidity and mortality.

Questions

1. Histologic characteristics of aGVHD can include the following except:
   A. Interface dermatitis with apoptotic keratinocytes
   B. Apoptosis along outer root sheath of hair follicle
   C. Acute folliculitis
   D. Perivascular dermatitis with basal epidermal vacuolization

2. The differential diagnosis when considering grade II skin aGVHD includes all of the following except:
   A. Stevens-Johnson syndrome
   B. Drug reaction
   C. Eruption of lymphocyte recovery
   D. Infection

3. Dermal eosinophils are present in which conditions?
   A. Drug eruption
   B. Erythema multiforme
   C. aGVHD
   D. A and C

Answers

1. Answer: C
2. Answer: A
3. Answer: D

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5 Leukemia Cutis and Hematologic Malignancies with Cutaneous Manifestation

Adam James Robin and Cecilia C. S. Yeung

Clinical History

In this chapter we present a case of leukemia cutis in an 18-year-old male with a history of monoblastic acute myeloid leukemia (AML) (SAB M5) in second remission. The patient received a matched unrelated donor transplant and had an acute episode of GI GVHD with diarrhea (confirmed with GI biopsy) on day 28, which resolved after treatment with prednisone, budesonide, and beclomethasone. Bone marrow aspirate on day 28 showed no morphologic or flow cytometric evidence of residual acute myeloid leukemia. Beginning on day 30, the patient had developed non-pruritic diffuse subcutaneous lesions over his chest and scalp, which were progressively worsening. Especially prominent on his chest was a raised nodule at several centimeters in diameter with associated erythema at the base of the lesion. Because of the concern of leukemia cutis, a skin punch biopsy of a scapular lesion was performed on day 37 (Figs. 5.1 and 5.2). Immunohistochemical studies of the cellular infiltrate are illustrated in Fig. 5.3. A bone marrow aspirate and biopsy 13 days later showed no morphologic or flow immunophenotypic (with a sensitivity

A. J. Robin
Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

C. C. S. Yeung (✉)
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: cyeung@fredhutch.org

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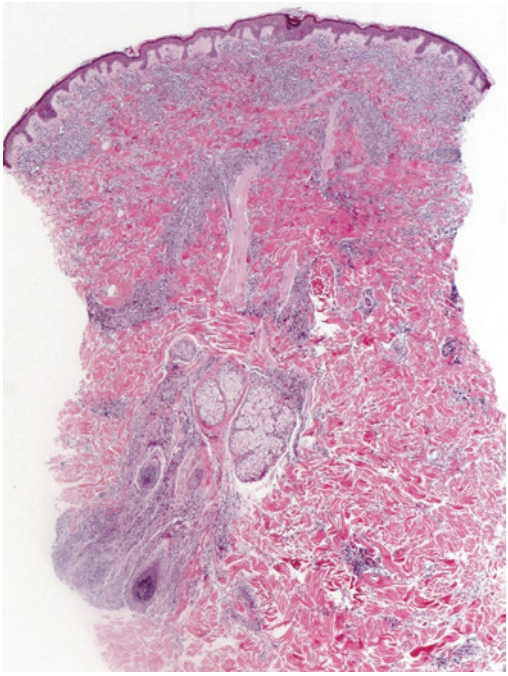

Fig. 5.1 An H&E-stained section shows heavy infiltration of the upper and mid-dermis by immature cells most compatible with leukemic blasts

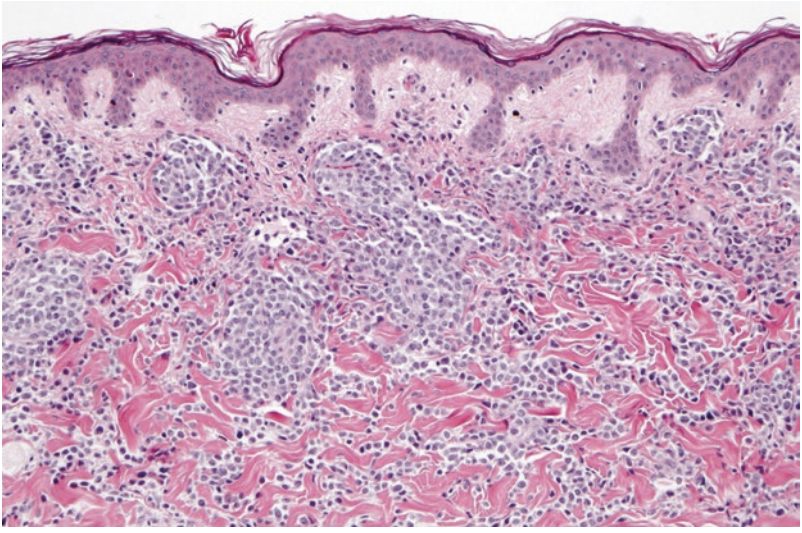

Fig. 5.2 There is a grenz (clear) zone between the dense dermal perivascular monomorphous infiltrates and the overlying epidermis

of detection of 10^{-3}–10^{-4}) evidence of residual acute myeloid leukemia. However, a marrow aspirate and biopsy from day 80 showed 72% monoblasts, which were confirmed by flow cytometry to have a similar immunophenotype to this patient's original leukemia (Fig. 5.4).

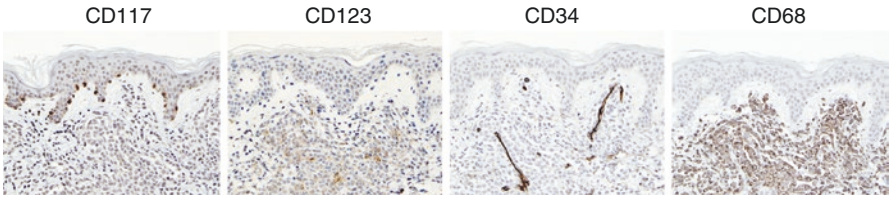

Fig. 5.3 Immunohistochemistry staining of the cellular infiltrate is positive for CD68 (monocytes), weakly positive for CD123 (a marker for plasmablastic lymphoma), and negative for CD34 and CD117 (surrogate for CD34). Typical leukemia markers such as CD34 and CD117 are commonly lost in leukemia cutis

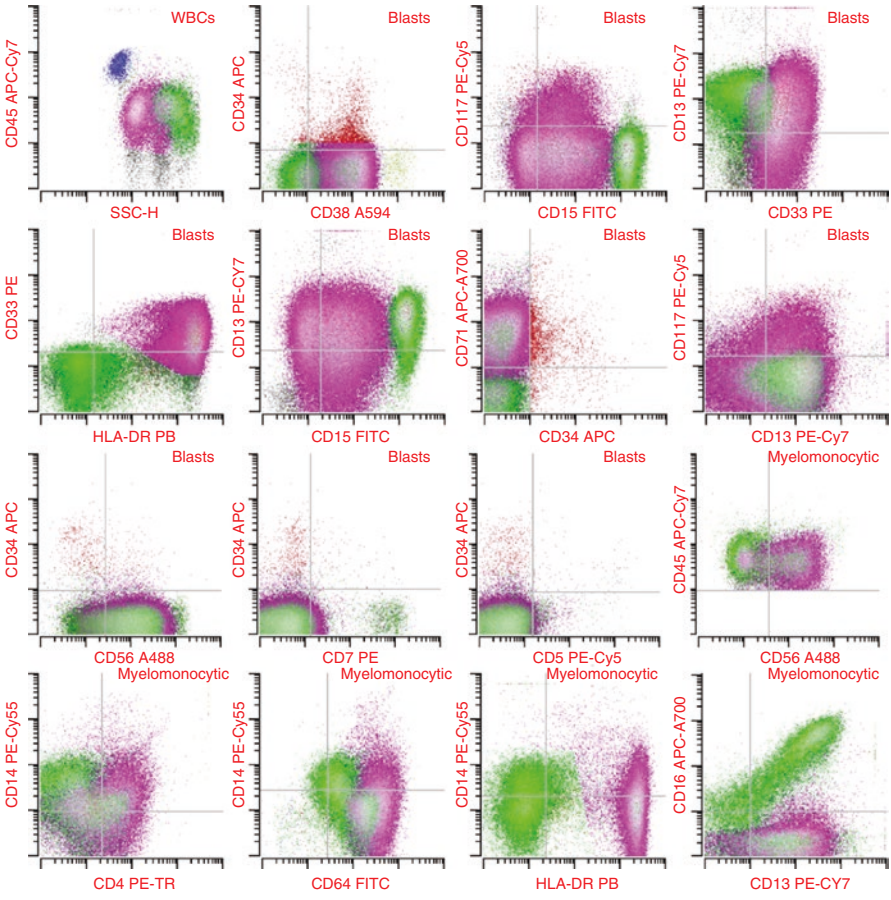

Fig. 5.4 On day 80, an abnormal immature blast population (magenta) in the patient's marrow was detected by flow cytometry that represented 50.5% of the white cells. The blasts abnormally expressed CD4, CD14 (low), CD15, CD34 (absent), CD45 (low), CD56, CD64, CD117 (absent), and HLA-DR (high) with normal expression of CD13, CD33, CD38, CD71, and CD123 without CD5, CD7, CD16, or CD19. This finding is consistent with persistent/recurrent acute myeloid leukemia of monocytic lineage

Diagnosis

Skin with leukemia cutis of AML with monocytic differentiation

Key Pathology Features

- Monotonous infiltrate
- Presence of a grenz zone
- Potential loss of original myeloid blast immunophenotypic markers
- Lack of epidermotropism

Differential Discussion

Following HSCT for hematopoietic malignancy and new-onset rashes with a mononuclear dense dermal infiltrate, diagnosis of leukemia cutis must be excluded. Our patient has leukemia cutis of AML (lesions are generally referred to as chloromas) with monocytic differentiation, which may have morphologic features overlapping with those of acute graft-versus-host disease (aGVHD). The infiltrate in leukemia cutis will be monomorphic, commonly have a grenz zone (a clear zone between the epidermis and the perivascular dermal infiltrate), and lack the prominent epithelial changes seen in GVHD. Our patient's biopsy showed dense perivascular collections of cells, but the leukemic infiltrate can also form a dense mass infiltrating the dermal collagen.

Leukemia cutis occurs in approximately 10% of myeloid leukemia, most commonly with monocytic subtypes and generally has poor prognosis [1]. Lesions of leukemia cutis can have varied appearances ranging from single to multiple violaceous-, red-, or skin-colored lesions. Often skin biopsies may arrive in pathology with other differentials [2]. The skin is rarely the only site of involvement in leukemia. In 50–90% of acute leukemia patients, there has been a prior established diagnosis of leukemia. The term chloroma is often used to encompass extramedullary manifestations of leukemia and refers to the green sheen seen on fresh-cut surfaces of the neoplastic tissue due to myeloperoxidase. This special feature provides stronger support for leukemic involvement of cutaneous sites [3].

Challenges in Diagnosing Leukemia Cutis in the Post-transplant Setting

Leukemias with monocytic differentiation more commonly involve the skin [4]. Often when myeloid leukemia involves the skin, characteristic expression of CD34, MPO, and CD117 is lost while maintaining expression of antigens more indicative of monocytic differentiation, such as CD68, CD163, and CD4 [4].

Benet et al. demonstrated that by using a combination of CD68, CD33, and MPO they could detect 100% of myeloid leukemia cutis in their study of 173 specimens [5]. In a smaller cohort, Cronin et al. had similar findings and showed that often the immunophenotype changed between the blasts in the marrow and the skin [2]. The mixed infiltrate of lichenoid GVHD may show varying degrees of monocytes and lymphocytes, which overlap with leukemia cutis (see Chap. 6). Further complicating the problem is that in many instances, when a skin biopsy is performed a sample for flow cytometry was not taken (flow cytometry allows us to identify immunophenotypic abnormalities that may confirm the neoplastic nature of an infiltrate). This often complicates the diagnosis of leukemia cutis, especially if the blasts have lost the characteristic progenitor markers of CD34 and CD117. Without flow cytometric data, it can be very difficult to distinguish with certainty between a monocytic infiltrate and monoblastic leukemia infiltrate. It is recommended that when there is strong clinical suspicion for leukemia cutis, a fresh biopsy of skin should always be collected and submitted in Roswell Park Memorial Institute medium or similar cell culturing media for flow cytometry.

GVHD with Abundant Monocytes

One morphologic presentation of aGVHD in skin biopsies when obtained near the onset of a rash is an abundance of monocytes within a pleomorphic infiltrate. Key features of florid GVHD not seen in leukemia cutis include epidermotropism with apoptosis and the absence of a grenz zone. Nishiwaki et al. (2009) performed a study of early skin biopsy near the onset of skin GVHD using IHC markers for T lymphocytes (CD3) and monocytes (CD163). They found that biopsies with dense cutaneous infiltrates in GVHD were monocyte-predominate. Moreover, they were associated with steroid-refractory GVHD and poorer overall survival in aGVHD [6]. A subsequent study by the same group shows that treatment with dexamethasone may ameliorate the effects of the macrophages on exacerbating GVHD [7]. Terakura et al. (2015) performed a case-controlled study using a CD163 semiquantitative score for dermal macrophage infiltration. They found a significant association between cases with many macrophages and increased risk of death. Nonetheless, the association between high macrophage infiltrate and severe GVHD or increased risk of death was not strong enough to apply as an independent prognostic indicator [8]. Further studies are needed to elucidate the effect that heavy macrophage infiltration has on the severity of GVHD.

Other Hematologic Malignancies Involving the Skin

A number of hematologic malignancies may present in the skin concurrently with GVHD, including AML. Clinically, the cutaneous T-cell malignancies mycosis

fungoides or Sézary syndrome (MF/SS) may mimic features of GVHD. Patients can present with scaly rashes, multiple plaques, or raised lesions. Early stages are typically limited to the skin and are often initially misdiagnosed as psoriasis. The disease has a predominantly indolent course in most patients and eventual disease dissemination to the lymph nodes and marrow. Ulceration and erythroderma can also be presenting features of the cutaneous lesions. The neoplastic infiltrate in the skin of these patients is epidermotropic and comprises small- to medium-sized atypical lymphocytes with cerebriform nuclei. In approximately a quarter of patients, circulating atypical lymphocytes are seen in the peripheral blood. Large cell transformation is generally associated with progression of the disease, but rare stage 1 cases will feature significant large cells morphology at diagnosis. The workup of MF/SS can be challenging, and often definitive diagnosis of a T-cell lymphoma is not possible in the early stages. Having a clinical suspicion of GVHD in the post-transplant setting can further confound the situation. The immunophenotype of MF/SS is generally CD4 positive although up to 20% are CD8 positive. There is often loss of one or more of the remaining T-cell antigens including CD2, CD3, CD5, and CD7. Molecular studies for T-cell clonality are not required for diagnosis and may in certain cases be misleading as inflammatory skin disorders as well as GVHD can show clonal signatures. In the patient scenario, where a clonal T-cell molecular signature is known from another definitive lesion, a comparative T-cell clonality study in the skin can be helpful in confirming skin involvement by T-cell lymphoma.

MF/SS comprise approximately half of the cutaneous T-cell lymphomas. Other common T-cell lymphomas that present in the skin include primary cutaneous γδ T-cell lymphoma NOS, primary cutaneous CD30+ lymphoproliferative disorders (lymphomatoid papulosis and cutaneous anaplastic large cell lymphoma), peripheral T-cell lymphoma, NOS, as well as other provisional entities highlighted in the updated 2016 WHO [9]. Less likely to confound the diagnosis of GVHD are B-cell lymphomas as these are generally associated with infiltrates that are more dense and monotonous, with some identifiable morphologic features (such as nodular proliferative pattern, sheets of large atypical cells, or plasmablastic morphology) or immunohistochemical markers. B-cell lymphomas which may involve the skin include primary cutaneous follicular center lymphoma, cutaneous marginal zone lymphoma, primary cutaneous diffuse large B-cell lymphoma leg type, and plasmablastic lymphoma.

Teaching Points

1. Leukemia cutis most commonly occurs with AML with monocytic differentiation.
2. AML leukemia cutis commonly loses expression of canonical markers CD34, MPO, and CD117, retaining CD68, CD4, and CD163, taking on an immunophenotype very similar to monocytes.

3. Morphologic features of aGVHD may include a dense inflammatory infiltrate that can have a high monocytic component which may overlap with leukemia cutis.
4. The features distinguishing GVHD from leukemia cutis are:
   (a) GVHD may include more frequent apoptotic basal keratinocytes; apoptotic change is not a feature of leukemia cutis.
   (b) Leukemia cutis has more monotonous dense infiltrate.
   (c) Leukemia cutis has a grenz zone between the infiltrate and the epidermis.
5. Immunophenotyping of dermal macrophages with IHC demonstrates a range of macrophages from few to many. Cases of aGVHD with increased dermal monocytes may be associated with more severe disease, treatment refractoriness, and increased risk of death.

Questions

1. A 30-year-old woman presents on day 50 post-transplant with a diffuse rash over her arm, back, and chest. The biopsy performed on the left arm shows a monocytoid cell-rich infiltrate with expression of CD68 and CD163. What would your differential include?
   A. GVHD
   B. Leukemia cutis
   C. Infection
   D. Macrophage activation syndrome
   E. All of the above

2. The first manifestation of relapsed acute leukemia may be in an extramedullary location.
   A. True
   B. False

3. When leukemia involves the skin, what markers are commonly lost?
   A. CD34
   B. CD117
   C. CD68
   D. CD123
   E. A and B
   F. All of the above

Answers

1. Answer: E
2. Answer: A
3. Answer: E

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Lichenoid Inflammatory Phase of Chronic Skin GVHD

6

Oliver H. Chang, Marie E. Perrone, Adam James Robin, and Howard M. Shulman

Clinical History

A 69-year-old man with MDS/AML received a HSCT from a DQ mismatched unrelated donor. He received MMF and cyclosporine for GVHD prophylaxis. On day 40 he developed a diffuse, erythematous, maculopapular skin rash primarily on his chest, face, abdomen, and back, with sparing of extremities. The rash exhibited discrete lesions clinically worrisome for a herpetic eruption. No changes had occurred in the medications prior to the onset of the rash. Marrow biopsies and flow cytometry performed 2 weeks before and 2 weeks after the skin biopsy showed no evidence of relapse. Punch biopsy of the rash was performed on day 45. The workup for both herpes simplex and zoster were negative by IHC and virologic tests. The biopsy contained changes most consistent with early lichenoid GVHD

O. H. Chang
Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

Department of Anatomic Pathology/Dermatopathology, VA Puget Sound Health Care System, Seattle, WA, USA
e-mail: ochang@uw.edu

M. E. Perrone · A. J. Robin
Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA
e-mail: perronem@uw.edu

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

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

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

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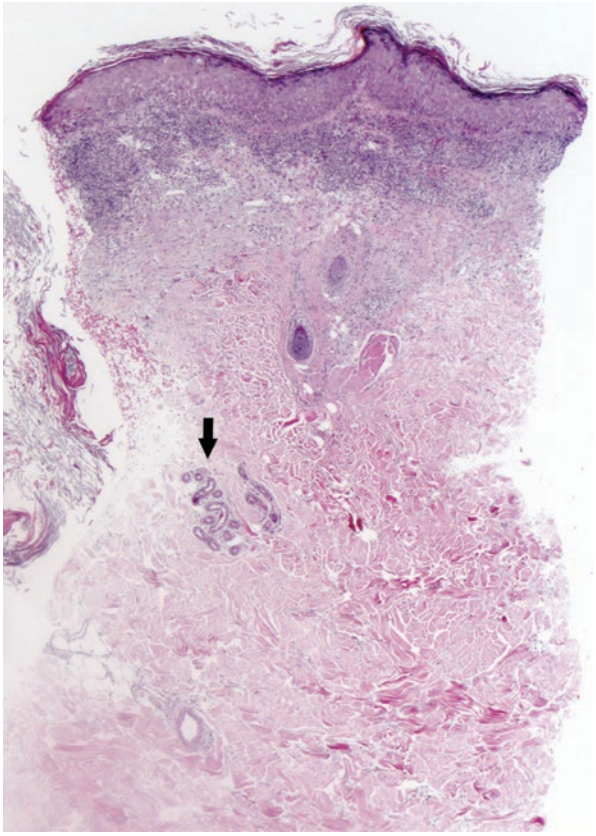

Fig. 6.1 Low-power view of skin biopsy on day 45. The biopsy has a lichen planus-like appearance with a thickened (hyperplastic/acanthotic) epidermis. The papillary dermis contains a dense band-like dermal infiltrate. The chronic infiltration extends down around the dermal adnexa, the hair follicles in the papillary dermis, and a cluster of eccrine glands (arrow) in the mid-reticular dermis

(Figs. 6.1 and 6.2). He was started on a course of high-dose prednisone and maintained on cyclosporine and MMF. When evaluated in dermatology clinic on day 80, he had erythema on his face and ears, white adherent oral plaques, and morbilliform papules on the torso and extremities. The palms and soles were spared. He was discharged on day 115 on treatment protocols for his cGVHD. He subsequently developed late-onset gut GVHD in the colon, superimposed on mild oral and skin cGVHD without sicca. He received IV prednisone and extracorporeal photopheresis. At 2 years, he experienced a flare of gut GVHD, requiring continuous low-dose prednisone and methotrexate. At 5 years he developed fasciitis in his arms and leg, with focal areas of stable sclerosis on the chest, forearms, and back. There was some impairment of mobility in the wrist. There was no sicca in the mouth or eyes, but he had a subtle lichenoid lesion on the lower lip.

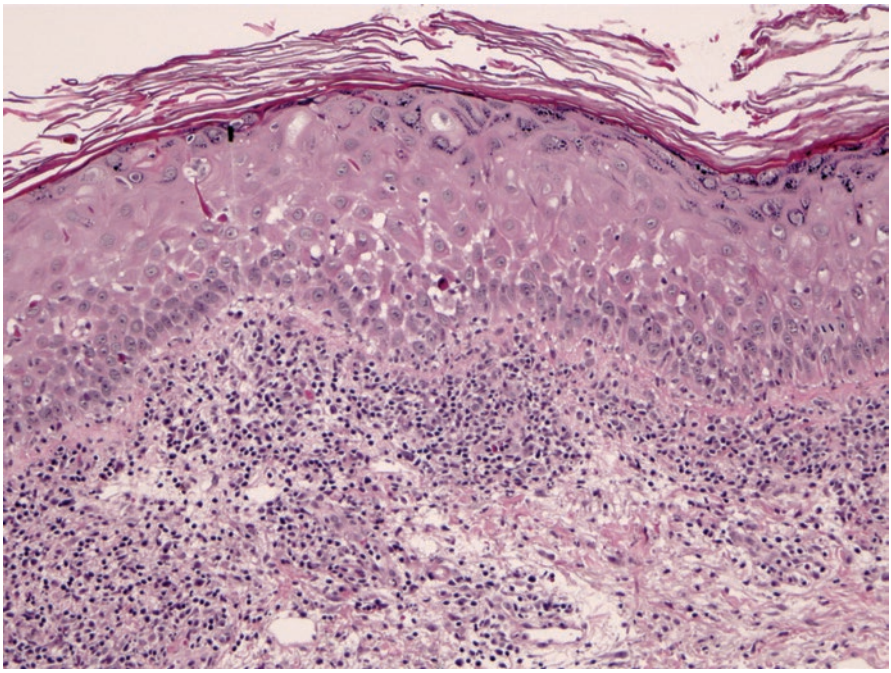

Fig. 6.2 Higher-power view displays the lichenoid epidermal alterations of hyperkeratosis (thickened stratum corneum), increased granular cell layer, hypergranulosis, and spongiosis, with edematous separation between the keratinocytes. Within the epidermis are scattered intraepidermal lymphocytes and apoptotic hypereosinophilic keratinocytes. The top portion of the papillary dermis lacks a cell-free zone (grenz zone) or subepidermal blister formation. The underlying dermal infiltrate contains a mixture of histiocytes, lymphocytes, and occasional eosinophils

Diagnosis

Lichenoid dermatitis favor early manifestation of inflammatory/lichenoid phase of chronic GVHD disease with generalized involvement of skin, mouth, and sicca syndrome.

Key Pathology Features

- Epidermal hyperkeratosis, hypergranulosis, and acanthosis (hyperplasia)
- Apoptotic keratinocytes along basilar layer and within lower stratum spinosum with disruption of epidermal melanin unit (Fig. 6.3)
- Band-like superficial dermal infiltrate of histiocytes, lymphocytes, and occasional eosinophils (Fig. 6.3)
- Sawtooth shortened rete ridges (Fig. 6.4)

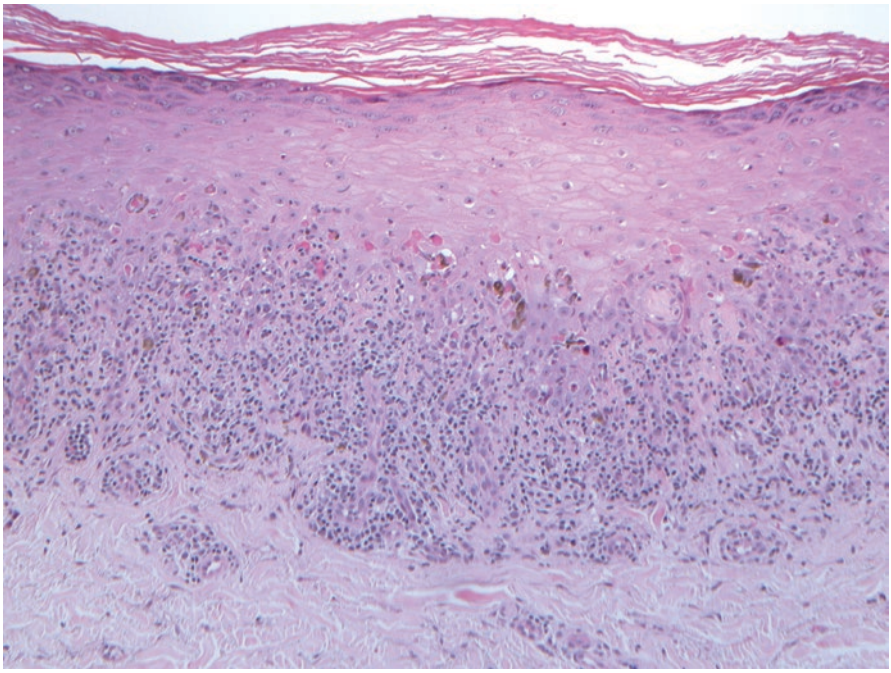

Fig. 6.3 Epidermal features of lichenoid cGVHD: Day 147 biopsy shows marked lichenoid acanthosis with marked apoptosis of basilar keratinocytes and disruption of epidermal melanin unit with melanin in stratum Malpighian and marked band-like dermal inflammatory infiltrate

- Inflammation, apoptosis, and eventual destruction of dermal adventitial appendages, pilosebaceous units, and eccrine ducts and deeply located eccrine glands (Fig. 6.4)
- Absence of sclerotic alterations in reticular dermis in early phase of lichenoid cGVHD (Fig. 6.4)

Differential Discussion

When an allogeneic recipient presents with a lichenoid rash, biopsies are recommended to confirm cGVHD if clinical signs are confined to internal organs or if assessment of GVHD activity is obscured by prior changes. In a tertiary referral center, 7% of the patients without prior histologic confirmation of GVHD had been incorrectly diagnosed and treated for cGVHD instead of other dermatitides [1], e.g. transient acantholytic dermatosis (Grover disease) [2]. In the polypharmacy milieu of HSCT, drug reaction is always a possibility and would be suspected if there were additions to the patient's medication regimen before the development of a rash.

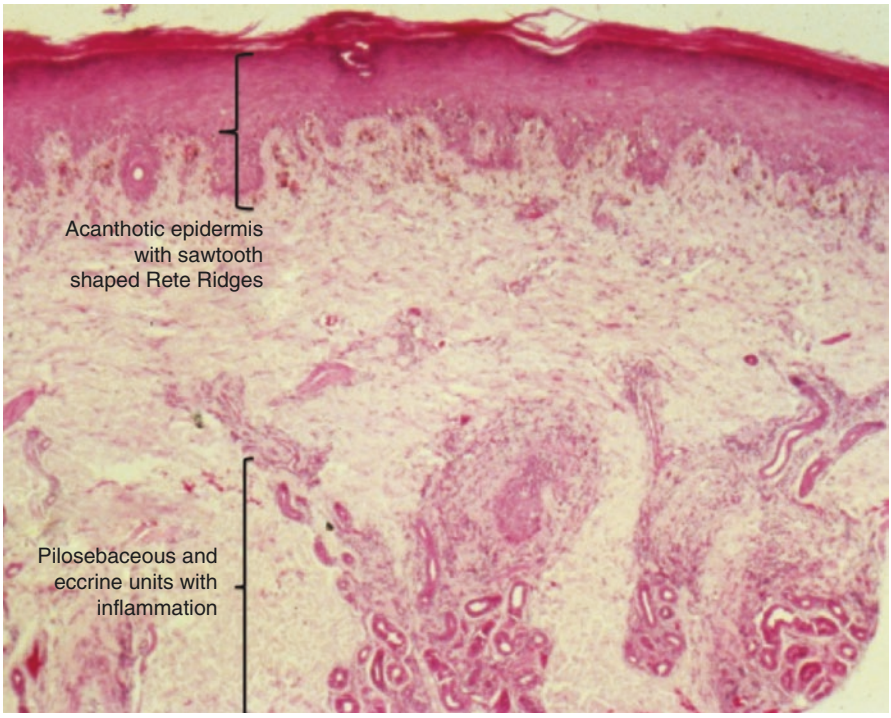

Fig. 6.4 Full thickness punch biopsy contains all of the histologic features of early lichenoid cGVHD. Epidermis has hyperkeratosis, hypergranulosis, and acanthosis with basilar apoptosis and sawtooth-shaped shortened rete ridges. A dense band-like chronic infiltrate fills the papillary dermis and extends down along the adventitial surrounding pilosebaceous units and eccrine glands

Severe drug reactions resulting in lichenoid changes or erythema multiforme with extensive acantholysis along the basal layer can be difficult to distinguish from GVHD. Morphologic features favoring cGVHD are lichenoid epidermal changes and inflammation with apoptosis within adnexal structures, in particular the eccrine glands and follicular outer root sheath (Chap. 3). In some patients, the initial appearance of cutaneous cGVHD is triggered in areas of sun exposure or within a viral exanthem (Chap. 17). The presence of blood eosinophilia and eosinophils in the skin biopsy does not rule out GVHD, as both occur in acute and chronic GVHD [3, 4].

Cutaneous cGVHD has a variety of appearances as acral keratotic papules (Fig. 6.5), a scaly erythematous rash (Fig. 6.6), papulosquamous plaques (Fig. 6.7), erythroderma, keratosis pilaris, dermatomyositis-like rash, keratitis folliculitis, ichthyosiform (fish-scale-like) dermatitis, lichen sclerosus et atrophicus, psoriasiform eruption, and morphea/fasciitis [5–9] (Chap. 7). Many of these gross appearances are illustrated in color photographs in Hymes et al. [10] and the textbook *Chronic Graft Versus Host Disease: Interdisciplinary Management* [11].

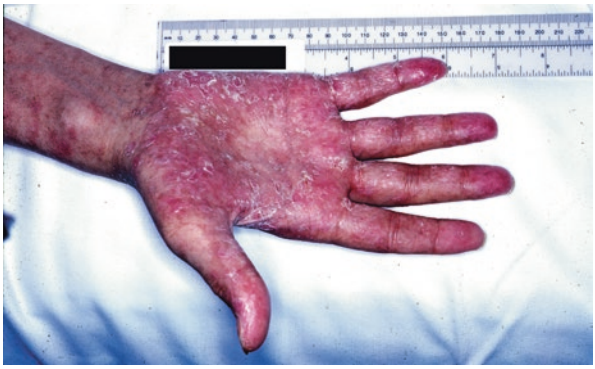

Fig. 6.5 Caption day 131 the erythematous palmar surface has raised scaly papules. The erythema is due in part to the dense palmar concentration of involved eccrine glands

The initial classification of skin cGVHD was based on the gross and histologic findings in a cohort of 19 Seattle patients transplanted in the 1970s. CGVHD was described as a pleomorphic biphasic entity separated into an early stage with extensive/generalized involvement, a localized stage, and a later-occurring sclerotic stage. Today, cGVHD is conceptually viewed as a continuum with an earlier inflammatory (lichenoid) stage and a later fibrotic (sclerotic or sclerodermatous) stage [12, 13]. CGVHD affects approximately 30–40% of all long-lived HSCT survivors [14]. After excluding leukemic relapse in long-lived survivors, it is the major cause of morbidity and mortality, evident by their frequency in many of this book's case histories. The 2005 NIH consensus classification of cGVHD distinguished aGVHD from cGVHD based on the presence of specific histologic and clinical manifestations rather than the empiric guidelines of events that occur after day 100 (see Table 1.1 of Chap. 1 and Table 2.1 of Chap. 2).

Lichenoid cGVHD damages or destroys a spectrum of epithelia resulting in alopecia areata, dystrophic nails (Figs. 6.8, 6.9, and 6.10), dyspigmentation, sicca syndrome (Chap. 17), and mucositis of oral mucosa, esophagus (Chap. 12), conjunctiva, and anogenital region. All phases of cGVHD as well aGVHD may be present concurrently at different biopsy sites. The usual progression of changes in refractory lichenoid cGVHD includes epidermal atrophy, effacement of rete ridges, injury to dermal adnexa, and pandermal sclerosis (Figs. 6.11 and 6.12). Serial skin biopsies that demonstrate progressive dermal fibrosis—even if inflammation and apoptotic changes are minimal to absent—indicate active GVHD. Immunosuppressive treatment may lead to an arrest in dermal fibrosis, resulting in poikiloderma without pandermal sclerosis (Figs. 6.13, 6.14, and 6.15).

The NIH consensus conferences also recognized an overlap syndrome of late-onset acute skin and gut GVHD (LOaGVHD) superimposed on existing cGVHD. LOaGVHD can present as either a persistent, de novo, or recurrent entity. It develops in 11% of survivors after 2 years [15]. Our patient described above had LOaGVHD. There are no histologic features which distinguish classic aGVHD from LOaGVHD [16]. The majority of clinical studies indicate that LOaGVHD

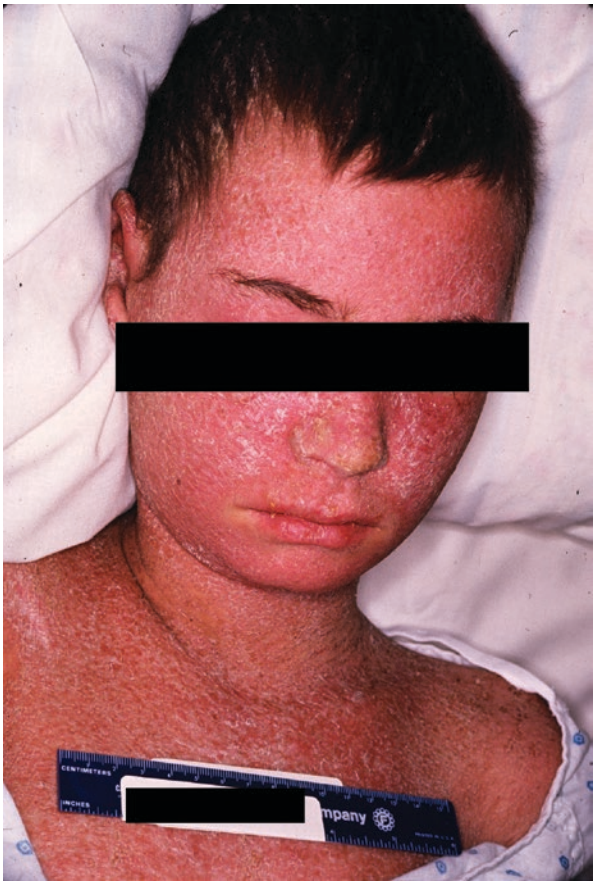

Fig. 6.6 On day 150 in this patient, early inflammatory/lichenoid cGVHD manifested as a scaly, erythematous rash

portends a worse prognosis [15, 17, 18]. There are some biological differences between aGVHD and LOaGVHD. Holtan et al. found elevated circulating angiogenic factors at the onset of LOaGVHD that were threefold higher than in classic aGVHD and not elevated in cGVHD [16]. Austrian investigators found that the T cells isolated from classic acute, lichenoid cGVHD and sclerotic cGVHD skin lesions had differences in the T cells isolated from the biopsies in their phenotypic, cytokine expression, and functional characteristics [19].

In summary, cGVHD encompasses a wide range of clinical presentations and carries significant morbidity and mortality in allogeneic recipients. The skin is the most commonly affected organ. Lichenoid GVHD represents the predominate variant of cGVHD, presenting with lichen planus-like lesions with erythematous or violaceous papules/plaques. Microscopic findings may mimic lichen planus but are often nonspecific. The differential diagnosis includes (but is not limited to) drug reaction, viral exanthem, erythema multiforme, and connective

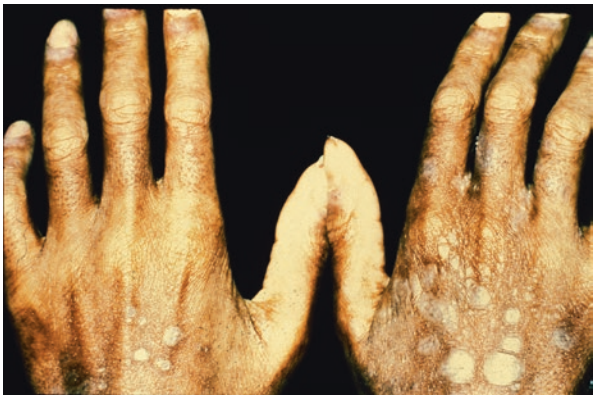

Fig. 6.7 Lichen planus-like plaques on the back of hands

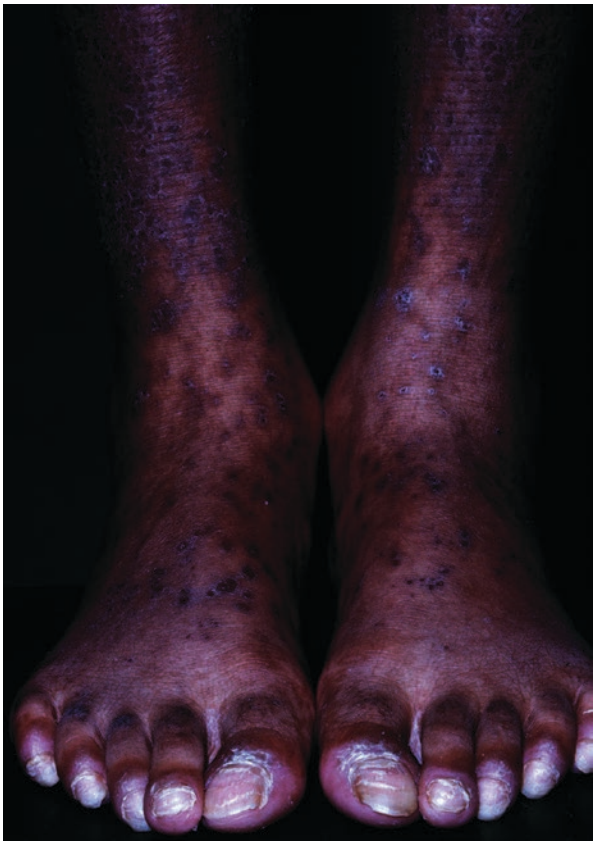

Fig. 6.8 Feet with dystrophic toenails

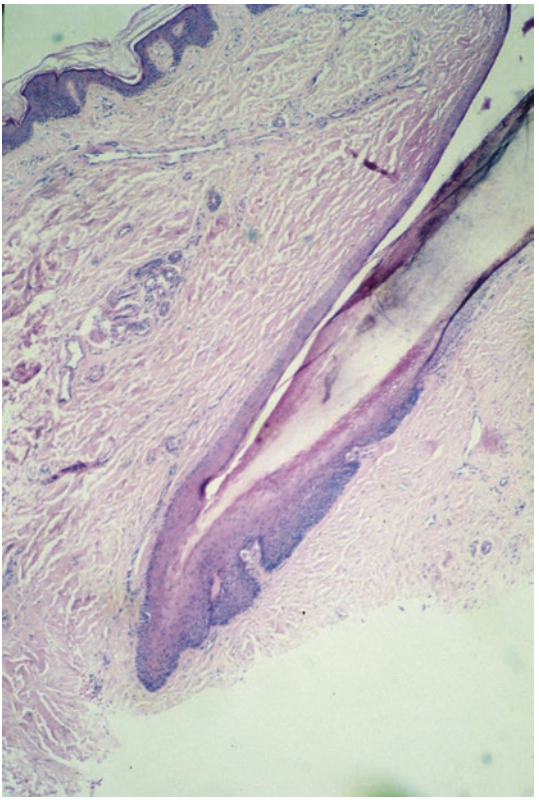

Fig. 6.9 Low-power view of nail bed

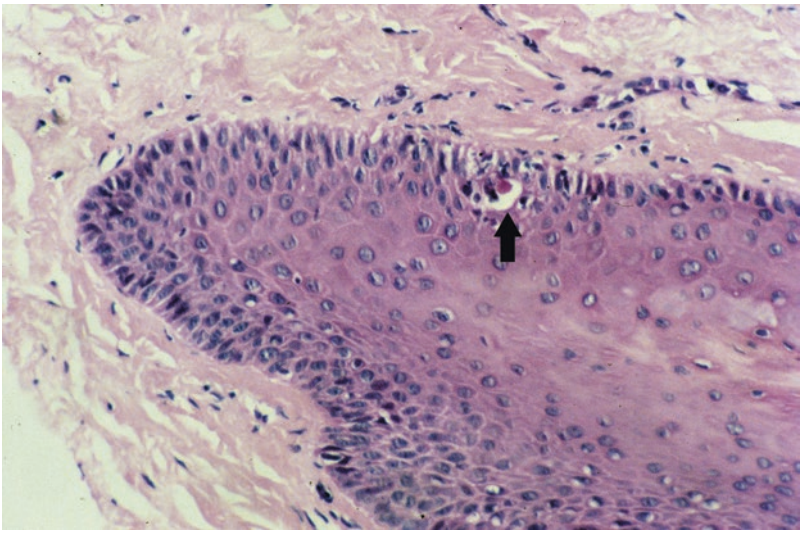

Fig. 6.10 High power shows apoptosis in the root matrix area responsible for nail growth (arrow)

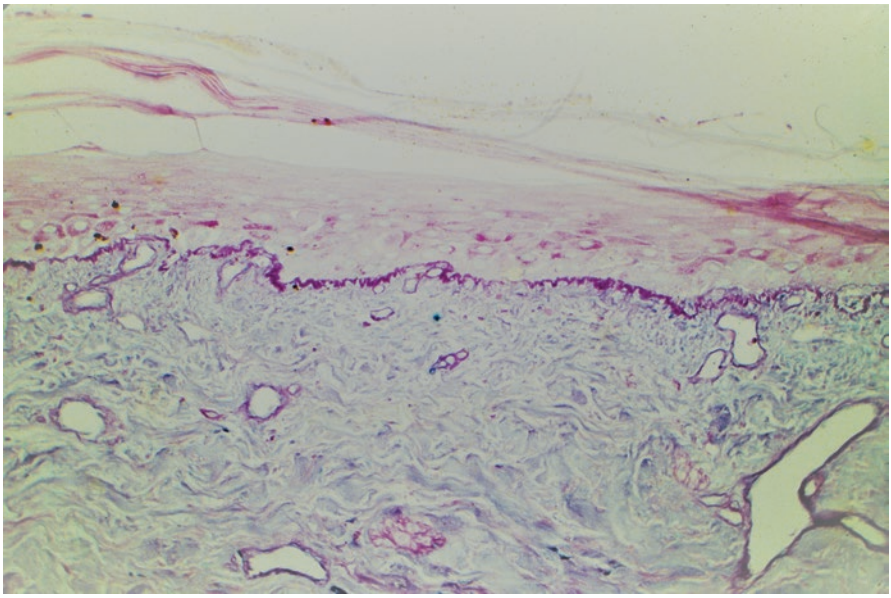

Fig. 6.11 Epidermis has flattened keratinocytes, thickened irregular basement membrane zone, and loss of rete ridges. Papillary dermis is fibrotic with ectatic venules. PAS-alcian blue stain

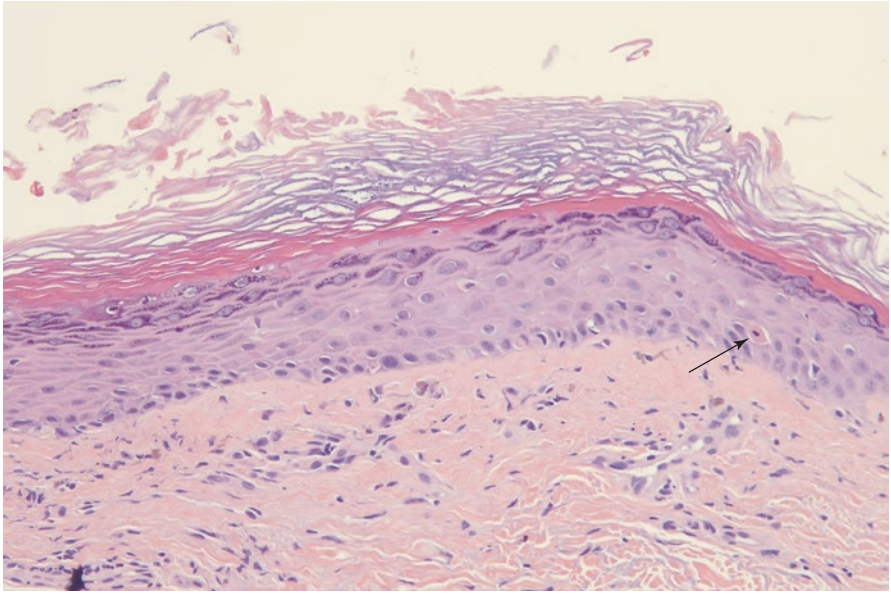

Fig. 6.12 Day 201 active lichenoid cGVHD has hyperkeratotic acanthotic epidermis with apoptosis (arrow) along straightened epidermal border. The fibrotic papillary dermis lacks band-like inflammation

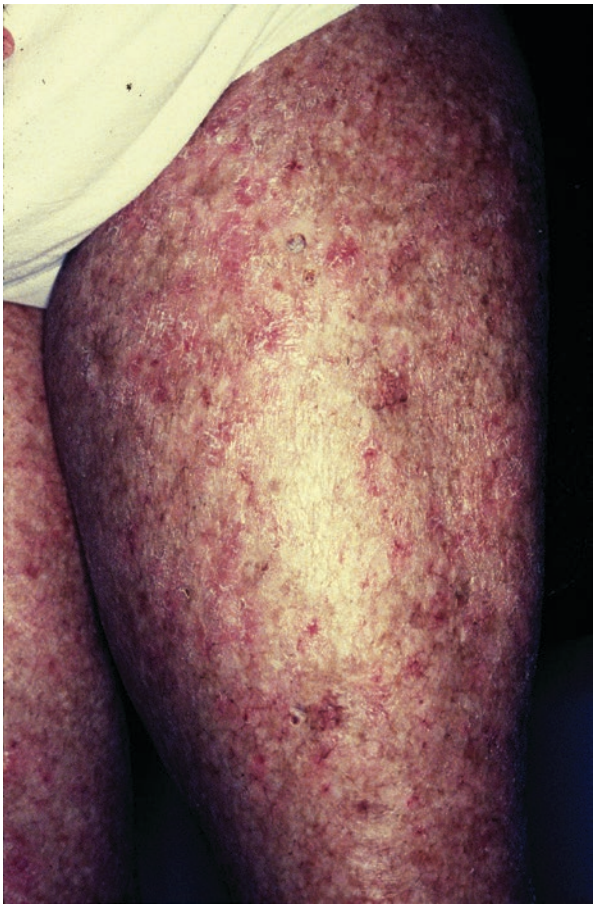

Fig. 6.13 Poikiloderma on day 307. Hyper- and hypopigmentation of the thin epidermis with some underlying fibrosis and vascular ectasia

tissue disease. If unresponsive to a variety of IS treatments [20], skin manifestations may progress to a sclerotic phase, characterized by clinical findings of scleroderma, morphea, or fasciitis. In the current milieu, clinicians are less inclined to obtain skin biopsies for histologic evaluation of cGVHD if the clinical features satisfy the NIH clinical diagnostic criteria [15]. If clinical lesions are heterogeneous, multiple biopsies are encouraged, as different phases of cGVHD may be present. LOaGVHD represents an important category in which there is presence of aGVHD in a patient with cGVHD. This entity signifies a worse prognosis[15, 17, 18].

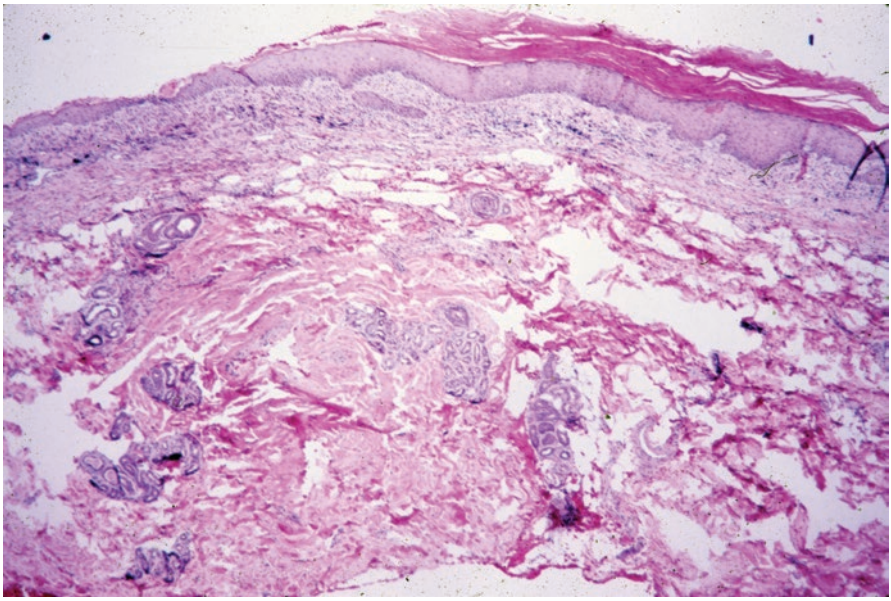

Fig. 6.14 Skin biopsy from day 610 shows transition in epidermal changes. On the left, the epidermis is atrophic, while on the right side, the epidermis has lichenoid changes with hyperkeratosis and acanthosis. Both regions lack rete ridges and abut an edematous papillary dermis. Eccrine glands are preserved but lack surrounding fat. Pilosebaceous units are absent

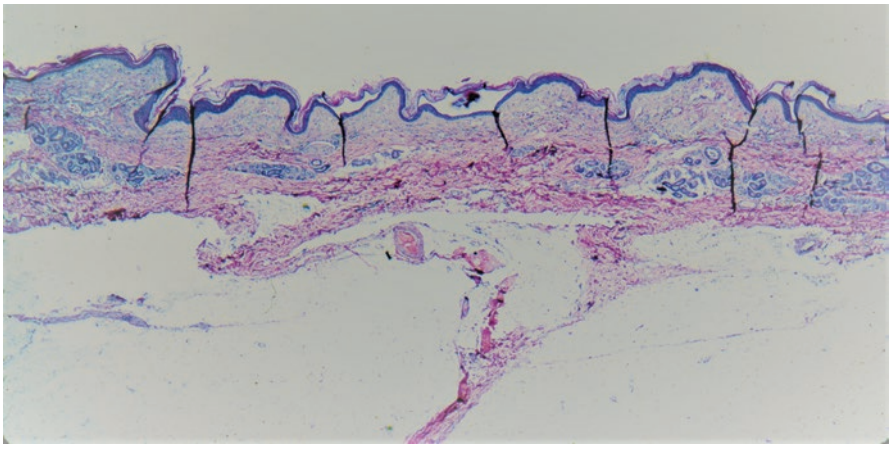

Fig. 6.15 Poikiloderma on day 610 after prolonged treatment with steroids. Epidermal and dermal atrophy and edematous papillary dermis has increased pigmentation and vascular telangiectasia. The eccrine glands are spared, and there is no sclerosis in the lower reticular dermis

Teaching Points

- The onset of cGVHD may be progressive, as a continuation of aGVHD, recurrent after a quiescent hiatus of inactive aGVHD, or de novo occurring without any prior aGVHD.
- The onset of cGVHD may first be triggered in an area of skin with sunburn or a viral exanthem.
- The cutaneous changes of cGVHD in the lichenoid phase may not be uniform.
- AGVHD and cGVHD both damage the epidermis, pilosebaceous units, and sweat ducts (acrosyringium).
- Inflammation and fibrous encasement of eccrine units and surrounding fat and dermal nerves indicate cGVHD.
- With IS treatment, manifestations such as the lichenoid band-like infiltrate are lessened, and destruction of eccrine units may not occur.
- Concurrent skin biopsies obtained at different sites may have different phases of cGVHD.
- The overlap syndrome refers to aGVHD with skin and gut involvement concurrent with cGVHD.
- Skin biopsies, regardless of type, should be full thickness in order to demonstrate alterations to the dermal collagen, eccrine glands, and subcutis.
- cGVHD activity is histologically recognized by finding inflammation and/or apoptosis of targeted structures. It may be difficult to distinguish static changes, epidermal atrophy, destruction of dermal adnexa, and fibrosis without inflammation or apoptosis, from active GVHD.
- Nails are often destroyed by cutaneous cGVHD directed at the generative proliferation zone of the nail plate. Features of nail cGVHD are similar to those seen in lichen planus and include atrophy, longitudinal ridging, and ulceration.

Questions

1. What features separate acute from chronic cutaneous GVHD?
2. How do you classify patients with cutaneous cGVHD who subsequently develop superimposed diarrhea, hematochezia, and biopsy-proven gut GVHD?
3. What changes occur in lichenoid cGVHD skin histology after IS treatment?

Answers

1. Answer: Lichenoid epidermal changes, inflammation of the eccrine glands (syringitis), and dermal fibrosis
2. Answer: Overlap syndrome in which the aGVHD component can have a persistent recurrence or late de novo appearance.
3. Answer: Usually inflammation and apoptosis are greatly diminished to absent. Dermal fibrosis may be arrested. The typical picture includes poikiloderma, with

dyspigmentation epidermal atrophy and telangiectasias. There is often atrophy of the dermis secondary to prolonged exposure to steroids.

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Cutaneous Chronic GVHD: Sclerodermatous and Morpheic Variants

7

Teresa S. Hyun and Howard M. Shulman

Clinical History

The patient is a 13-year-old boy who was transplanted for thalassemia major. On posttransplant day 17, he developed a mild aGVHD of the skin that resolved with steroids by day 21. At 6 months, he had de novo onset of generalized cGVHD involvement of the skin, hair, and nails, oral-ophthalmic sicca, and dyspigmentation. When first seen in our clinic at year 3, he had sclerodermatous skin with diffuse hidebound sclerosis, leukoderma or hyperpigmentation, and alopecia. There were contractures associated with ulcers involving his wrists, elbows, knees, and ankles. His immunosuppresive (IS) treatment included prednisone, thalidomide, cyclosporine, cytoxan, and azathioprine with waxing and waning responses (Figs. 7.1 and 7.2). Serial skin biopsies obtained after day 832 displayed some differences in the extent and depth of dermal sclerosis. All biopsies showed epidermal atrophy, destruction of sampled pilar units, and most lacked eccrine glands (Fig. 7.3). The day 948 skin biopsy had signs of ongoing cGVHD activity with a rare apoptotic change in the atrophic epidermis and some chronic inflammation.

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

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

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

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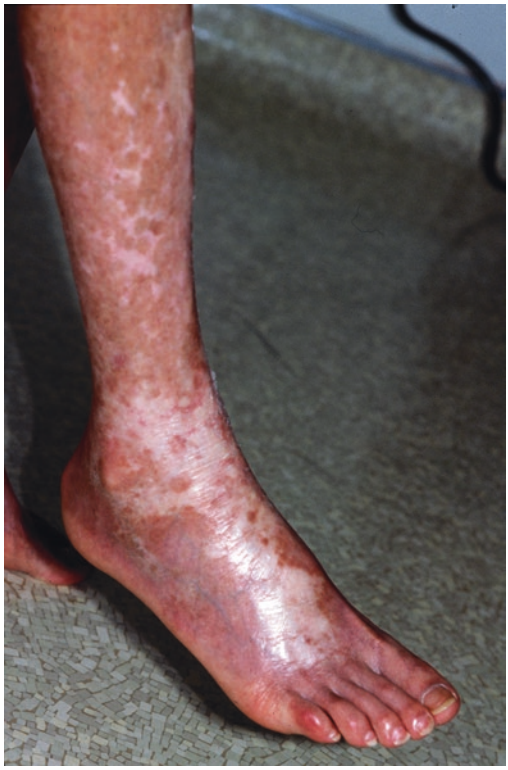

Fig. 7.1 Sclerotic cGVHD in the lower extremity has atrophic skin with hyperpigmentation and leukoderma. The skin is hidebound and has contracture around the ankle joint

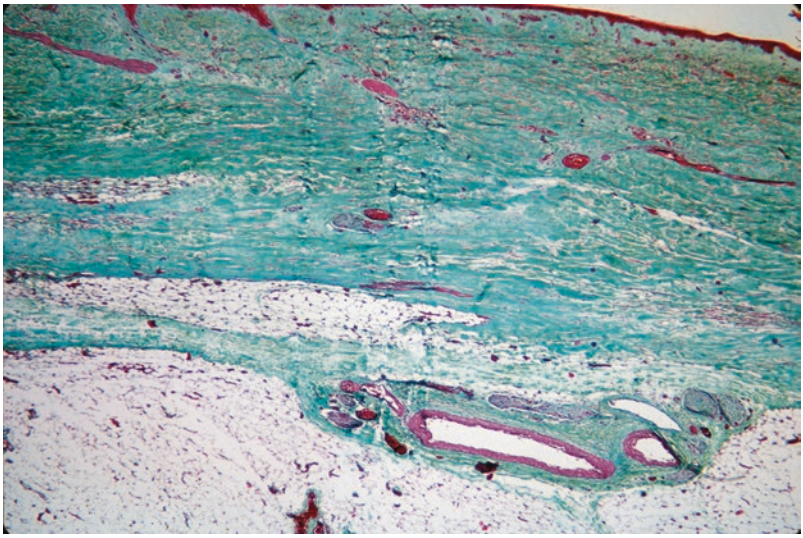

Fig. 7.2 Trichrome stain of a biopsy from sclerodermatous skin. The skin has pandermal sclerosis, which extends into the subcutis and envelopes an artery. The sclerotic collagen has straightened homogenized (waxy) bundles. The pilar units are destroyed, leaving behind only arrector pilorum. The eccrine glands, now repositioned into the upper dermis, are surrounded by fibrosis

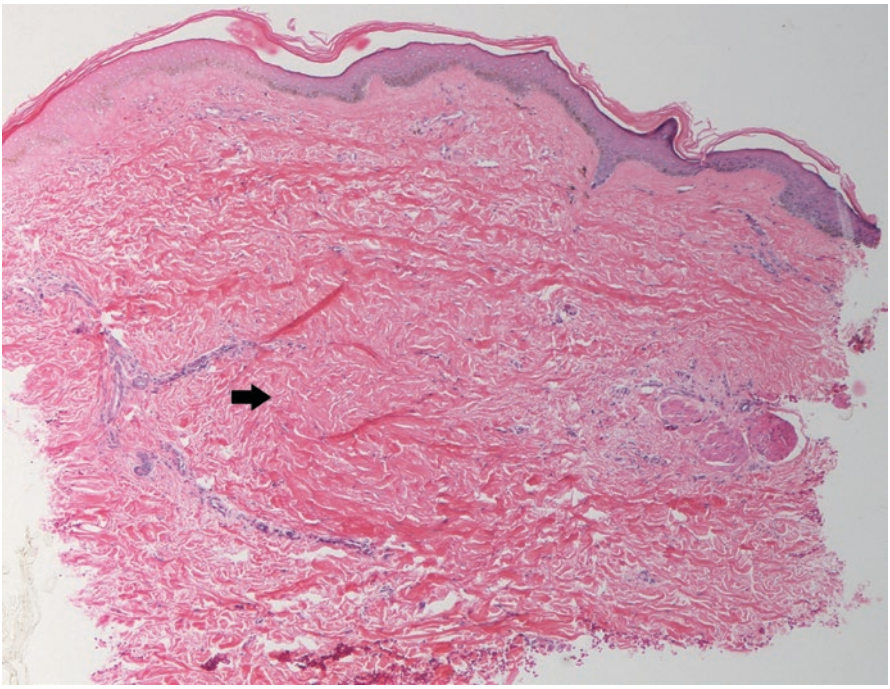

Fig. 7.3 Morpheic cGVHD (H&E stain). The mildly hyperkeratotic epidermis has a heavily pigmented and straightened basal layer without rete ridges. The papillary dermis is fibrotic, and there are no dermal appendages. There is no associated inflammation. The reticular dermis contains straightened collagen bundles in its upper portion. A large irregular, nodular area of deep sclerosis is in the mid- to lower dermis (arrow). At the edge of the section lies a cluster of arrector pylori. Biopsy from other sites had more uniform sclerosis

Diagnosis

Chronic GVHD of the skin, cutaneous sclerosis (CS) type

Key Pathology Features

- Lichenoid cGVHD progresses downward from the papillary dermis to subcutis.
- Straightening, thickening, and homogenization of collagen bundles (sclerosis) throughout the reticular dermis, eventually leading to pandermal sclerosis.
- Loss of adipose tissue surrounding the eccrine units (entrapment), atrophy of the adnexal structures, and entrapment of nerves and arteries in subcutis.

- Panniculitis along the dermal-subcutaneous interface with fibrotic widening of the dermis.
- Morphea starts as focal intradermal areas of sclerosis in the mid- or lower reticular dermis without involvement of overlying epidermis or adnexa.
- Over time, morpheic sclerosis extends upward to papillary dermis until there is pansclerosis.
- Eosinophilic fasciitis has edema and fibrosis of the deep fascia and subcutaneous septa with a mixed inflammatory infiltrate.
- Different phases of cutaneous cGVHD can occur synchronously.
- Rate of progression may be halted by IS treatment.

Discussion

Sclerotic cGVHD affects approximately 20% of patients with cGVHD [1]. It causes substantial disability and reduces quality of life but does not increase the probability of recurrent malignancy. There is some data to suggest that cGVHD patients have lower rates of relapse, attributed to the higher graft-versus-leukemia (GVL) effects [2]. Sclerotic cGVHD is defined clinically by the NIH consensus criteria as sclerosis, contractures, and/or fasciitis [3]. The histologic evolution in the skin to the sclerotic state is a continuum. We have arbitrarily included this evolution into this chapter even though it clearly begins soon after the onset of cGVHD. There is no clear milepost that indicates when a biopsy should be reclassified from lichenoid to sclerotic phase of cGVHD. The evolution to pandermal sclerosis may be modified by IS, and variability exists from different biopsy sites.

There are two separate pathways of cGVHD that lead to dermal sclerosis. The most common pathway proceeds from the lichenoid inflammatory stage and follows the downward extension of dermal fibrosis toward the subcutis (Fig. 7.4) [23]. Within several weeks after the onset of cGVHD, the dermal-epidermal border becomes straightened with loss of the rete ridges, and pilosebaceous units are destroyed. Fibrous remodeling begins in the papillary dermis with variable numbers of fibroblasts and sometimes with an increase in microvessels (Fig. 7.5). These changes may not be uniform throughout the skin (see Fig. 6.14). The normal reticular dermal curlicue pattern of collagen bundles is replaced by straightened and thickened fibers that acquire a waxy, homogenized appearance (Fig. 7.6). The fibrosing process envelops the eccrine units and their surrounding adipocytes. Within the subcutis, a lobular panniculitis develops along the dermal-subcutis interface as well as along the fascial septa (Fig. 7.7). This results in additional downward collagenous expansion of the reticular dermis (Fig. 7.6). Arteries and nerves formerly located in the subcutis become incorporated within the dense collagen in the expanded dermis (Fig. 7.2). The clinical consequences of the thickened fibrotic dermis are stiff hidebound skin, frozen joints and contractures (Fig. 7.8), chronic ulcers (Fig. 7.1), anhidrosis from loss of sweat glands, and peripheral neuropathies [4] (Fig. 7.9).

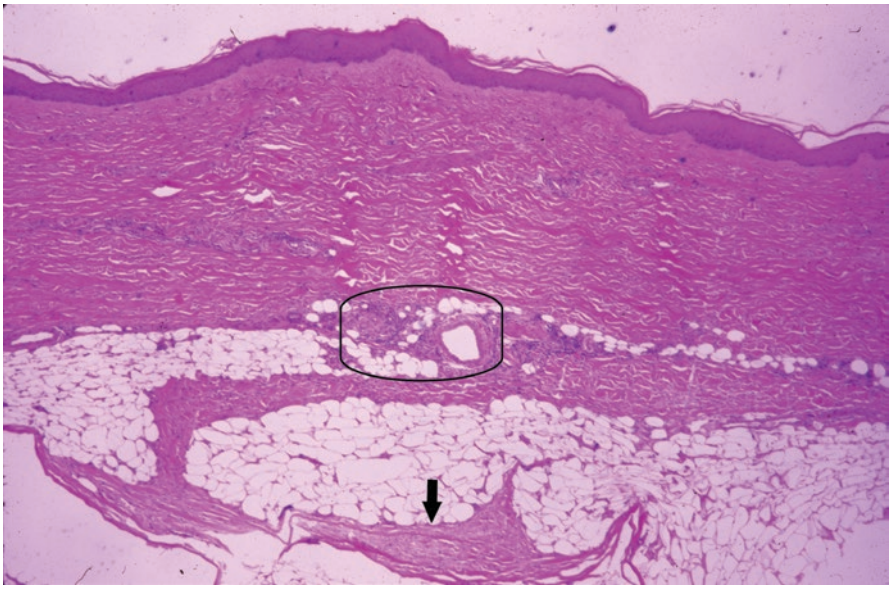

Fig. 7.4 The skin from day 457 shows transition from lichenoid to sclerotic phase. The epidermis has acanthosis, while dermal sclerosis has now extended into subcutis with formation of fibrotic septa (arrow). Dermal follicles are destroyed. A cluster of eccrine glands and an adjacent artery is enveloped by fibrosis (circle)

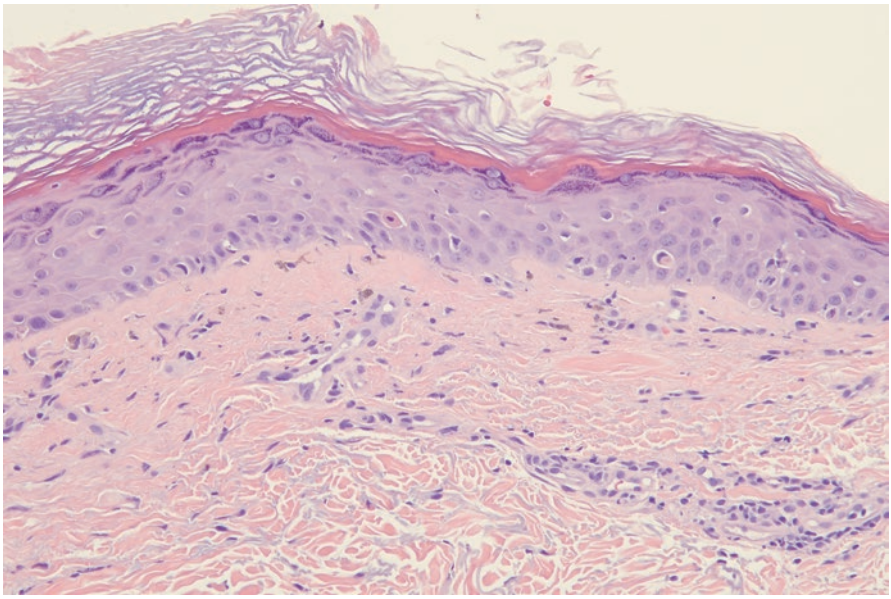

Fig. 7.5 Skin biopsy following IS treatment from day 200. The lichenoid epidermis has apoptotic bodies. The partially fibrotic papillary dermis contains fibroblasts and irregular venules

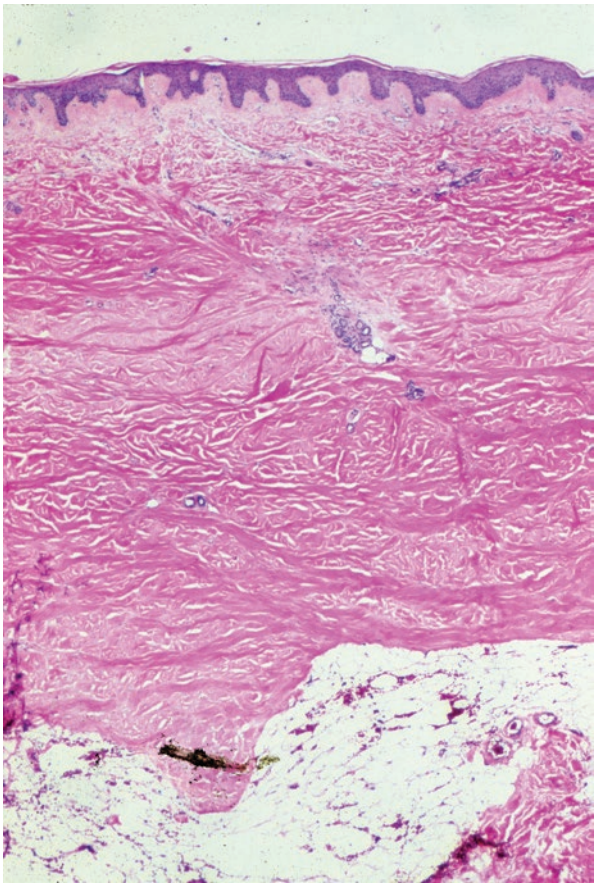

Fig. 7.6 Skin biopsy shows the transformation of dermal collagen bundles into elongated straightened bundles. The entrapped sweat gland is located in the mid-dermis due to additional collagen laid down below it

The second pathway leading to sclerosis in cGVHD is morphea. It was originally classified as localized cGVHD but is now regarded as a precursor that evolves to deep cutaneous sclerosis. Morphea stereotypically begins as areas of dyspigmentation or leukoderma with variable degrees of underlying induration (Fig. 7.10). A full-thickness skin biopsy demonstrates focal nodular fibrosis in the deeper reticular dermis with little or no inflammatory involvement of the epidermis (Fig. 7.11). The distribution sometimes corresponds to areas compressed by clothing (Fig. 7.12). The overlying epidermis usually has minimal to no apoptosis or vacuolar change. With the passage of time, the fibrosis involves the entire breadth of the dermis. The lesions may coalesce, may become more indurated, and may be fixed to the underlying tissues. The development of sclerotic cGVHD may include a mixture of morphea and evolution from a lichenoid inflammatory state to sclerosis, often presenting simultaneously.

The third pathway, eosinophilic fasciitis (EF), can be regarded as a form of morphea involving the deep fascia and subcutaneous septa with edema, fibrosis, and

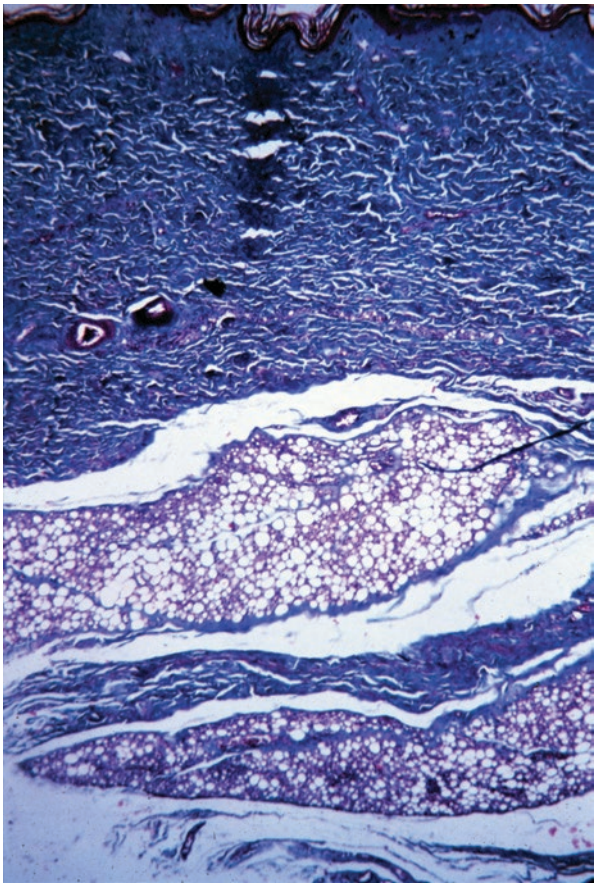

Fig. 7.7 Lobular panniculitis in the subcutis beneath the sclerotic dermis

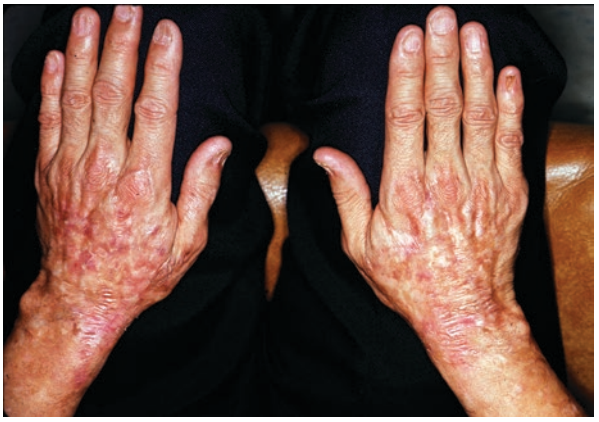

Fig. 7.8 A dorsal view of sclerotic hands shows the generalized form of sclerosis with hidebound skin and dystrophic nails

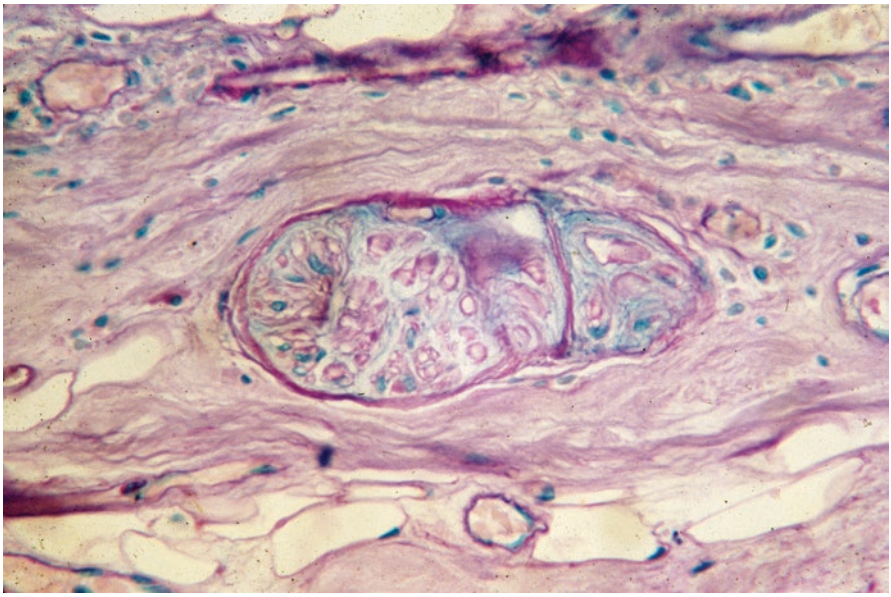

Fig. 7.9 A small nerve is surrounded by dense fibrosis (PAS, Alcian blue)

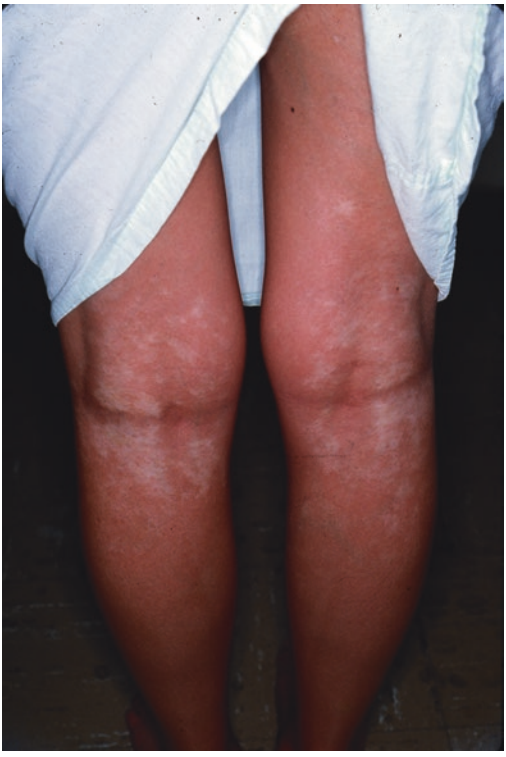

Fig. 7.10 Early manifestation of morphea 1 year posttransplant. Patient developed areas of rippled skin with leukoderma and irregular dyspigmentation

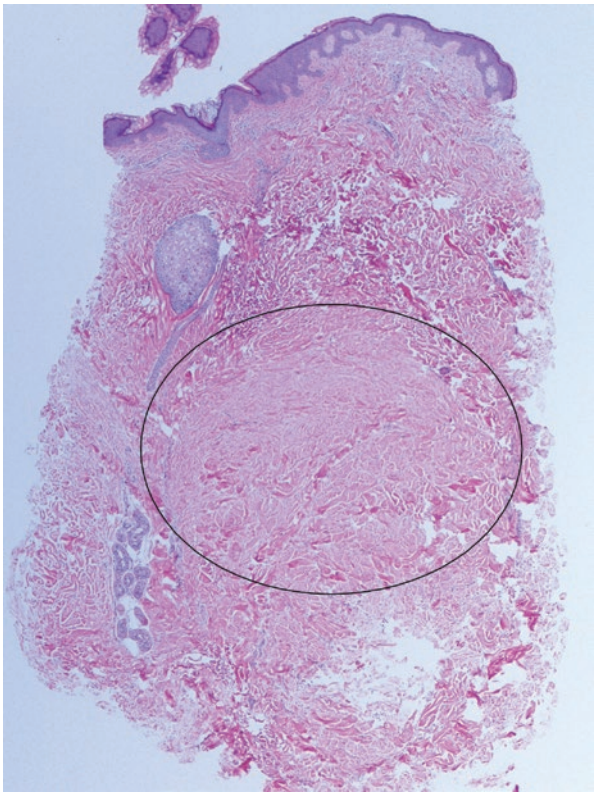

Fig. 7.11 Histologic features of morphea. In the lower reticular dermis is a nodular focus of homogenized collagen (circle). The epidermis, upper dermis, and adnexa are not yet affected

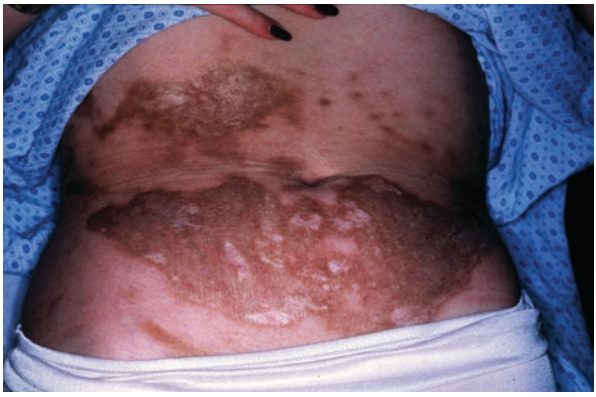

Fig. 7.12 Advanced morphea from a patient with cGVHD showing an irregular, deeply pigmented band-like area of sclerosis whose initial presentation was in the zone of compression from underwear waistband

chronic (especially eosinophilic) inflammation. Clinically, EF presents as a depressed, linear scar with a rippled, cellulite appearance. Lastly, some patients with sclerosis may present without a preceding lichenoid, morpheic, or fasciitis phenotype.

Differential Diagnosis

Fully developed sclerotic cGVHD closely resembles progressive systemic sclerosis (PSS). In the context of post-allogeneic HSCT, the differential diagnosis includes fibrosing dermopathy related to gadolinium exposure used in imaging studies [5]. Other possibilities, such as fibrosis from total body irradiation, do not occur with doses given for pretransplant conditioning (range, 200 cGy–1000 cGy). Lichen sclerosus atrophicus (LSA)-like changes in the anogenital region are a manifestation of cGVHD and should not be considered a separate entity (Chap. 12) [5].

Pathobiology

The marked clinical and histologic resemblance of sclerotic cGVHD (both types) to PSS (Figs. 7.13 and 7.14) prompted murine and human studies to study the potential mechanisms of PSS and determine if cGVHD was a suitable model. PSS has reduced small vessels and distinctive structural changes in the dermal microvasculature as visualized on nailbed capillaroscopy that differ from cGVHD [6–8]. Two methodicologically different human HSCT studies designed to evaluate the density of papillary dermal microvasculature in cGVHD produced contradictory results. Biedermann et al., using *Ulex europaeus* agglutinin to identify vessels in cGVHD, found loss of the papillary dermal microvessels accompanied by activated T cells. They hypothesized that the alloreactive donor T cells targeted endothelium causing loss of vessels, reduced perfusion, hypoxia, and subsequent fibrosis [9]. Fleming et al. compared the findings in papillary dermal vessels identified by CD31 and VE-cadherin from the skin of normal controls and PSS and cGVHD patients. Fleming found that (1) PSS biopsies have significantly fewer dermal vessels than cGVHD or normal controls; (2) canonical endothelial markers for VE-cadherin and vWF were significantly decreased in PSS but not in cGVHD; and (3) some cGVHD biopsies contained areas of vascular endothelial proliferation not present in the PSS biopsies [10].

Recent preclinical and clinical studies have focused on the cascade of events that lead to fibrosis in cGVHD which can be likened to abnormal wound healing with excessive collagen formation. The genesis of fibrosis involves the interactions of T17-helper cells and cytokines and the prolonged uncontrolled B-cell activation of macrophages [11]. The activated macrophages

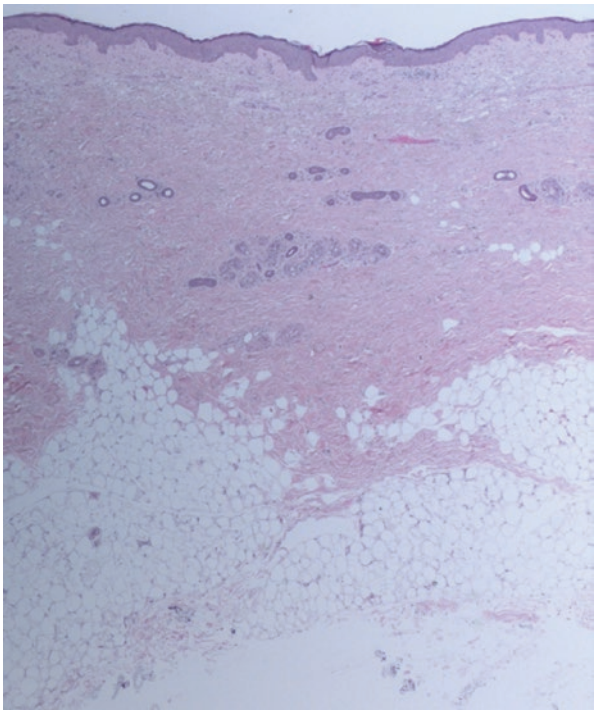

Fig. 7.13 H&E stain of a skin biopsy from a patient at Day 10 post-autograft for PSS. Pandermal sclerosis with entrapment of eccrine units and extension of fibrosis into subcutaneous fat. The dermal alterations are similar to those illustrated by Fig. 7.14, which illustrates pandermal sclerosis from cGVHD in a trichrome stain

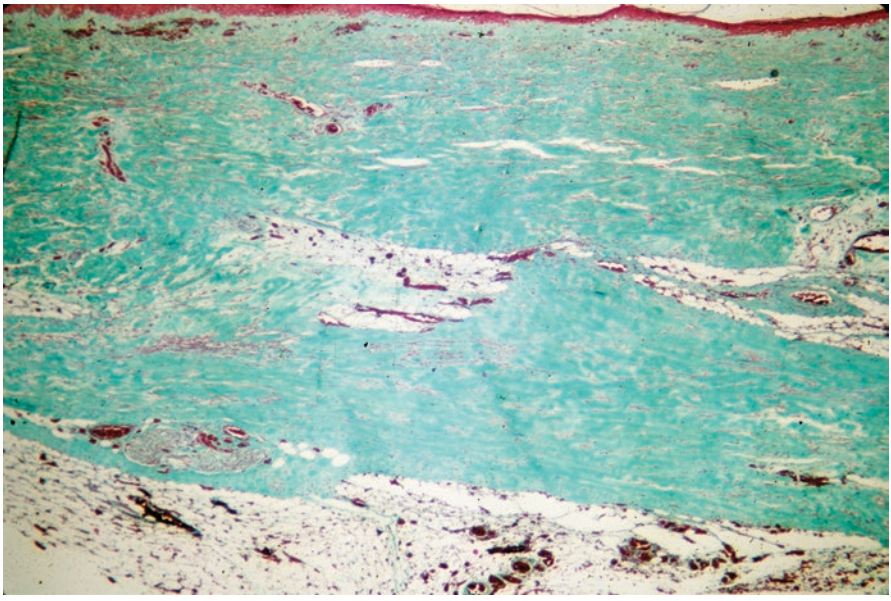

Fig. 7.14 Pandermal sclerotic pattern from cGVHD (trichrome stain), which is morphologically very similar to Fig. 7.13

produce TGF-β and PDGF-α, which signal fibroblasts to differentiate into molecular heat-shock chaperone 47-positive (HSP47+) myofibroblasts. These in turn are driven to synthesize excess collagen [12, 13]. High levels of IgG antibodies against cell surface antigens expressed in organs affected by cGVHD have been detected in patients who develop cGVHD. These antibody levels decreased following therapies such as prednisone and extracorporeal photopheresis [14]. Genetic components associated with PSS as shown by single nucleotide polymorphisms (SNPs) are also associated with the risk of sclerotic cGVHD. The SNPs common to both support the role of the adaptive immune system, specifically B-cell activation, T-cell antigen receptor response, and HLA-DP-mediated antigen presentation in the pathogenesis of these disorders [15].

These studies have led to the identification of cellular and molecular mediators of cGVHD, with the potential of new therapeutic targets including IL-17A, CSF-1, Janus kinases inhibitors, and vitamin A-coupled liposomes carrying HSP47, a molecular chaperone responsible for HSP47+ myofibroblastic activation of collagen synthesis. Studies by Sato et al. and Yamakawa et al. suggest that myofibroblastic collagen synthesis can be blocked via a vitamin A-coupled liposome carrying siRNA-coupled HSP47, thus blocking HSP47+ expression on myofibroblasts [13, 16, 17]. Trials to block or reverse sclerosis have included ibrutinib, imatinib, inhibiting the profibrotic morphogen hedgehog [18], and pirfenidone to block macrophage infiltration and TGF-β production [19]. Evaluation of these trials has mainly relied on clinical parameters such as the modified Rodnan skin score and range of motion maneuvers. Biopsies of sclerotic skin may be unable to distinguish active changes from static preexisting changes unless there is apoptotic or chronic inflammation [20]. Two prior studies have validated histologic scoring schema on H&E-stained sections to follow development and/or regression of dermal sclerosis after autologous HSCT for PSS [21, 22]. Our modification using the Nash grading scheme (Table 7.1) could be utilized in clinical trials to evaluate the dermal changes. The interpretation requires a full-thickness biopsy. Ideally the biopsy or multiple biopsies sampled simultaneously should be taken from indurated and/or inflammatory areas, avoiding areas close to the tendons. Serial skin biopsies taken after treatment for comparison should be from the original location because of the variableness of skin changes in different locations.

To summarize, the diagnosis of sclerotic cGVHD should include the classical features of one of three clinical forms: lichenoid, morpheic, or eosinophilic fasciitis. Skin biopsies are important to clarify ambiguous rashes and to rule out infections or drug allergy masquerading as or coexistent with cGVHD. Lastly, they may be useful to verify any improvements in new experimental agents' efficacy in treating sclerotic cGVHD.

Table 7.1 Grading of dermal fibrosis in systemic sclerosis. Grading is based on the dermal breadth and distribution of fibrosis in H&E-stained biopsies

Dermal fibrosis grade	Description
0	No homogenization, but there may be atrophic thin, straightened collagen bundles with increased amounts of interstitial ground substance
1	Less than 25% sclerosis with residual foci; some residual straightening or eosinophilic collagen bundles may be present
2	Focal sclerosis – less than 50% overall
3	Incomplete homogenization with spaces between the collagen bundles with 50–75% sclerotic change
4	Deep dermal sclerosis that is >50% sclerotic but lacks pandermal sclerosis[a]
5	Pandermal sclerosis without obvious expansion of the lower reticular dermis with some sparing of perieccrine adipose tissue
6	Pandermal sclerosis with homogenization from the papillary to the reticular dermis; includes obvious widening of the reticular dermis below the eccrine coils into the hypodermis and formation of fibrous septa

[a]Modification by Teresa Hyun, M.D. Seattle Cancer Care Alliance, Seattle, WA
Nash et al. [22] (Modified with permission of the American Society of Hematology)

Key Teaching Points

- There is an approximately 20% incidence of sclerotic cGVHD in post-transplant survivors.
- Dermal sclerosis in cGVHD develops along three different pathways: lichenoid, morpheic, or eosinophilic fasciitis.
- The most common pathway arises from generalized lichenoid (inflammatory) type.
- Sclerosis proceeds downward and leads to the destruction of dermal adnexa and fibrous reorganization of dermal collagen bundles.
- Panniculitis along the dermal-subcutis interface results in additional collagen added to the deep dermis.
- Fasciitis results from inflammation and edema along the deep facial septa.
- Morphea consists of geographic areas with a dyspigmented epidermis with focal sclerotic nodules in the mid-reticular dermis. Inflammation is minimal. Morpheic sclerosis extends upward to involve the entire dermis.
- Histopathology of sclerotic cGVHD changes over time and varies from location.
- IS treatment may block the progression to complete pandermal sclerosis.
- Evaluation of sclerotic skin response to IS relies on the clinical evaluation. Biopsy of sclerotic skin may not be able to distinguish active changes from static preexisting changes without serial biopsies for comparison.
- Histologic schema to grade progression/regression or no change after anti-sclerotic treatment is under investigation.

Questions

1. What are the criteria for classifying a patient as having sclerotic cGVHD?
2. What are the two forms of sclerotic cGVHD?
3. Histologic features of cutaneous dermal sclerosis typically include the following except:
   A. Deep fibrosis along the fascial septa
   B. Entrapment of eccrine units
   C. Interface dermatitis
   D. Panniculitis along the dermal-subcutis margin
4. Studies of cutaneous sclerosis have concluded the following, except:
   A. SNPs show genetic similarities between PSS and cGVHD.
   B. Elevated ratios of IgG antibody levels to cell surface membrane antigens on GVHD targeted organs.
   C. Normal expression of canonical endothelial antigens on dermal microvessels.
   D. The evaluation of anti-sclerotic cGVHD in clinical trials can be based solely on skin biopsy.

Answers

1. Answer: They are the NIH clinical criteria of cutaneous sclerosis, fasciitis, and contractures. Clinical evaluations including range of motion, Rodnan skin mobility scores, and presence of palpable sclerosis or fasciitis or contractures.
2. Answer: Generalized sclerosis and morphea. They differ mainly in the distribution of sclerosis.
3. Answer: C. Morphea does not begin with inflammation in the epidermis or papillary dermis. Sclerosis is preceded by an inflammatory phase in the upper dermis with advanced stages of generalized sclerosis having minimal epidermal inflammation.
4. Answer: D. The evaluation of cGVHD for treatment relies on clinical parameters such as the modified Rodnan skin score and range of motion maneuvers since biopsy of sclerotic skin may not be able distinguish active changes from static preexisting changes.

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8 Early Changes of Gut GVHD: Differential Diagnosis and Criteria for Crypt Cell Apoptosis

Cecilia C. S. Yeung, David W. Woolston, and Howard M. Shulman

Background Information

Since the early days of hematopoietic stem cell transplantation (HSCT), the finding of apoptotic crypt abscesses in rectal biopsies has been used to diagnose GVHD in the gastrointestinal tract [1]. However, the overlap in histological presentation between GVHD, gut infections (Chap. 11), and/or drug toxicities creates a vexing problem when interpreting gut biopsies. Evaluating biopsies from the early post-transplant period is particularly problematic due to the histological overlap from persistent pre-transplant conditioning toxicity. To address these questions, a human-subject review-approved protocol studied the changes in serial rectal biopsies of 13 patients transplanted for leukemia/lymphoma. All study biopsies were fixed in Bouin's solution and stained with H&E and Alcian Blue. Suction rectal biopsies were obtained 4–8 cm proximal to the dentate line. Biopsies were obtained pre-transplant between days 7 and 10, between days 17 and 21, and on approximately day 35 or at the onset of clinical GVHD as defined by secretory diarrhea, elevated

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

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

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

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

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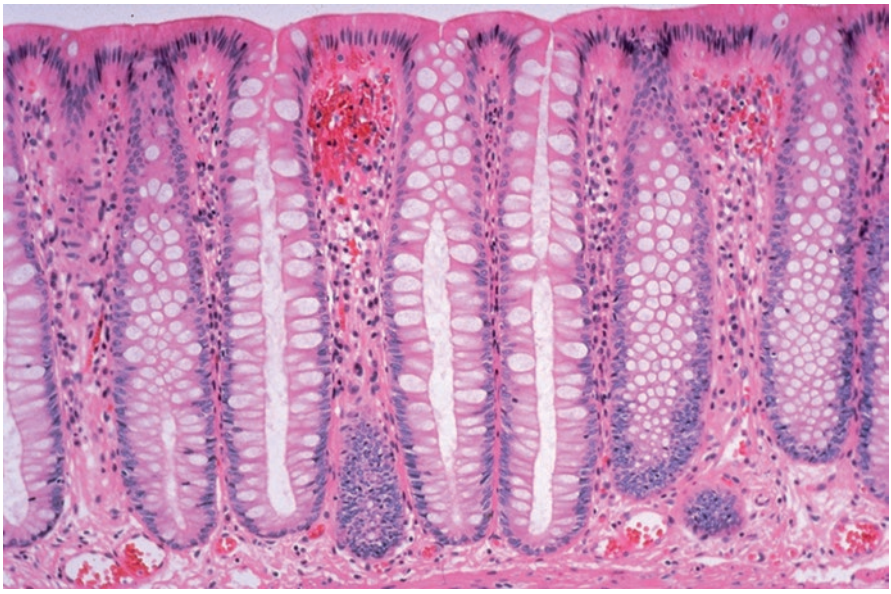

Fig. 8.1 Normal colonic mucosa has straightened crypts with scattered cells in the lamina propria

bilirubin, and skin GVHD (thus verifying multi-organ GVHD) [2]. All of the study patients received high-dose myeloablative conditioning. At least three patients had sequential rectal biopsies. The pre-transplant biopsies in all 13 patients were normal (Fig. 8.1).

Between days 7 and 10, all the patients' biopsies show diffusely damaged abnormal mucosa. Interim biopsies around days 14–18 showed improvement which varied in the extent of residual damage including 3 patients whose biopsies had only rare degenerative crypt cells (enterocytes). Three out of 13 patients developed typical clinical findings of acute GVHD with skin rash and secretory diarrhea without any associated infectious etiology and had abnormal rectal biopsies consistent with early GVHD. The case history from one of these three patients, whose biopsies illustrate the continuum of changes, is described below.

Clinical History

This 30-year-old was transplanted for AML in relapse after a myeloablative conditioning with cytoxan and 10 GY TBI. Research biopsy on day 7 showed diffusely damaged mucosa with extensive crypt dropout, cytologic nuclear atypia with enlarged nucleoli, loss of mucous, apoptotic crypt abscesses containing cellular debris, diffusely increased mononuclear cell infiltrate, and focal microhemorrhages (Fig. 8.2). Marrow engraftment occurred on day 14. Rectal biopsy on day 16 (Fig. 8.3) showed marked improvement with regenerative crypts with increased mitotic figures with mild architectural distortion. The crypt cells had mild nuclear

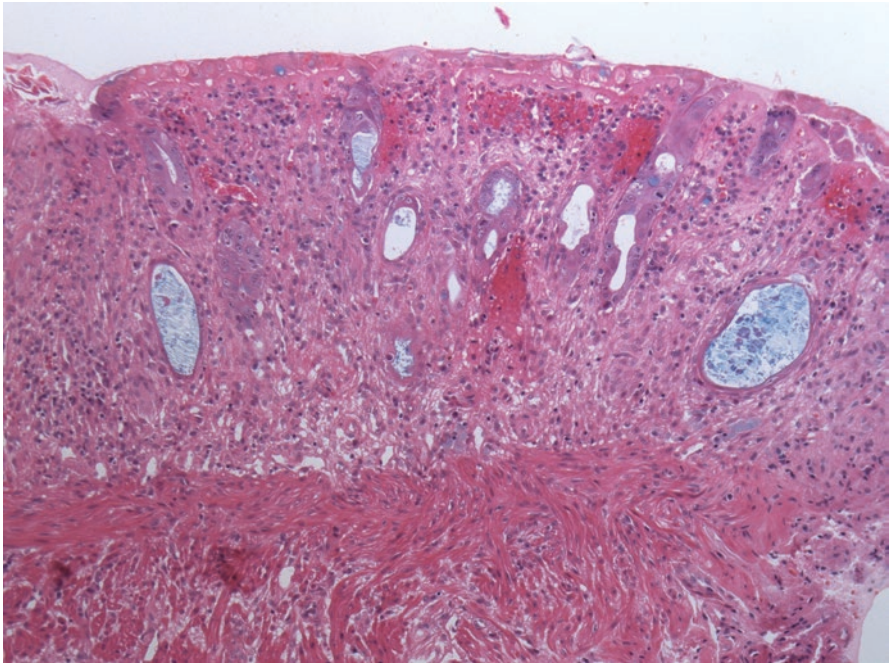

Fig. 8.2 Day 7 rectal biopsy contains extensive crypt destruction with mucosal ulceration. Residual dilated crypts are surrounded by markedly edematous lamina propria with increased cellularity and microhemorrhage

dyspolarity and some goblet cell mucous. Apoptosis was markedly reduced. There are still a few cystically dilated degenerative crypts devoid of any epithelium. The lamina propria was less cellular, and the surface epithelium was intact. He developed skin GVHD on day 24. A gut biopsy on day 30 was normal; a repeat skin biopsy on day 35 had marked changes of GVHD with numerous apoptotic keratinocytes and lymphocytic infiltration of the epidermis. Gut biopsy on day 36 showed a rare apoptotic crypt cell consistent with GVHD (Fig. 8.4). The degenerative enterocyte was located along the abluminal side of the laterobasal region of the crypt (the progenitor cell zone). The apoptotic cell had an enlarged clear zone containing karyolytic nuclear debris. The "exploding crypt cell" was thus defined to be the earliest identifiable change attributed to GVHD. Thereafter, the patient developed severe diarrhea with up to 20 brown to green stools/day. He initially received high-dose prednisone and was switched to an alternate-day regimen. Diarrhea, abdominal cramps, nausea, and vomiting persisted. An upper GI showed separation of bowel loops, luminal narrowing, and effacement of mucosal folds with multiple mucosal ulcerations involving the entire jejunum and ileum, along with dilution of the barium by excessive secretions and rapid transit time. The D-xylose test (testing for malabsorption) was abnormal. A rectal biopsy obtained 1 day after the radiology examination was normal. Hyperalimentation was started with lessening of his gut symptoms of abdominal cramping and frequency of stools. He remained on IS and subsequently developed severe sclerodermatous chronic GVHD with oral-ocular

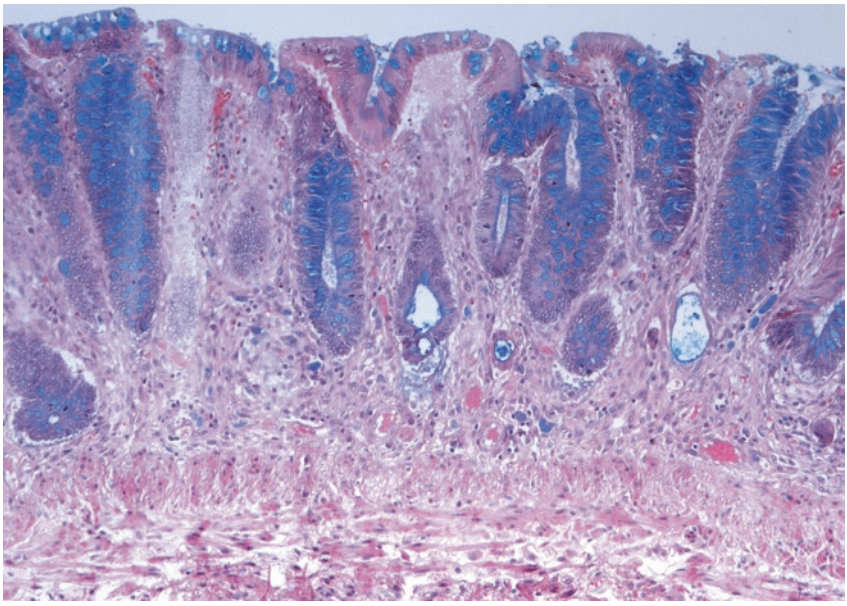

Fig. 8.3 Day 16 rectal biopsy: the mucosa surface has reepithelialized, and regenerative crypts are producing mucin. Residual cystically dilated crypts at the base of the lamina propria show apoptotic change. The lamina propria is still edematous and hypercellular

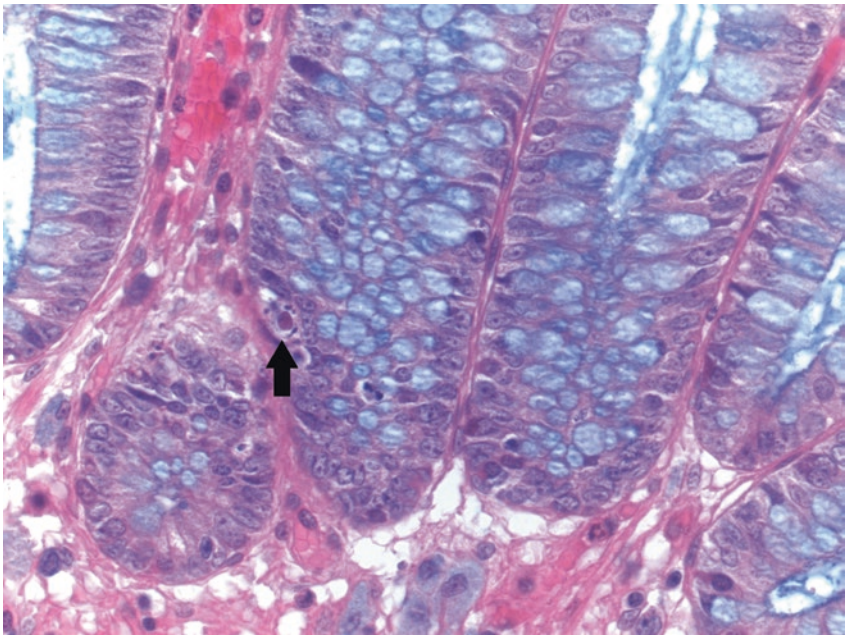

Fig. 8.4 Day 36 rectal biopsy: an isolated apoptotic crypt cell is located at the lateral basal position in otherwise normal crypts (arrow)

sicca and obstructive pulmonary function, all refractory to secondary IS modalities. During the third year post-transplant, he developed pancreatitis complicated by an infected pancreatic pseudocyst with the development of gastric cutaneous fistulas. The patient died after two unsuccessful surgical attempts to stem the problem.

Diagnosis

Refractory gut GVHD most marked in the ileum

Key Pathology Features

1. The conditioning chemoirradiation toxicity on gut GVHD is diffuse, in contrast to the initial focal involvement of GVHD.
2. Cytotoxic conditioning changes consist of crypt cell degeneration, nuclear atypia of crypt colonocytes, crypt abscess, crypt dropout, dilated crypts lined by flattened epithelium containing debris, decreased mucus, and surface micro-ulcerations.
3. The first histologic manifestation of GVHD is individual enterocyte apoptosis in the crypt progenitor cell zones, the basal lateral portion of crypts, and the neck region in the stomach.
4. Though individual enterocyte apoptosis is a characteristic of GVHD, it is not pathognomonic. Other conditions, including infections (see Chap. 11), phosphate enema, and drugs including proton pump inhibitors, NSAIDs, and mycophenolate induce individual crypt cell degeneration. The criteria for exploding crypt cell identification are defined below.
5. Even in the presence of a floridly abnormal upper GI series, the rectal biopsy can be normal attesting to the focal nature and persistence of GVHD in the intestines.

Differential Discussion

In the 38 years since Epstein's seminal study was published [2], more carefully defined criteria for individual crypt cell degeneration (apoptosis), for apoptotic crypt abscess (destruction), and for mucosal denudation were spelled out by the NIH pathology consensus panel [3] (Table 1.1 in Chap. 1) and a 2015 consensus study of histologic diagnostic criteria for gut GVHD by Kreft et al. [4]. Standardization of these criteria is important because the interpretation is based on an inconsistent spectrum of histologic changes. Interpretation is affected by differing fixation and staining procedures that can cause confusion with nuclear debris or lymphocytes. Because an apoptotic cell exists for only 1–3 hours, several different appearances are possible in a single tissue section [5, 6]. The consensus criteria by Kreft et al. has helped standardize the definition of apoptotic crypt cells, which is particularly relevant when discussing minimal diagnostic criteria based on the number of apoptotic cryptenterocytes. It is also relevant when comparing methods and studies from separate institutions (Fig. 8.5).

Other caveats regard whether an apoptotic cell adjacent to a colonic lymphoid nodule is due to GVHD. Apoptosis along the surface epithelium is considered a normal feature unless the mucosa has complete destruction of the underlying crypts due to prolonged GVHD. Neither a single isolated nuclear fragment nor a single eosinophilic fragment is considered an apoptotic cell, as they can represent a part of a living lymphocyte or eosinophil. Adenomatous tissues should not be evaluated, as they typically contain apoptotic cells. Gastric G cells may show cytoplasmic clearing and should not be confused with apoptotic cells.

Crypt destruction and/or crypt abscess encompass several definitions by several sources [1, 3, 12]. The NIH consensus defines apoptotic crypt destruction as apopotosis of at least a third the circumference of a cross sectioned crypt with at least half the diameter of a normal crypt [4]. An exploding crypt is dilated, lined by a flattened epithelium, and filled with cellular debris [3]. Star et al.'s definition of a crypt microabscess requires ≥3 apoptotic bodies in the crypt wall, and is classified by its intraluminal cellular content—neutrophilic, eosinophilic, or apoptotic/mixed [12].

When our patient's study was conducted, all the leukemic patients had been conditioned with high-dose myeloablative regimens. Based on the serial findings, the

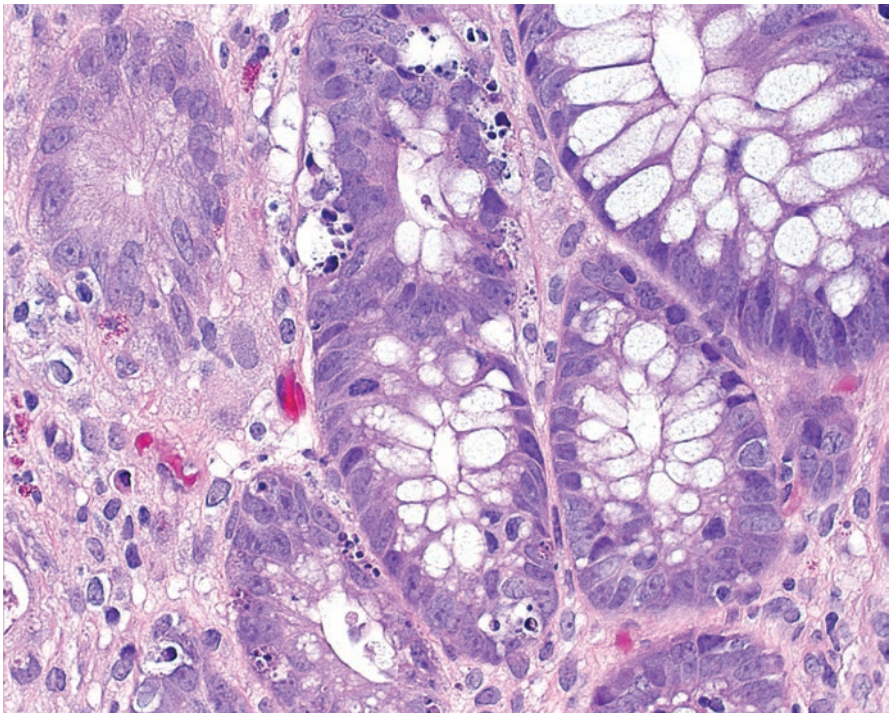

Fig. 8.5 Numerous apoptotic crypt cells have a cleared-out cytoplasm containing hyperchromatic, condensed nuclear debris

rectal biopsies showing crypt cell degeneration, crypt dilation, crypt abscess, and dropout could not be clearly separated from the effects of chemoirradiation and conditioning before day 20. This is similar to then-existing proviso, which disregarded GVHD in the differential for skin biopsies before day 20 based on a related study by Sale et al. [7]. This rule is no longer sacrosanct for several reasons. First, many patients receive less rigorous conditioning lessening the duration of chemotoxicity to the gut. Second, in those receiving grafts from mismatched or unrelated donors, the onset of GVHD may be earlier and more severe. Third, with the great variation in myeloproliferative conditioning regimens used in different centers, some regimens may actually result in more destructive and prolonged gut changes [8] (Fig. 8.6). High-dose myeloablative regimens may produce prolonged crypt destruction with the surface only lined by residual atypical cells, which may persist beyond day 30 (Figs. 8.7 and 8.8). In contrast to the diffuse abnormalities of conditioning toxicity, the early changes of GVHD are focal with crypt cell apoptosis adjacent to otherwise normal enterocytes (Fig. 8.9). As a note of caution, small numbers of apoptotic bodies are not specific to GVHD. Other such etiologies include those from normal cell turnover and drugs including NSAIDs and proton pump inhibitors [9]. Mycophenolate mofetil (MMF), a T-cell inhibitor commonly used for IS, can produce colitis and upper gut damage histologically resembling GVHD, e.g., focal ulcerations, apoptosis, and intense acute and chronic inflammation [10, 11].

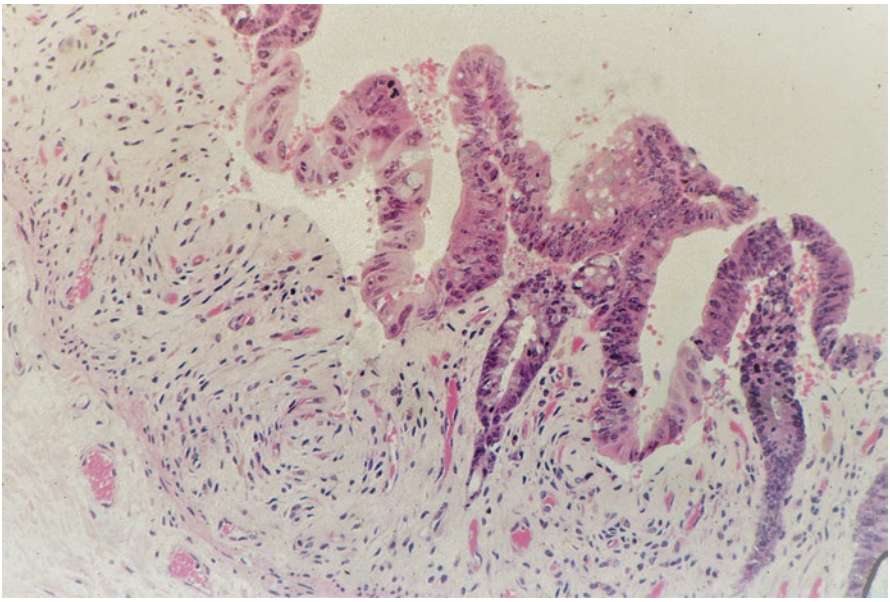

Fig. 8.6 Day 14 post-transplant after conditioning with high-dose ara-C: mucosa displays marked cytologic atypia on the surface and in the remaining damaged crypts, which are surrounded by markedly edematous lamina propria

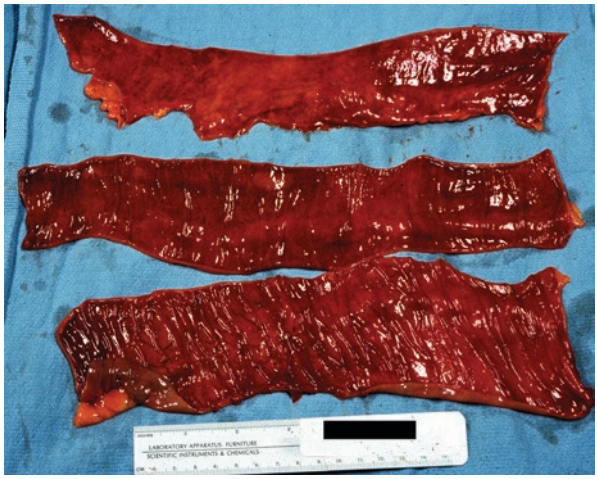

Fig. 8.7 Severe persistent enteritis from chemotoxicity 40 days post-autologous transplant after high-dose conditioning. Gross photo shows diffuse edematous and hemorrhagic mucosal surfaces

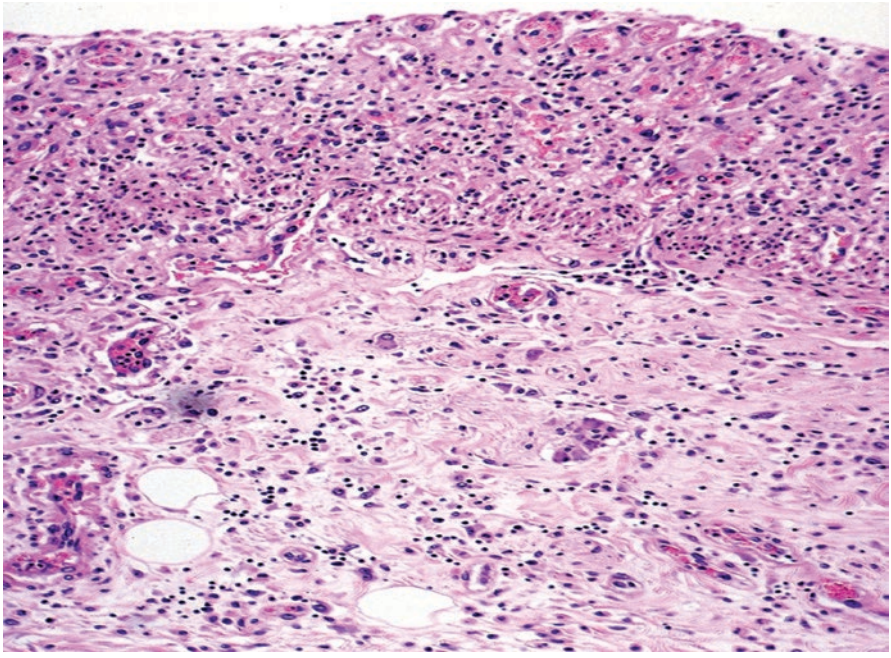

Fig. 8.8 Microscopic view of the same small bowel as in Fig. 8.7 shows ulceration with diffuse mucosal crypt destruction

One 2013 study documented the histopathological discrepancies between MMF colitis and GVHD: changes favoring MMF include greater eosinophil numbers (>15 per 10 HPF), lack of neuroendocrine cell aggregates in the lamina propria, and few apoptotic microabscesses (≥3 apoptotic bodies with apoptotic debris in the lumen) as useful indicators of MMF colitis [12]. Since these discriminating features

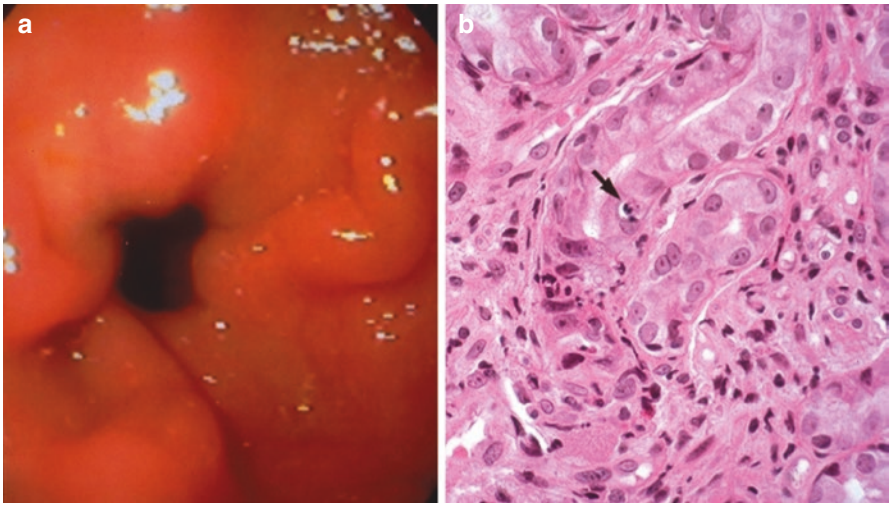

Fig. 8.9 Gastric mucosal biopsy of GVHD. Endoscopy (**a**) shows mildly edematous gastric mucosa. Biopsy (**b**) shows focal crypt cell apoptosis (arrow)

were garnered from a comparison of separate populations with GVHD/no MMF or MMF/no GVHD, it would be difficult to recognize gut MMF toxicity if the patient has concurrent GVHD (a similar problem occurred when trying to distinguish the gut toxicity of the antiviral drug brincidofovir from GVHD [13]). Furthermore, discriminating features drawn from a populational study are not inherently applicable to a single patient's case. In clinical practice, MMF colitis is distinguished from GVHD by the improvement of bloody diarrhea following dose reduction or withdrawal from MMF [14].

Infectious agents that produce changes and damage to the gut that could be confused with GVHD, especially CMV, are discussed in Chap. 11. Quantitation of individual apoptotic cells and how they are used in patient management as well as a discussion of grading will be discussed in Chap. 9. Chapter 10 describes the pathologic spectrum of severe unrelenting gut GVHD, the immunopathogenesis of the damaged crypt cell niche, and the contribution of vascular damage.

Teaching Points

1. The rectal biopsy, taken within the first 7–10 days post-full myeloablative conditioning, shows diffuse gut damage.
2. Following a full myeloablative conditioning, the standard practice then based on the Epstein study avoided obtaining gut biopsies, or else censored their interpretation, before day 20 to avoid confusion with residual effects of conditioning [2].

3. Strict adherence to this rule is no longer germane since the advent of newer, less gut-toxic conditioning agents and the use of reduced intensity conditioning regimens. Because of the possibility of hyperacute GVHD developing after a mismatched transplant, GVHD is a consideration after development of diarrhea between days 10 and 14.
4. Some preparative regimens have a longer effect on the cytologic and architectural changes of the mucosa.
5. Mucosal changes persisting after melphalan conditioning can produce "autologous GVHD" or "pseudo-GVHD," which resembles GVHD in allogeneic recipients [15], but it responds to steroids and does not lead to chronic GVHD.

Questions

1. How does the distribution of gut injury differ between chemoirradiation toxicity and early GVHD?
2. How long does the effect of conditioning toxicity of the gut persist before GVHD can be considered?
3. What other conditions besides GVHD can produce individual cell necrosis of crypt cells?

Answers

1. Answer: Gut damage after a myeloablative conditioning regimen produces diffuse changes throughout the mucosa with extensive crypt cell degeneration, destruction, and abscess formation. In contrast, the initial changes in GVHD are individual enterocyte apoptosis adjacent to normal crypts. Crypt abscess formation occurs later with widespread apoptotic changes.
2. Answer: This depends on the type of conditioning regimen, whether it is a full myeloablative, reduced intensity regimen, or with certain conditioning agents, such as melphalan. Previously, day 20 was considered the cutoff before a diagnosis of GVHD could be entertained. This is no longer applicable in the era of reduced intensity conditioning regimens or with mismatched donor transplants, where the onset of diarrhea between days 10 and 14 raises a serious concern for hyperacute GVHD. In contrast, after an autograft conditioned by melphalan, mucosal changes can persist for over 30 days.
3. Answer: In addition to the conditioning cytotoxicity, infections (Chap. 11); drugs such as mycophenolate, proton pump inhibitors, and NSAIDs; and exposure to a phosphate enema can all result in the formation of apoptotic changes.

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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.

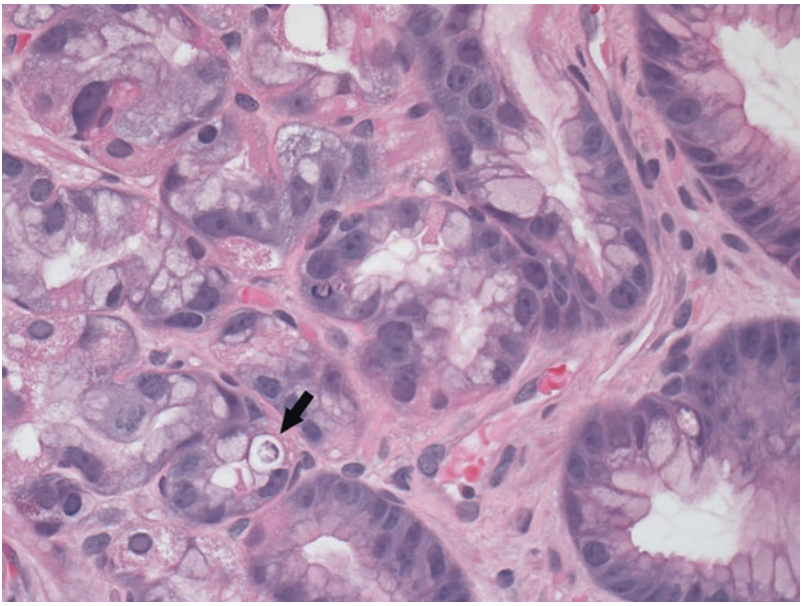

Fig. 9.1 Gastric antral biopsy from day 25. Adjacent to the normal glands are focal apoptotic bodies (one is indicated by the arrow), average 6 per tissue piece, Lerner-Sale grade I, Myerson grade 3

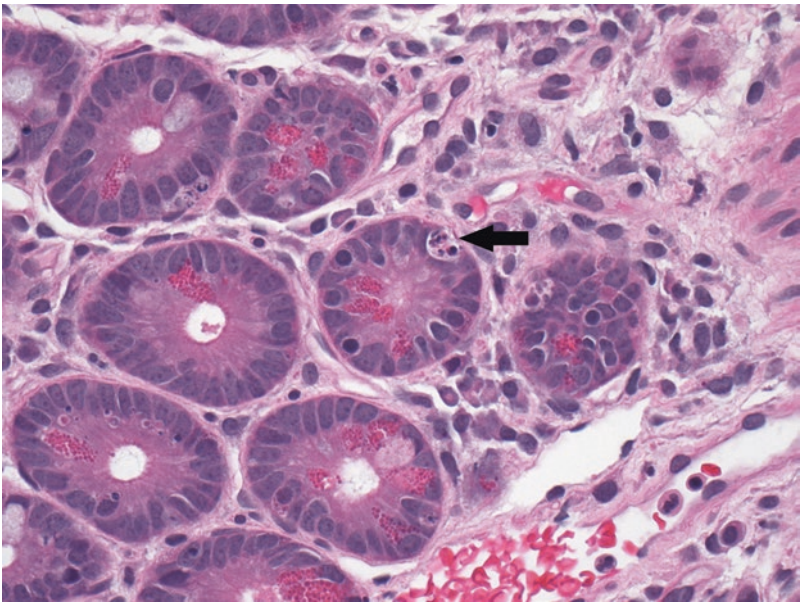

Fig. 9.2 Duodenal biopsy from day 34 demonstrating crypts have many Paneth cells in contrast to Fig. 9.10 below. The arrow denotes an apoptotic body

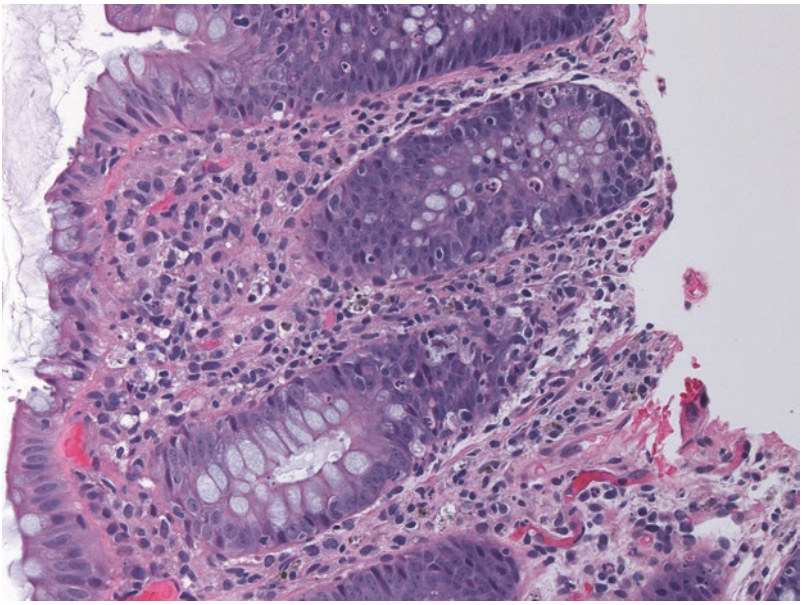

Fig. 9.3 Colon biopsy from day 59 has a marked mixed inflammatory infiltrate in the lamina propria with lymphocytes and many scattered neutrophils infiltrating crypts, neutrophilic cryptitis. The distinction of apoptotic activity from the inflammatory cells is challenging. Because many neutrophils are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

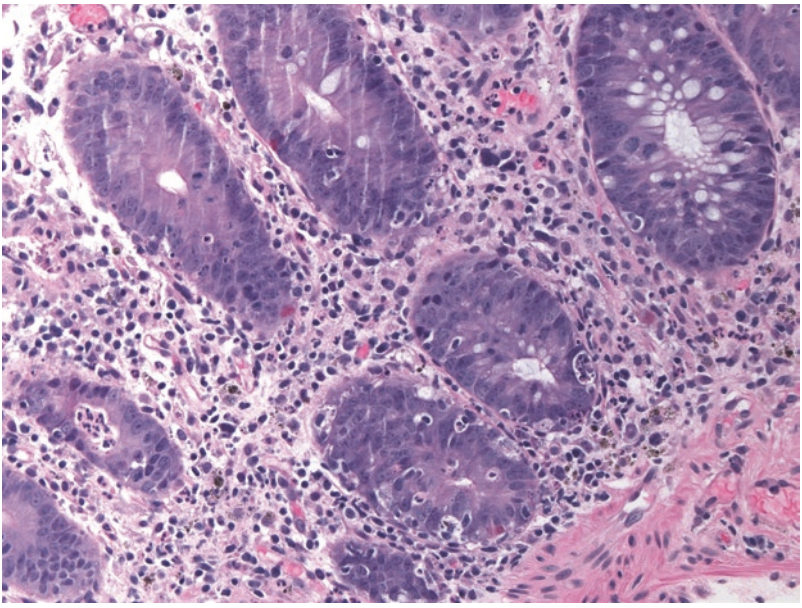

Fig. 9.4 A second view of the same colon biopsy as in Fig. 9.3 shows similar inflammatory infiltrate in the lamina propria, including an early neutrophilic crypt abscess, though less overall apoptotic activity. There is neither apoptotic crypt microabcess as defined by Star et al. [29] nor crypt destruction 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

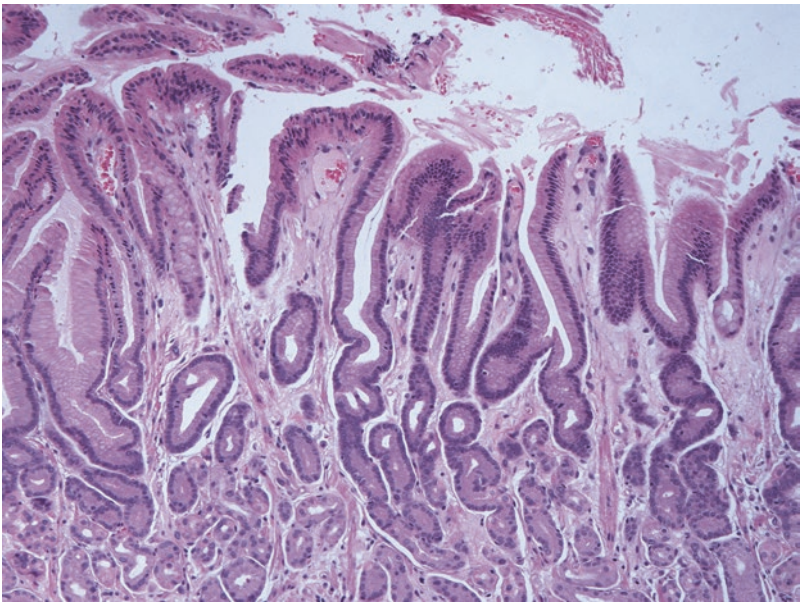

Fig. 9.5 GAVE: Low power of the gastric antrum shows edematous gastric fronds containing dilated capillaries and smooth muscle bundles which extend perpendicular to the muscularis mucosa toward the villus surface

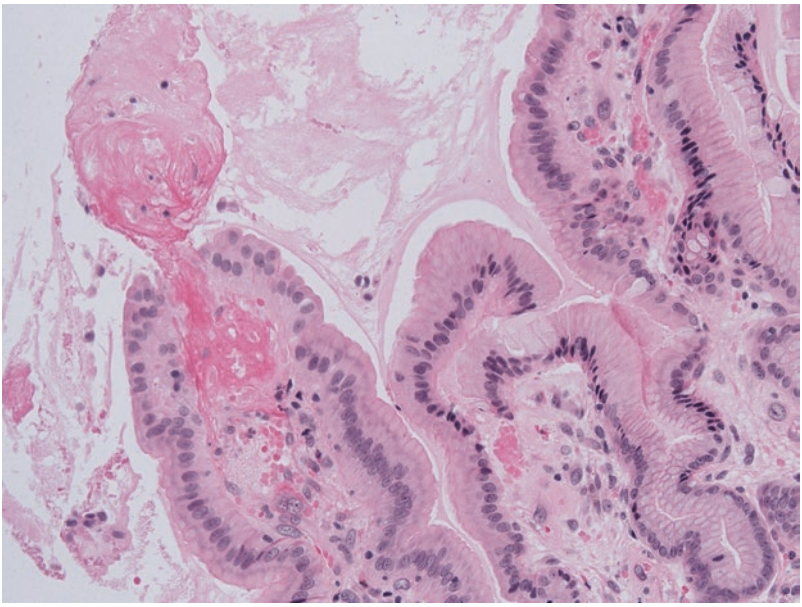

Fig. 9.6 GAVE: Higher power view of another biopsy shows a ruptured dilated capillary with extravasation of red blood cells onto the surface

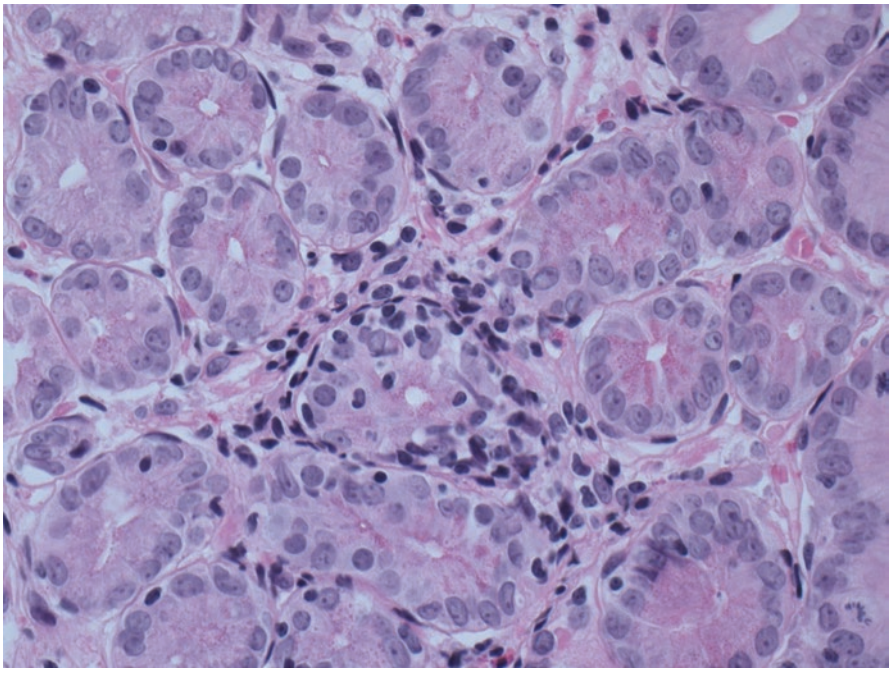

Fig. 9.7 This figure illustrates focally enhanced gastritis (FEG). A cross-sectioned gastric gland is encircled and infiltrated by a lymphoid infiltrate without accompanying apoptotic change. FEG has an increased association with GVHD, but is not a diagnostic indicator for GVHD [3]

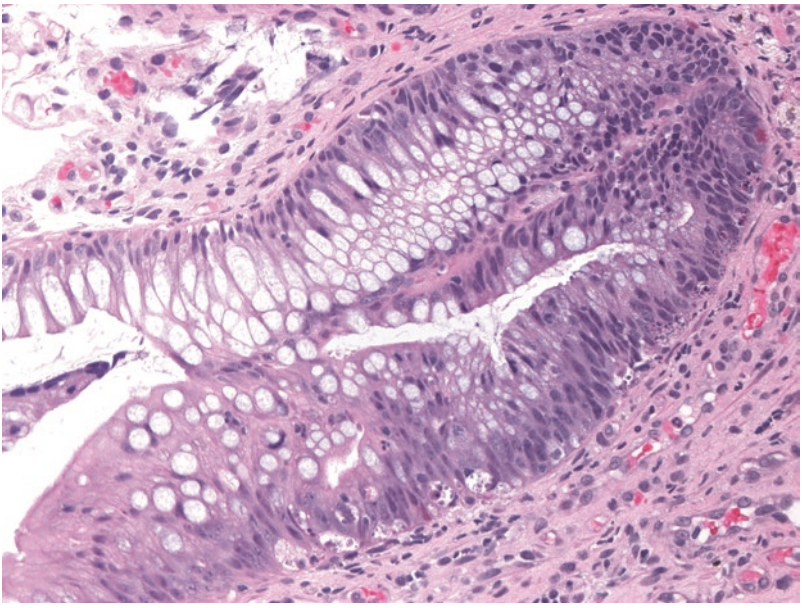

Fig. 9.8 Colonic biopsy from day 166 demonstrates enlarged, irregular regenerative crypts with confluent apoptosis consistent with prior crypt destruction. Lerner-Sale II, Myerson grade 5

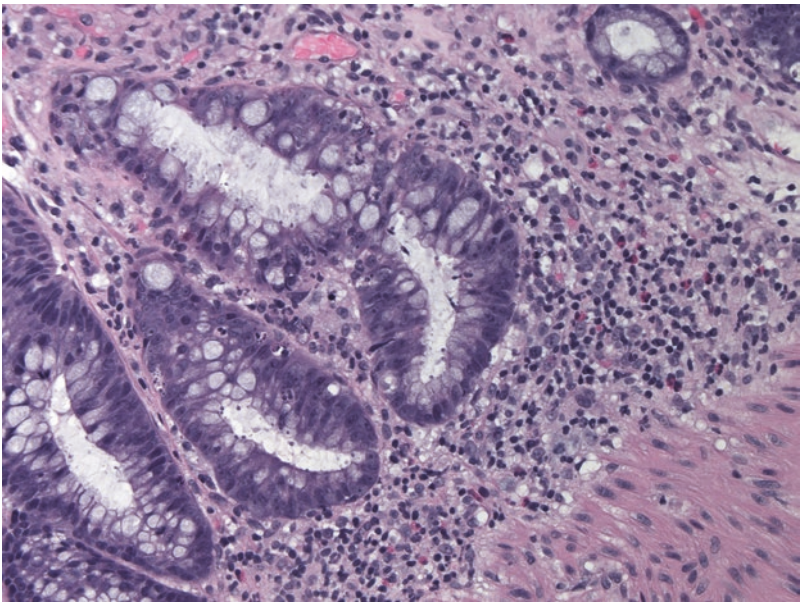

Fig. 9.9 Active colonic GVHD on day 201. The marked inflammatory content in the lamina propria includes a number of eosinophils and neutrophils which are contributory to the cascade of injury caused by GVHD [1, 2]

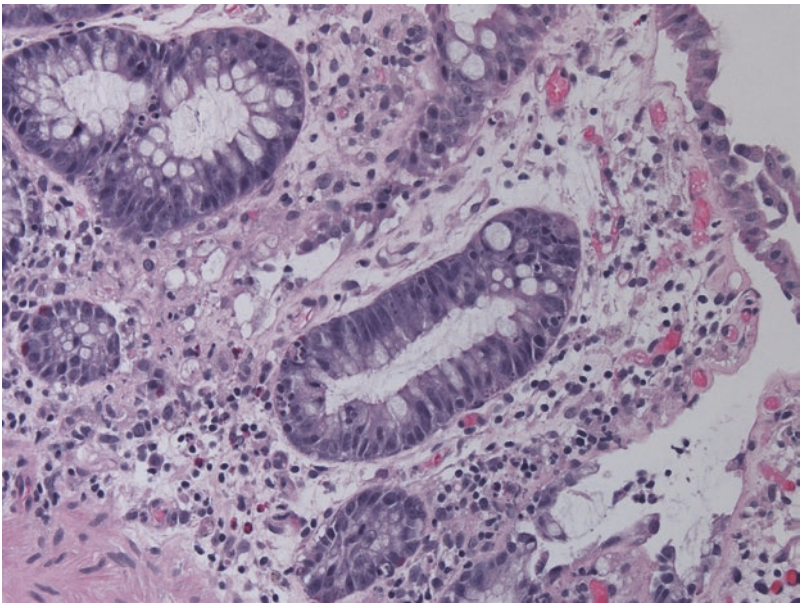

Fig. 9.10 Damaged ileal crypts in the day 201 biopsy have nuclear hyperchromatism, loss of villus architecture, and flattened surface epithelium without goblet cells. Crypts lack Paneth cells (in contrast to Fig. 9.2), a feature associated with a poor outcome [4]. The dropout of crypts is Lerner-Sale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

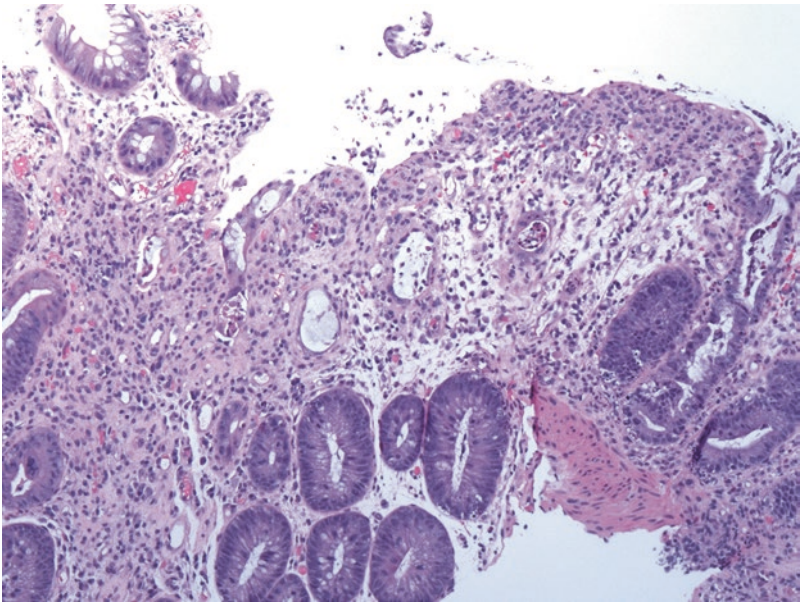

Fig. 9.11 Colonic mucosa on day 201 displays spectrum of severe mucosal damage, from exploding crypts lined by flattened crypt epithelium whose lumina contain apoptotic debris, to crypt destruction with mucosal ulceration. Lerner-Sale grades II–IV, Myerson grade 5

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

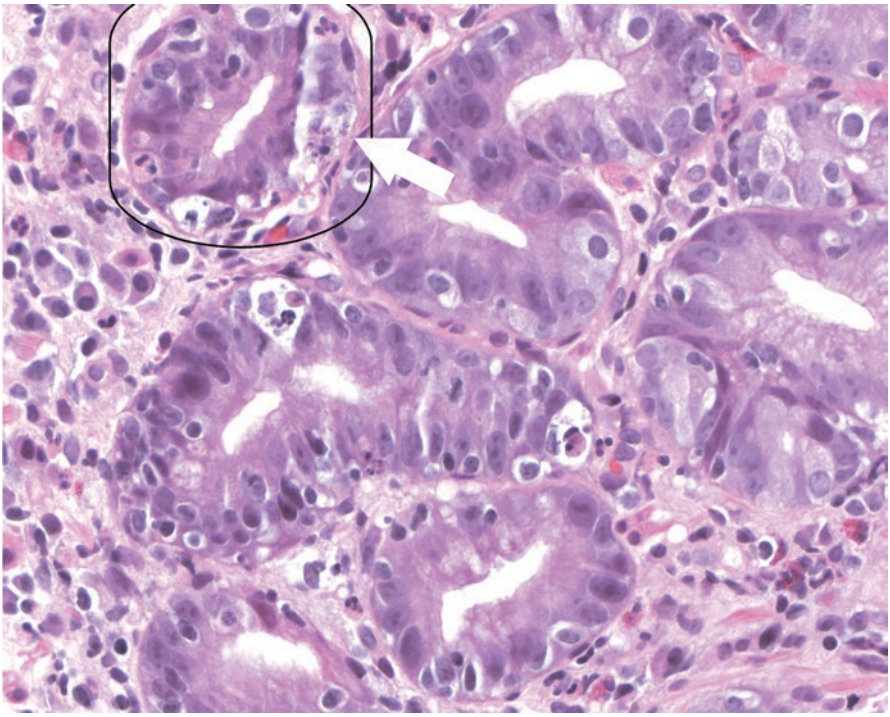

Fig. 9.12 Severe GVHD in colon day 86 with numerous apoptotic bodies. The top, leftmost crypt (circled) shows apoptotic crypt destruction involving >1/3 of the wall (white arrow). Though this field would be classified as Lerner-Sale grade II, this understates the severity of activity, which would be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

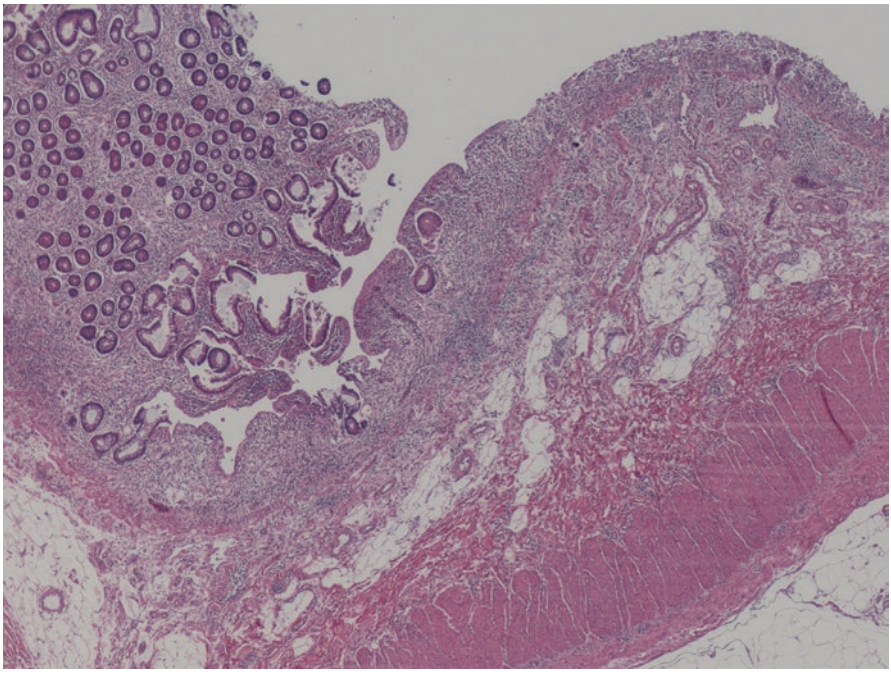

Fig. 9.13 Low-power view of the intestine demonstrates the patchy distribution of GVHD. Ulcerated segments complete mucosal destruction of crypts adjacent to areas with architectural disarray and small irregular crypts

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

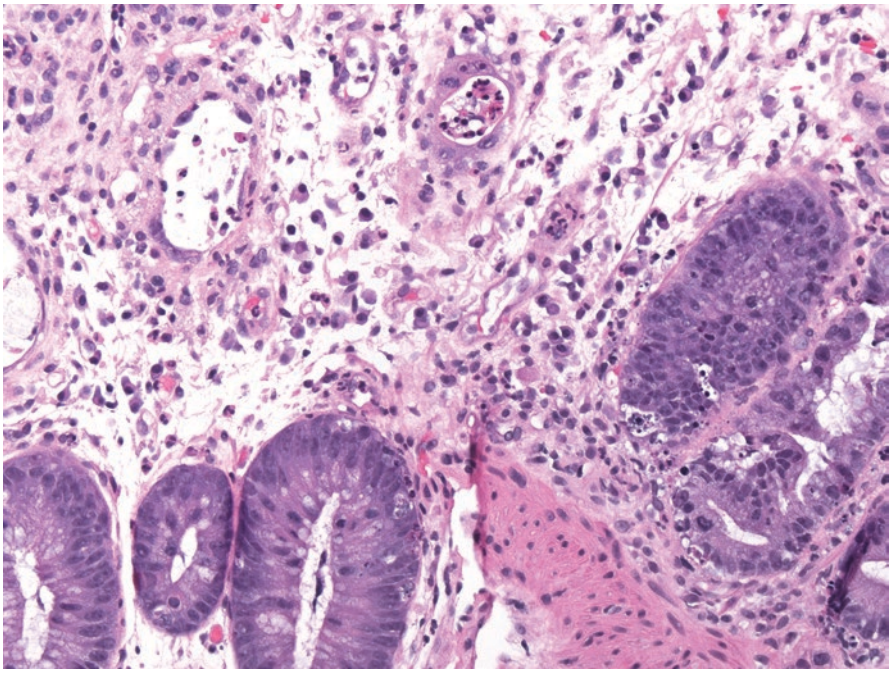

Fig. 9.14 Higher magnification of Fig. 9.11 demonstrates crypt abscesses containing, eosinophils and/or apoptotic debris. Extensive apoptosis is present in some residual crypts

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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Pathobiology of Fatal Gastrointestinal GVHD

10

Howard M. Shulman and David W. Woolston

The two patient scenarios described below illustrate two different sequelae of intractable gut GVHD.

Patient A

Clinical History—Patient A

A 12-year-old received a matched sibling allograft for aplasia. On day 53, she developed a skin rash and bloody diarrhea, proven by biopsies to be GVHD. Despite treatment with prednisone, ATG, and azathioprine, gastrointestinal problems persisted with vomiting of bilious material, urgent diarrhea with intermittent blood, severe abdominal pain and tenderness, and inability to eat or drink. X-ray imaging studies with barium and modern techniques using CT, magnetic resonance enteroscopy, and microbubble ultrasound demonstrated pan-intestinal involvement with edema, hypervascularity, and segmental narrowing of the bowel lumen [1–3] (Figs. 10.1, 10.2, and 10.3). Because of the inability to eat and to fully assess her small bowel, an exploratory laparotomy was performed. Diffuse thickening of the bowel and the mesentery was notable in the small intestines, as was alternating segments of dilatation and narrowing. 127 cm of midgut was resected (Figs. 10.4, 10.5, and 10.6). The patient suffered a cardiac arrest the following day (day 208 post-transplant).

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

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

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

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

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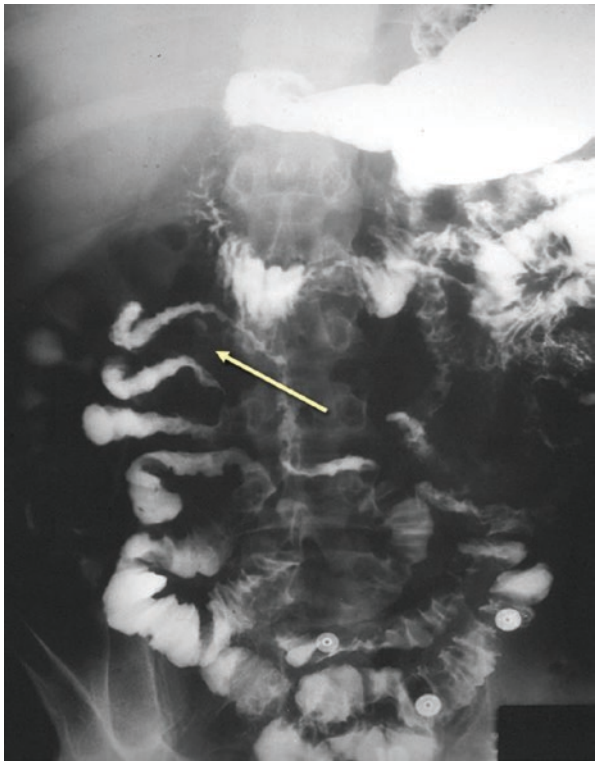

Fig. 10.1 Barium X-ray of acute GI GVHD demonstrating massive edema in the small intestine (arrow)

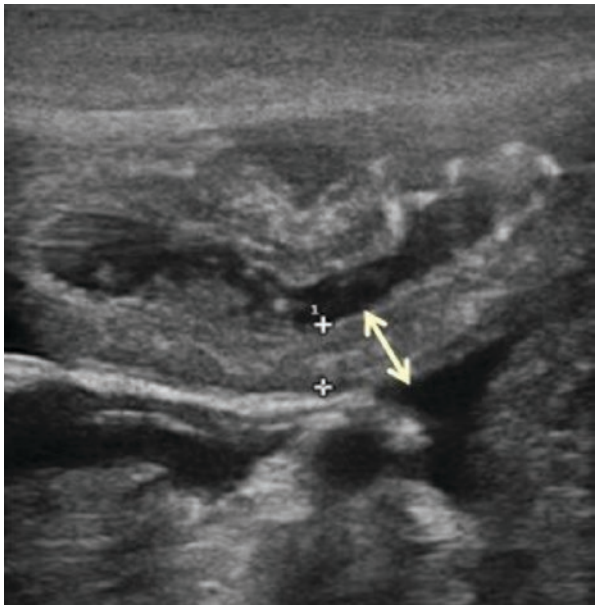

Fig. 10.2 Abdominal standard ultrasound findings in acute GVHD. Arrows indicate increased bowel thickness from mucosal edema

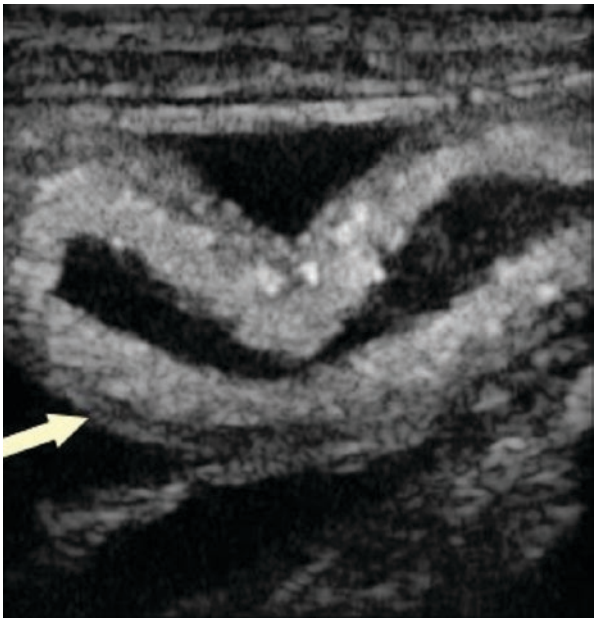

Fig. 10.3 Microbubble ultrasound arterial phase with complete enhancement from mucosal to serosal layer (arrow)

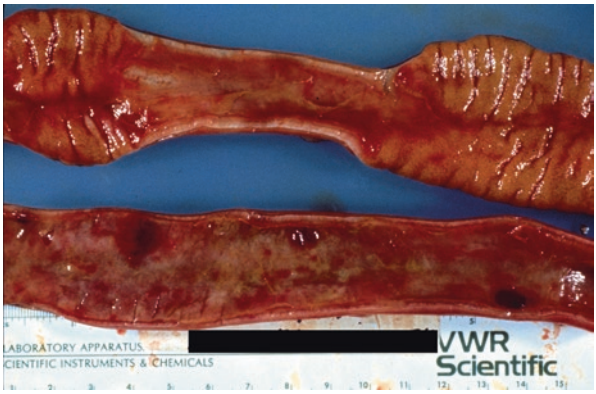

Fig. 10.4 Contains two segments from the intestinal resection. The top segment shows a narrow, ulcerated segment adjacent to dilated segments with linear ulcerations in the edematous mucosa. The lower segment in the photo shows diffuse ulceration with complete destruction of the mucosal layer overlying the edematous submucosal layer

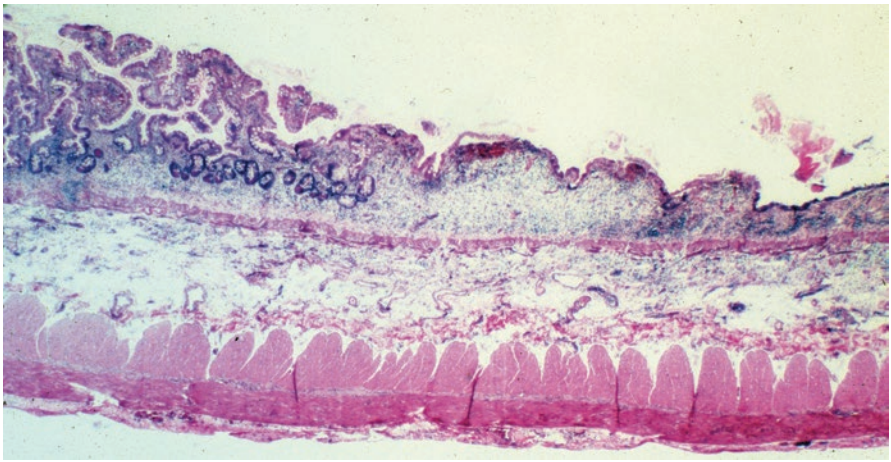

Fig. 10.5 Histologic section taken at the junction of the ulcerated and dilated segment with relatively preserved mucosa adjacent to ulcerated zone overlying an edematous submucosa

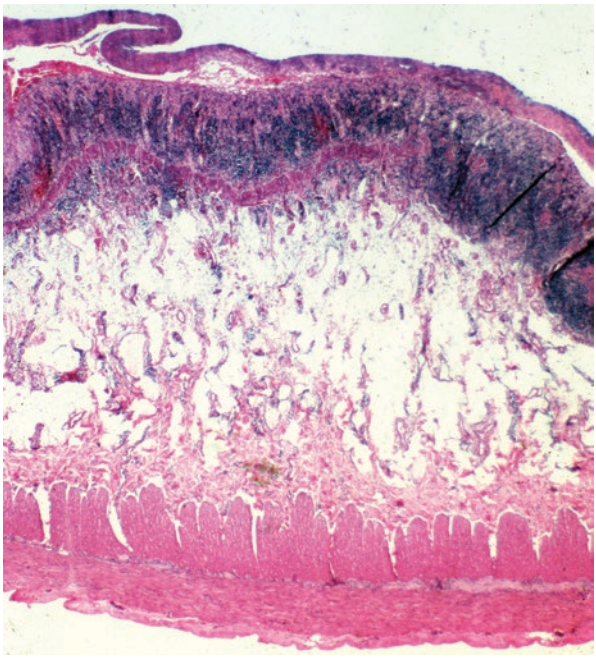

Fig. 10.6 Section taken from an area of ulceration shows a dense lymphocytic infiltrate occupying the muscularis mucosa covered by a thin strip of surface epithelium or fibrin. Beneath the infiltrate is a markedly edematous submucosa

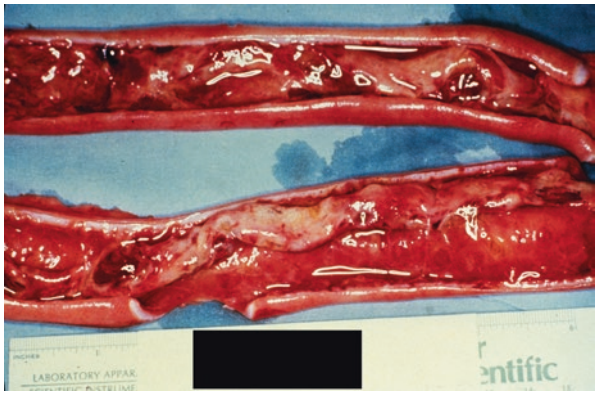

Fig. 10.7 Surgical resection taken from another patient with fatal GVHD contains a necrotic cast of sloughed mucosa resulting from diffuse intestinal involvement by GVHD

Diagnosis—Patient A

Unrelenting lower gut GVHD with segmental intestinal ulceration with stenotic narrowing

Key Pathology Features—Patient A

1. Even in the presence of severe lower gut GVHD, the changes are not uniform.
2. Ulcerated stenotic and denuded segments with no residual crypts may alternate with dilated segments with partially intact mucosa.
3. Greenish-brown casts passed per rectum (Fig. 10.7). Casts of necrotic debris admixed with fibrin may fill the intestinal lumen.

Patient B

Clinical History—Patient B

A 16-year-old with refractory AML received an allogeneic HSCT from his matched HLA sibling. He developed presumptive skin aGVHD on day 18, verified by biopsy on day 25. He subsequently developed gut GVHD with a maximum clinical Glucksberg grade III [4]. The elevated liver tests, linked to an epidemic of acute hepatitis A, resolved. Following a quiescent period of activity, he developed treatment-refractory progressive extensive multisystem cGVHD. The skin manifestations included painful scaly red dermatitis on the palms and soles, diffuse hyperpigmentation, contractures, ulcerations, alopecia, dystrophic nails, and severe painful neuralgias attributed to dermal sclerosis. Other major morbidities included non-bloody diarrhea with malabsorption and steatorrhea. Endoscopic biopsy of

duodenal mucosa and pancreatic function tests were normal. He developed progressive cholestatic liver GVHD with ascites, total bilirubin of 22 mg/dl, and alk phos of 2200u. Liver biopsy showed marked bile duct damage and cholestasis (see Fig. 14.10 in Chap. 14). On post-transplant day 458, he died with failure to thrive and hepatic encephalopathy. The photomicrographs of bowel are from the autopsy (Figs. 10.8 and 10.9).

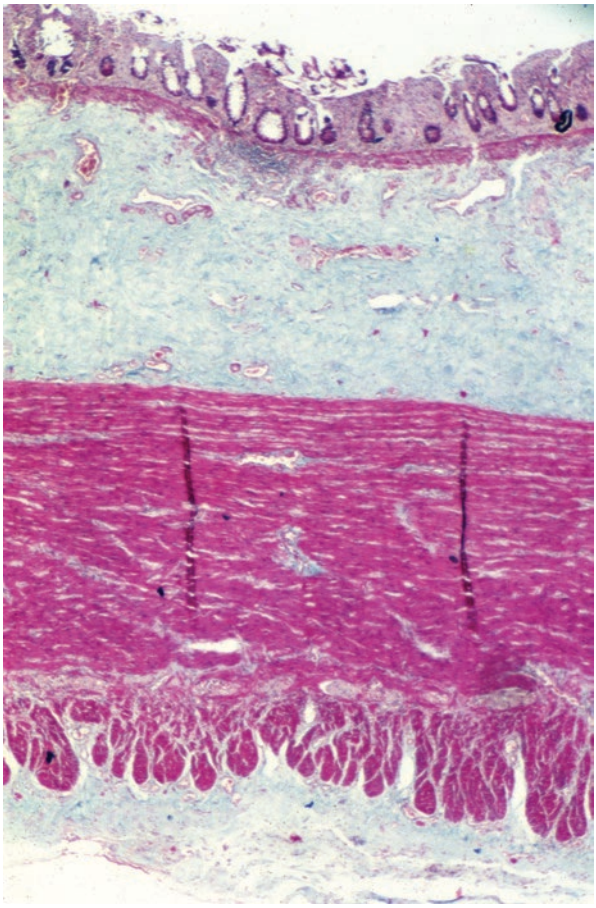

Fig. 10.8 Trichrome stain of colon autopsy shows fibrotic thickening of submucosa and lesser thickening of serosal layer

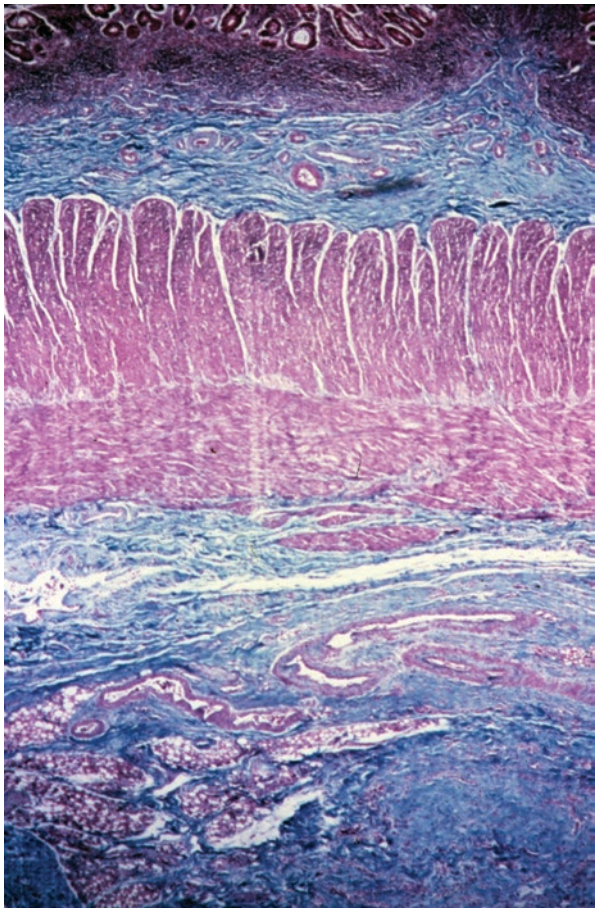

Fig. 10.9 In addition to submucosal fibrosis below the outer muscularis layer is ongoing panniculitis with fibrous organization of the periserosal mesenteric fat

Diagnosis—Patient B

Chronic GVHD with widespread submucosal and periserosal fibrosis

Key Pathology Features—Patient B

1. Dense fibrosis with thickening of the submucosal and periserosal layers.
2. All regions of upper and lower gut showed these fibrotic changes including the esophagus, intestines, and colon (Fig. 10.10).
3. The typical changes in long-standing gut GVHD are erythematous mucosal polypoid islands outlined by irregular ulcerations (Fig. 10.13 and Fig. 10.14). Microscopically, the mucosa consists of irregular misshapen crypts and thickening of the basal lamina.

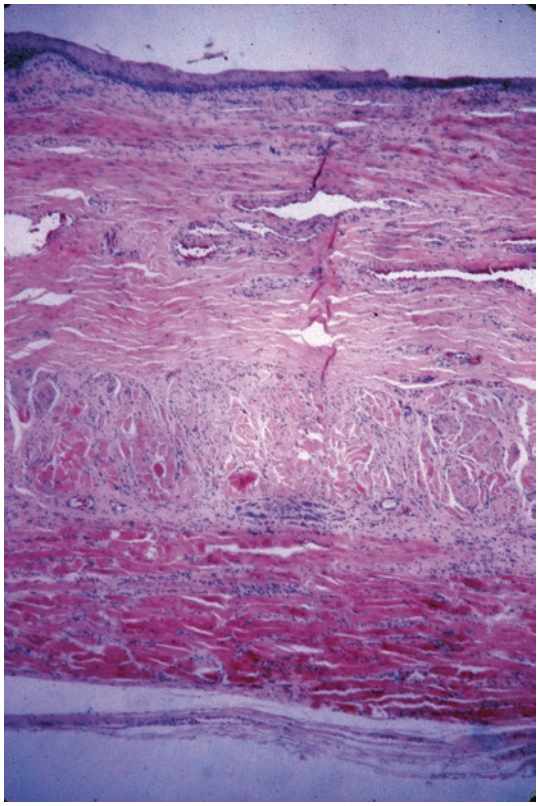

Fig. 10.10 Esophagus biopsy from day 350 autopsy from a patient with extensive cGVHD: beneath the intact squamous mucosa is a markedly thickened and fibrotic submucosa. The muscularis has inflammatory and degenerative changes

Differential Discussion

Patient A

Spencer et al. summarized the courses of 13 allogeneic recipients who underwent laparotomy for severe enteritis [5]. Widespread small bowel ulceration was present in all 13 patients. The etiologies included GVHD and/or opportunistic infections, toxicity from pre-transplant conditioning, and EBV-associated lymphoproliferative disorder (Figs. 10.11 and 10.12). Intestinal infections that were unrecognized before laparotomy were due to CMV, herpes simplex virus, adenovirus, and Torulopsis glabrata. 11/13 patients died in the perioperative period. Despite earlier and better diagnostic tools, severe intestinal GVHD still remains as a serious cause of morbidity and mortality.

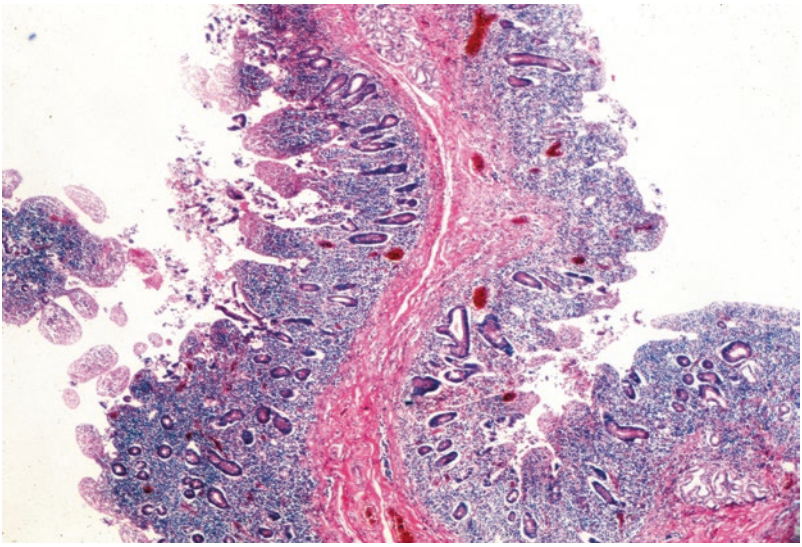

Fig. 10.11 EBV post-transplant lymphoproliferative disease. Dense infiltration of the intestinal mucosa

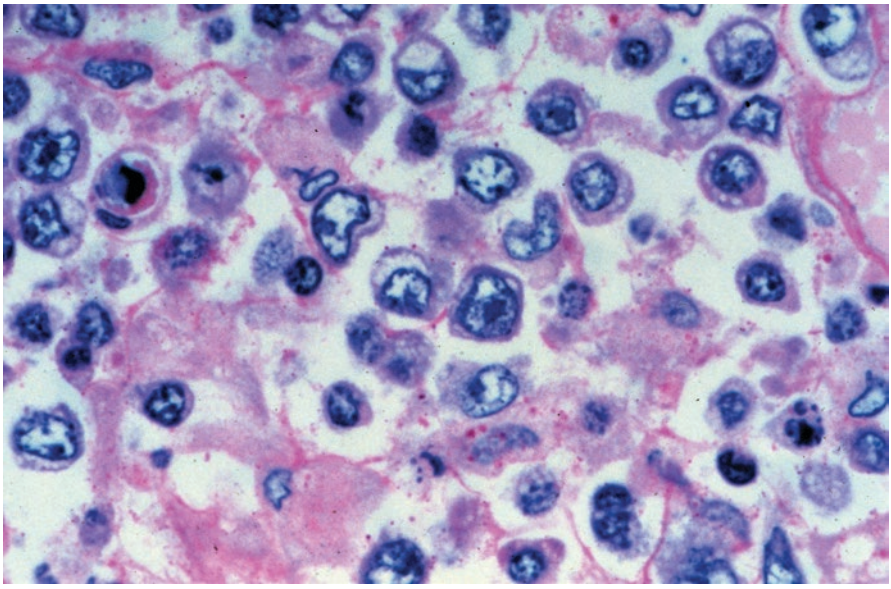

Fig. 10.12 Higher power of Fig. 10.11 shows the lymphoplasmacytoid cytology

Patient B

In contrast to the diffuse intestinal denudation in patient A, patient B had malabsorption and steatorrhea with atrophic (but relatively intact) mucosal surfaces but dense fibrosis throughout the lamina propria, submucosa, and periserosal mesentery. This appeared to be a true manifestation of his diffuse sclerodermatous cGVHD. The reason for the present rarity or absence of this complication is not clear, though it may be due to different immunosuppression (IS) agents that are now available, earlier intervention, or lack of autopsies in such patients. The typical fibrosis associated with chronic or persistent GVHD only involves the basement membrane below the surface epithelium. Chronic mucosal changes include architectural distortion, cystic change, cytologic atypia, loss of mucin, and loss of intestinal Paneth cells (Fig. 10.5) [6]. Because these changes do not occur in a chronologically discrete manner before or after day 100, the NIH Consensus no longer acknowledges day 100 as the temporal threshold between acute and chronic gut GVHD [7].

General Discussion

In this chapter, we describe two different irreversible courses of gut GVHD that can appear before or after day 100. Case 1 illustrates that damage from gut GVHD may not be uniform with severely damaged and ulcerated mucosa adjacent to relatively preserved segments of intestinal mucosa. The changes of persistent GVHD in the colon are more uniform (Fig. 10.13). Imaging studies, including CT and ultrasound, have pinpointed the location of the regions with the most pronounced damage [1–3].

The ileal predilection for the most severe denudation may be explained by the high concentration of lymphoid aggregates located in the ileum (Fig. 10.14) [8]. The high endothelial venules within the lymphoid aggregates are the entry portals for the lymphoid cellular effector cells of GVHD [9]. Unfortunately, in the past, surgical resection of localized severely damaged segments of gut had not been very successful [5].

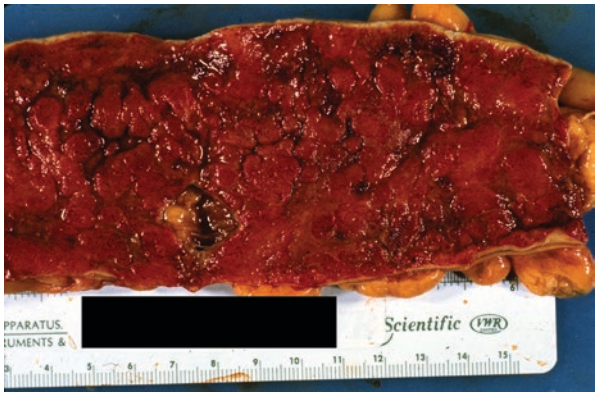

Fig. 10.13 After a protracted course of severe gut GVHD at autopsy, the colonic mucosa has an irregular edematous granular surface interspersed with linear ulcerations

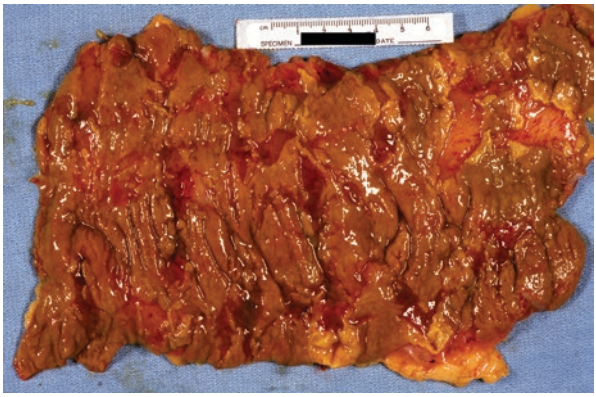

Fig. 10.14 Protracted fatal gut GVHD. The mucosa has edematous raised folds separated by irregular linear ulcerations

In a cohort of 1462 HSCT recipients in Seattle, the prevalence of severe grade 3–4 clinical gut GVHD was 7.9%. Mortality was high, with survival at 1 and 2 years, only 29% and 25% [10]. Other studies have noted an equally bleak outcome [11–13]. A variety of different modalities have been utilized for earlier recognition and stratification of patients at risk for a poor outcome. These modalities include biomarker data [14–18], stool volume, radiologic imaging, and endoscopic visualization. This information is used to guide early entry into experimental treatment protocols before mucosal barrier destruction, and subsequent intractable gut damage occurs.

An enigmatic question regarding steroid-resistant GVHD (SR-GVHD) is why do patients remain refractory despite various sustained high-dose IS regimens [19]? Histology of such patients typically shows little inflammation or apoptotic activity. A better understanding of the pathobiology of the crypt cell niche is needed to develop effective treatments to restore the mucosal barrier. The crypt niche of the gut has complex relationship between factors which sustain or inhibit growth (Fig. 10.15) [20]. Renewal of the intestinal crypt stem cells (ISC) depends on both the growth stimulatory effect of IL2 derived from lamina propria lymphocytes and the crypt Paneth cells adjacent to the ISCs. Paneth cells provide the growth factor REG3α and protective antimicrobial α-defensins [21]. This defense is aided by the protective layer of mucin, which shields the epithelial cells from exposure to commensal bacteria. Loss of Paneth cells, along with biochemical markers of REG3α in the duodenum, is correlated with a poor outcome. However, the loss of Paneth cells is not present at the onset of GVHD [6]. When these protective and stimulatory influences are destroyed by GVHD, commensal luminal bacteria cause bacterial translocation and, subsequently, TNF upregulation, amplification of inflammation, cytokine release, and infiltration by neutrophils which release toxic oxygen free-radicals into the crypt.

A number of studies focus on endothelial cells' (EC) participation in the genesis of GVHD. These studies note the markedly damaged capillary endothelium in intestinal GVHD manifest by swollen and/or denuded ECs, separation of ECs from their basal lamina, and patchy microhemorrhages [22]. Mucosal ECs are also damaged

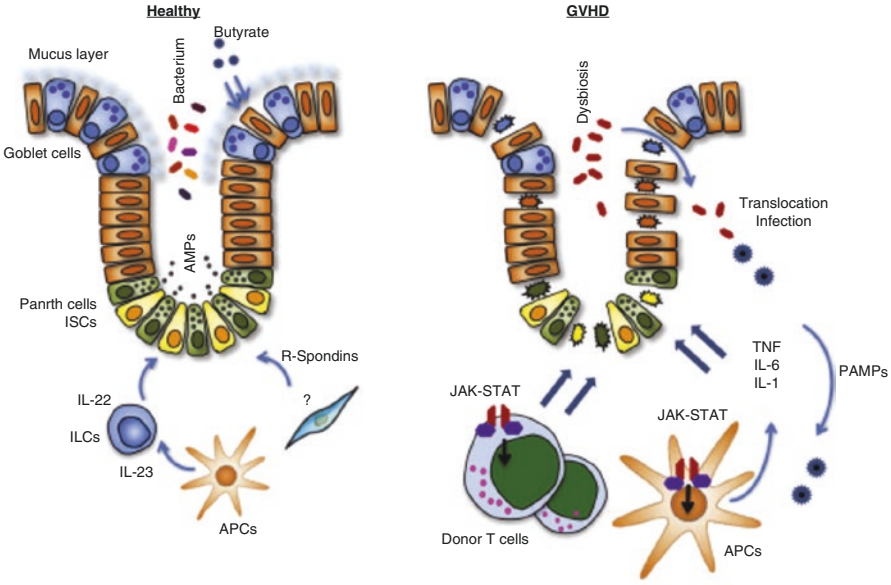

Fig. 10.15 The diagram above (from Biology of Blood and Marrow Transplantation. 2016:22(1):11–16) shows the contrast between the normal epithelium versus epithelium attacked by GVHD. The details of this process are described in the text

by the infiltration of neutrophils [23] and activated eosinophils [24]. A relevant question is whether angiogenic factors are an initiating cause or a secondary effect of GVHD [25]. Luft et al. noted that blood samples from patients with gut GVHD contained (1) elevated levels of angiopoietin-2 (ANG2) (antagonist to ANG1, which mitigates endothelial permeability and promotes blood vessel development and integrity [26]); (2) epithelial cytokeratin-18 (CK-18) (a marker of epithelial injury); and (3) decreased levels of thrombomodulin and vascular endothelial growth factor (VEGF) (a regulator of EC survival and blood vessel permeability) [27]. In a cohort of patients with SR-GVHD, the ANG2 levels were elevated even before transplant. Decreased or absence of VEGF and elevated ANG2 causes EC death. Furthermore, ANG2 sensitizes EC to inflammatory cytokines and has a role in the initiation of inflammation [28]. Other studies support that reduced thrombomodulin levels predict SR-GVHD [29] and increase non-relapse mortality [30]. In addition, a biomarker panel incorporating the values of ANG2, C-reactive protein, D-dimer, and thrombomodulin was significantly associated with poor overall survival [30]. Mir et al. found a pro-inflammatory and pro-thrombotic EC phenotype at the onset of aGVHD [31]. These studies support the concept that EC injury influences the genesis and propagation of SR-GVHD, although the relationships and interactions of the contributing elements require further study.

A recent pilot study found that lithium (Li^+) administration, if given soon after documentation of extensive endoscopic mucosal denudation or unresponsiveness after 5 days of steroids, could restore mucosal barrier function, greatly improving survival [32] (Figs. 10.16 and 10.17). Li^+ facilitates mucosal regeneration via

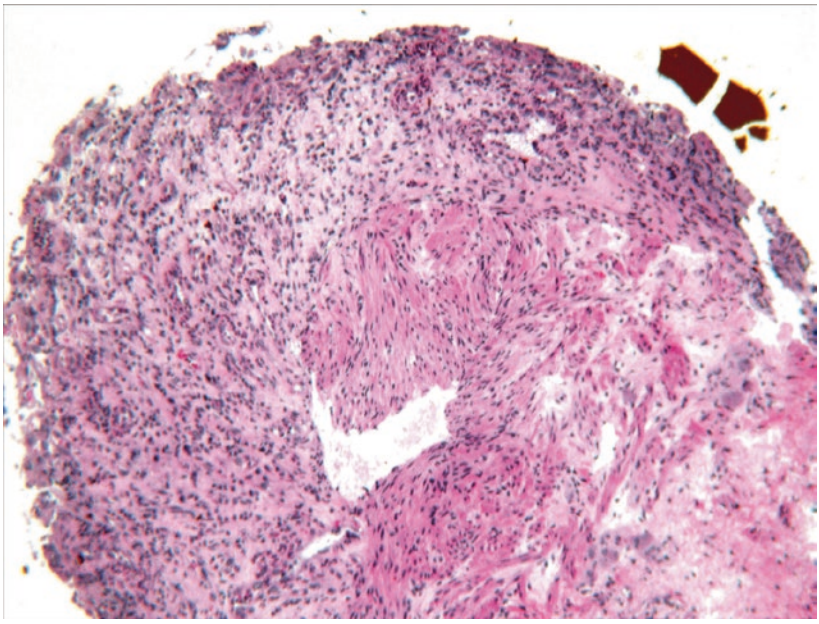

Fig. 10.16 Pre-lithium biopsy demonstrates complete mucosal destruction

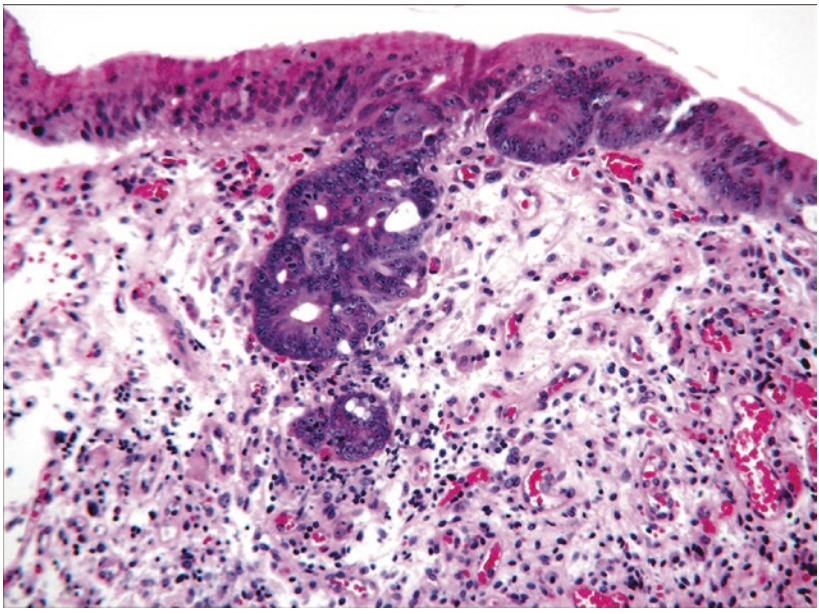

Fig. 10.17 Colonic mucosa post-lithium demonstrates regeneration of surface epithelium and a crypt surrounded by chronic inflammation, indicating some persistence of GVHD

induction of Wnt signaling, which blocks the inhibitory effect of GSK-3 (a regulator of cytosol β-catenin in the nuclear effector pathway). In addition to stimulating crypt stem cell proliferation and replication, Li^+ exerts an inflammatory effect by blocking GSK-3 stimulation of inflammatory cells [33, 34]. It should be noted that even with restoration of the mucosal barrier, IS must still be given to control the underlying GVHD response.

In conclusion, it is hoped that improved understanding of the mucosal barrier's pathobiology and the role of the microbiota and endothelium will translate into improvements in the management of gut GVHD.

Questions

1. Which region(s) of the gastrointestinal tract is/are most severely involved with persistent and refractory GVHD?
2. What are other causes of diffuse mucosal destruction?
3. What is the significance of loss of intestinal Paneth cells?

Teaching Points

1. Chronic persistent gut GVHD has several characteristic findings, the most common of which is diffuse mucosal ulceration, which may be segmental and alternating with dilatation. Other findings include fibrinopurulent casts composed of necrotic tissue. Intestinal perforation rarely occurs from severe GVHD.
2. In addition to GVHD, diffuse intestinal ulceration can result from several other causes: infections, drug and chemoradiation, toxicity, and post-transplant lymphoproliferative disorder.
3. Diffuse fibrosis of the submucosa and mesenteries is a rare complication associated with severe and prolonged chronic GVHD.
4. Following a diagnosis of gut GVHD, the risk profile for developing gut SR-GVHD can be predicted by several clinical features including the response to steroids, the persistent need for immunosuppression, diarrhea volumes, serum albumin, endoscopic assessment, panels composed of several biomarkers [35], and positive biopsies from the lower gut, even those of only Lerner-Sale grade $\geq$ I.
5. The pathobiology of SR-GVHD of the gut involves damage to the ISC niche with loss of the mucous barrier. The role of EC injury as a cause or effect of this damage is under investigation.
6. Loss of Paneth cells in tissue sections is associated with poor prognosis, though these changes occur later and thus are not useful in the early stages of gut GVHD [6].

Answers

1. Answer: The midgut, ileum, and colon
2. Answer: CMV enteritis, herpes simplex, and severe toxicity from conditioning chemotherapy (see Chap. 9)
3. Answer: Paneth cells produce the antibacterial granules defensin and REG3α, which provide growth factors for the intestinal stem cells. Elevated REG3α levels are associated with a poor outcome. Paneth cell loss is not present in early GVHD biopsies.

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Abdominal Pain and Diarrhea When It Is Not GVHD

11

David Myerson and Sahl Ali

Clinical History

The patient is a 45-year-old female post cord blood transplant for an immunodeficiency syndrome. Pretransplant complications included chronic autoimmune disease, malnutrition of chronic disease, bone marrow aspergillosis, and disseminated mycobacteria avium complex (MAC). Plasma PCR for adenovirus was negative. She was prepared with nonmyeloablative alemtuzumab, fludarabine, melphalan, and thiotepa and engrafted with cord blood stem cells. Donor engraftment was documented throughout. Her posttransplant course was complicated with persistent high-volume diarrhea. On day 31 posttransplant, endoscopic biopsies of the stomach, duodenum, and rectosigmoid colon all showed GVHD of mild histologic activity. This was initially treated conservatively. On day 84, endoscopic biopsies of the stomach, duodenum, and rectosigmoid colon were free of GVHD. On day 133, an esophagus biopsy demonstrated HSV esophagitis. She subsequently developed liver failure with ascites, attributed to MAC. On day 179, an endoscopic biopsy demonstrated candida esophagitis. The endoscopy was negative for adenovirus and CMV. On day 315, biopsies showed chronic gastritis, along with a normal-appearing duodenum, and a colon biopsy consistent with GVHD of mild histologic activity. She was then started on prednisone for GVHD. There was some improvement of symptoms, but a non-GVHD etiology was suspected to be the underlying cause,

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: dmyerson@fredhutch.org

S. Ali
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
e-mail: sahl@wustl.edu

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and prednisone was tapered. On day 366, continuing pain and diarrhea led to another set of biopsies, which demonstrated GVHD of mild histologic activity in the duodenum, with possible viral inclusions. Immunohistochemistry demonstrated focal adenovirus infection. Brincidofovir treatment was initiated for the adenovirus. However, a rapidly progressive monomorphic posttransplant lymphoproliferative disorder (PTLD) intervened.

Pathology Images (Figs. 11.1, 11.2, and 11.3)

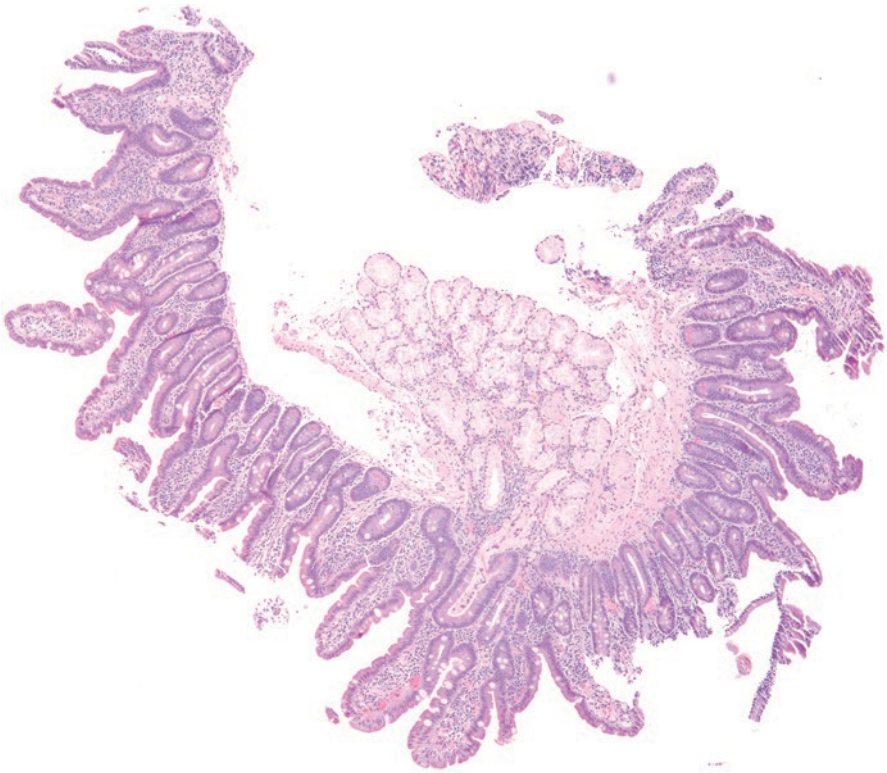

Fig. 11.1 Architecture is normal, with the villi on the low side of the usual 3–5:1 villus/crypt ratio, a frequent finding posttransplant

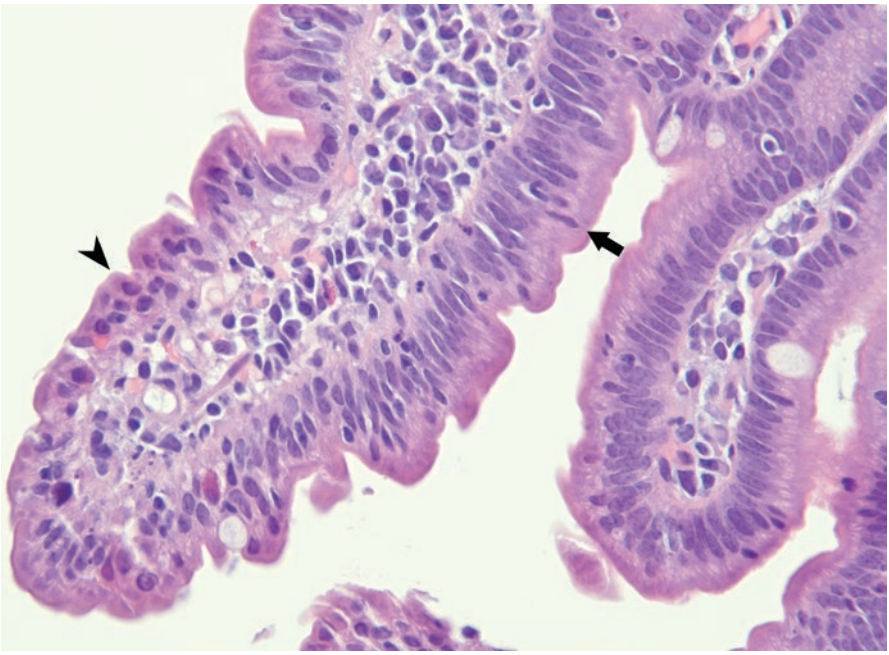

Fig. 11.2 The figure shows minimal findings with a few slightly enlarged, slightly misarranged nuclei with rare, ill-defined inclusions. There are minimal irregularity of nuclear arrangement, loss of polarity (arrow), minimal nuclear enlargement and rounding (arrowhead), and possible hyperchromatic smudge-like nuclear inclusions

Diagnosis

Adenovirus duodenitis

Key Pathology Features

Loss of polarity and "piling up" in the epithelium
Nuclear atypia with slight enlargement and rounding
Hyperchromatic smudge-like nuclei

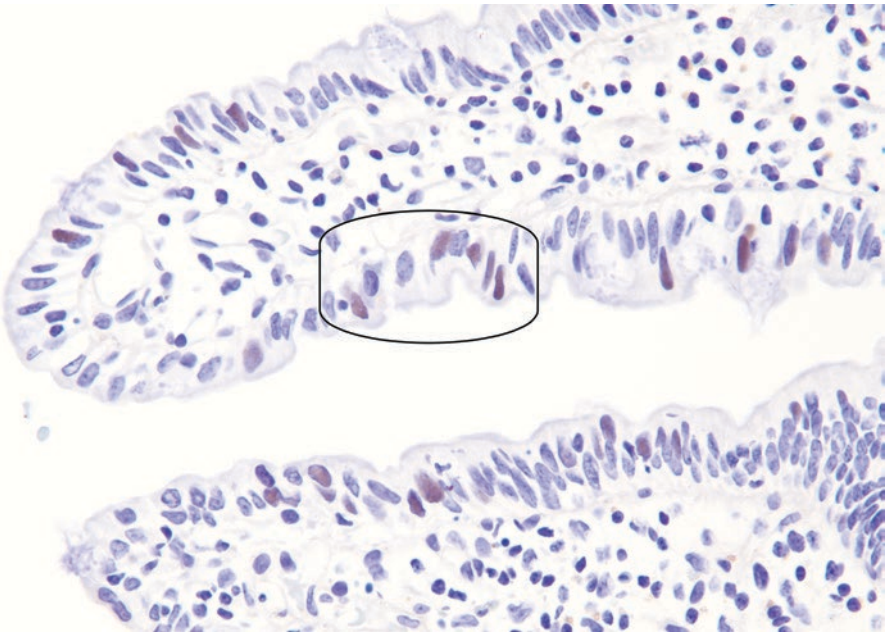

Fig. 11.3 Adenovirus immunohistochemistry shows many cells staining brown with variable intensity, the nuclear staining diagnostic of adenovirus (Cell Marque Adenovirus 20/11&2/6). The affected cells tend to be the atypical ones with hyperchromatic smudge-like nuclei. IHC shows a focus with slightly swollen nuclei, slightly misarranged and piled up, containing several adenovirus-infected cells (circle). Many infected cells, however, are not morphologically distinguishable from normal [1]

Differential Discussion

Although plasma PCR was not performed for this patient's adenovirus, it is usually positive in cases of adenovirus of the GI tract. Another case of more intense adenovirus is shown in Figs. 11.4, 11.5, 11.6, and 11.7. The patient was 40 days posttransplant for CLL. Informed by a positive shell vial culture assay, adenovirus IHC was performed. Ultimately disseminated adenovirus developed, with plasma PCR positivity up to $10^{5.7}$ copies per ml, with associated hepatitis and nephritis, the latter the most typical presentation of adenovirus posttransplant [2].

CMV is a frequent cause of pain or diarrhea after transplant. Although antiviral prophylaxis or treatment has reduced the incidence, CMV is still a frequent cause of gastroenteritis and colitis. CMV infection may be associated with large "cytomegalic" Cowdry type A inclusions, often appearing as an "owl's eye" because of artifactual shrinkage from fixation. CMV may also be characterized by cytoplasmic inclusions. Since CMV does not infect stratified squamous epithelium, the esophagus is usually spared in a focal infection [3]. Infection of the stomach is usually in

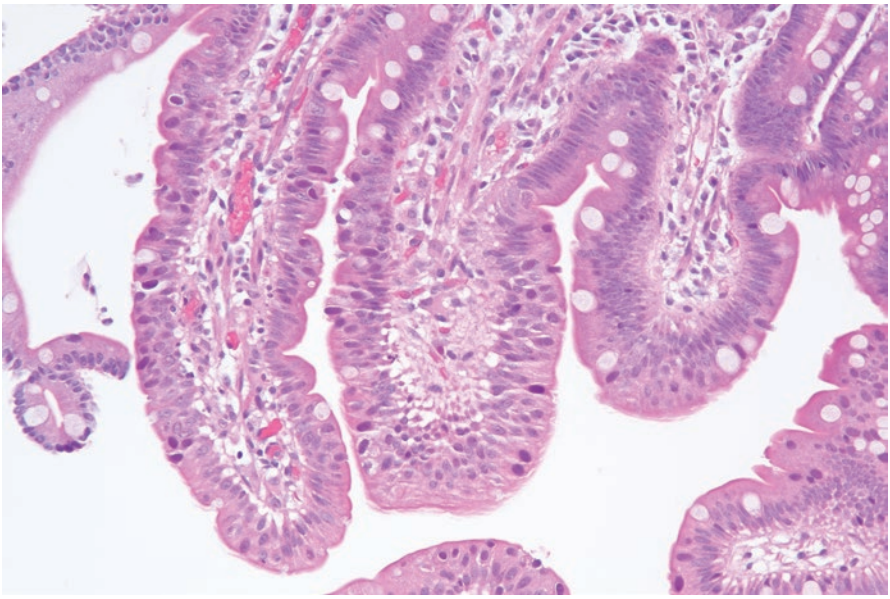

Fig. 11.4 Mid-power view of a heavily infected epithelium. Here, many foci of adenovirus are present, but the findings on H&E alone are not striking. Inflammation is minimal. There are atypical cells with plump hyperchromatic nuclei and loss of polarity. There are cells with slight atypia, and without atypia, that are infected as well

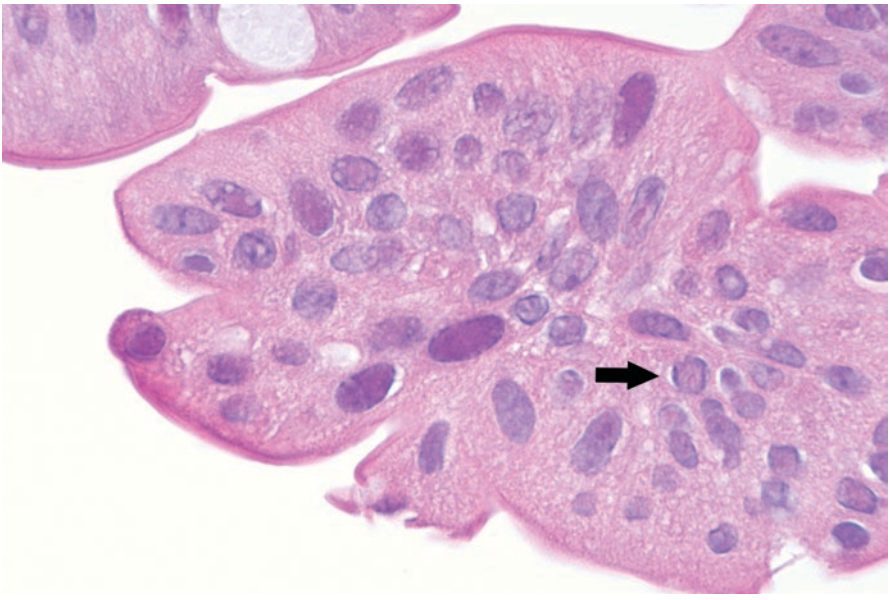

Fig. 11.5 High-power (100×) view of a focus on H&E. Various inclusions are appreciated, some appearing similar to the Cowdry type A inclusions of herpes, with a ring of marginated chromatin (arrow). Viral inclusions of adenovirus are also variously described as hyperchromatic with irregular "geographic" boundaries or "smudged"

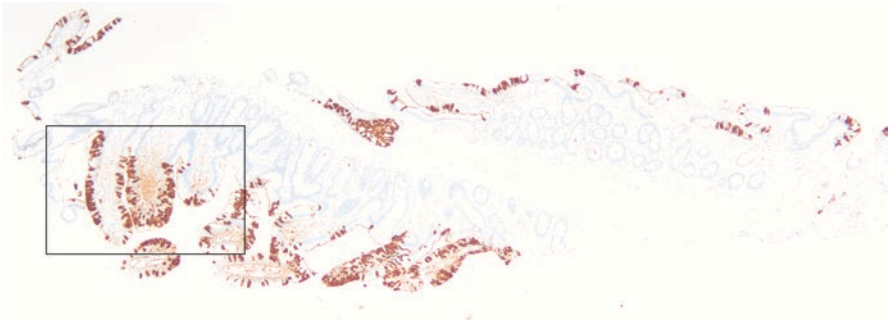

Fig. 11.6 Low-power view showing a near-normal villous architecture. However, this duodenum is heavily infected with adenovirus, as demonstrated by decoration by IHC

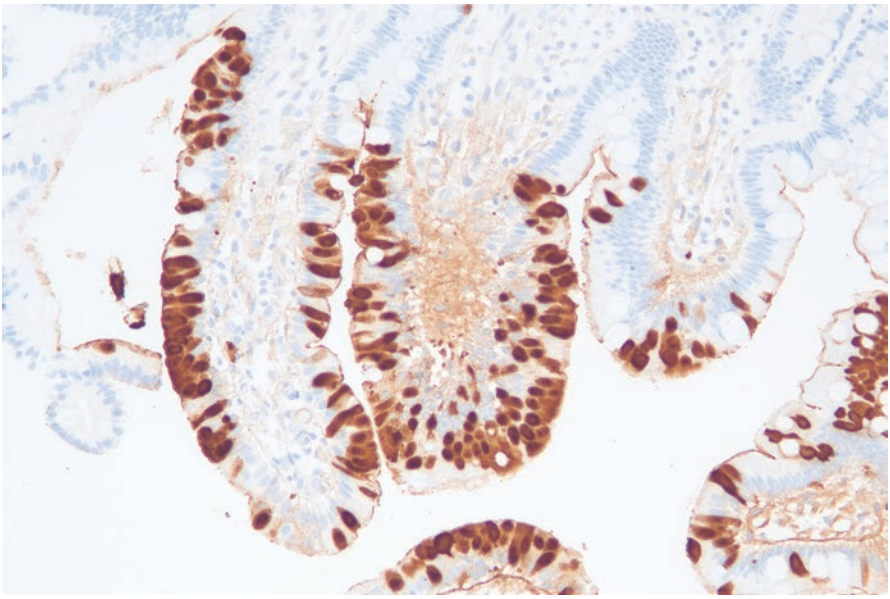

Fig. 11.7 Mid-power view of the outlined section in Fig. 11.6 with adenovirus IHC, showing virtually all the enlarged nuclei associated with adenovirus

the epithelial cells rather than the lamina propria (Fig. 11.8), but intestinal infection is often in the underlying lamina propria, with endothelial cells and reactive myofibroblasts in granulation tissue the frequent target (Fig. 11.9). The presence of CMV does not exclude concurrent GVHD, quite the reverse, as CMV has long been known to be associated with GVHD [4]. As CMV and all other herpesviruses are latent viruses, present for life once infected, the presence of low-titer PCR positivity in a posttransplant patient does not definitively indicate active infection. A minimal finding of CMV by IHC, however, is worrisome.

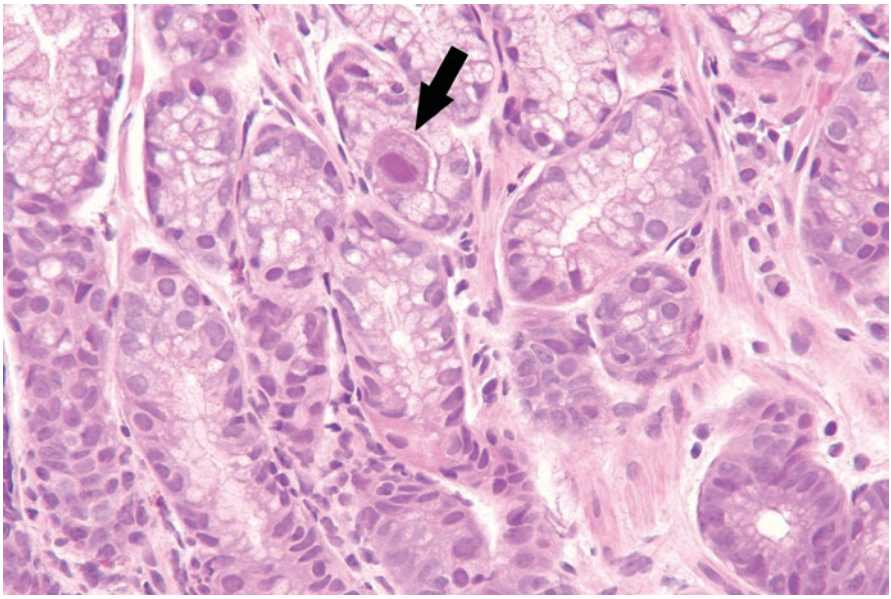

Fig. 11.8 This stomach shows a typical large amphophilic CMV "cytomegalic" nuclear inclusion in an epithelial cell (arrow)

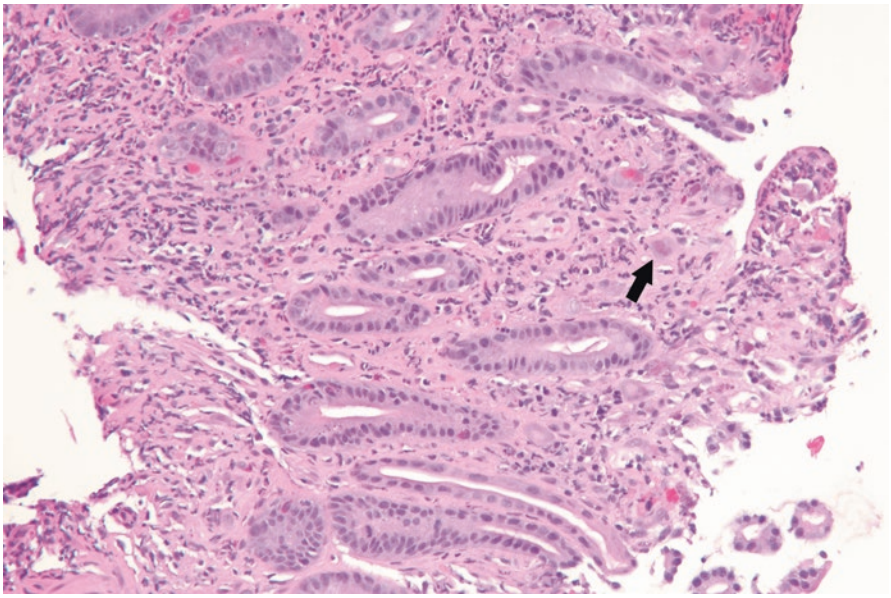

Fig. 11.9 CMV-associated ulcer with mixed inflammation in a stomach with foveolar hyperplasia. Here, the CMV-infected cells are in the lamina propria. One with a smudgy amphophilic inclusion near the ulcerated surface is indicated (arrow). IHC for CMV showed widespread positivity

Fungal infection may occasionally cause gut symptoms. This may be due to localized *Candida* or *Aspergillus*, a *Zygomycetes*, or another mold. Gut molds more typically occur in the background of disseminated infection. Pictured is a case of zygomycosis with *Rhizopus oryzae* in a 38-year-old only 10 days post-second transplant for an immunodeficiency disease. The mold disseminated to the gut through a mycotic embolus in the celiac axis artery, pictured (Fig. 11.10). It should be noted that fungus cannot be reliably speciated by morphology in tissue sections [5].

Another common cause of diarrhea is pseudomembranous colitis, usually caused by *Clostridium difficile*. In our patients, this is the most common cause of bacterial colitis (Fig. 11.11).

Typhlitis (neutropenic enterocolitis) occasionally occurs and is caused by gut flora with expansion of the submucosa by large expanses of bacteria. It is frequently fatal but may be successfully treated on occasion (Fig. 11.12).

Protozoa may cause diarrhea, as in a normal population. Here, a 68-year-old patient 785 days posttransplant exhibits *Giardia lamblia* in the duodenum (Fig. 11.13).

Chronic GVHD may present as a thickened terminal ileum, similar to Crohn's disease. Rarely, *Yersinia enterocolitica* may also induce a thickened terminal ileum, with ulceration (Figs. 11.14 and 11.15).

Posttransplant lymphoproliferative disorder (PTLD) may also present as a thickened terminal ileum. This is a donor-derived, EBV-associated, lymphoid proliferation which may be detected by in situ hybridization for EBER (EBV-encoded RNA). Sometimes, PTLD presents as pseudopolyps in the terminal ileum (Fig. 11.16).

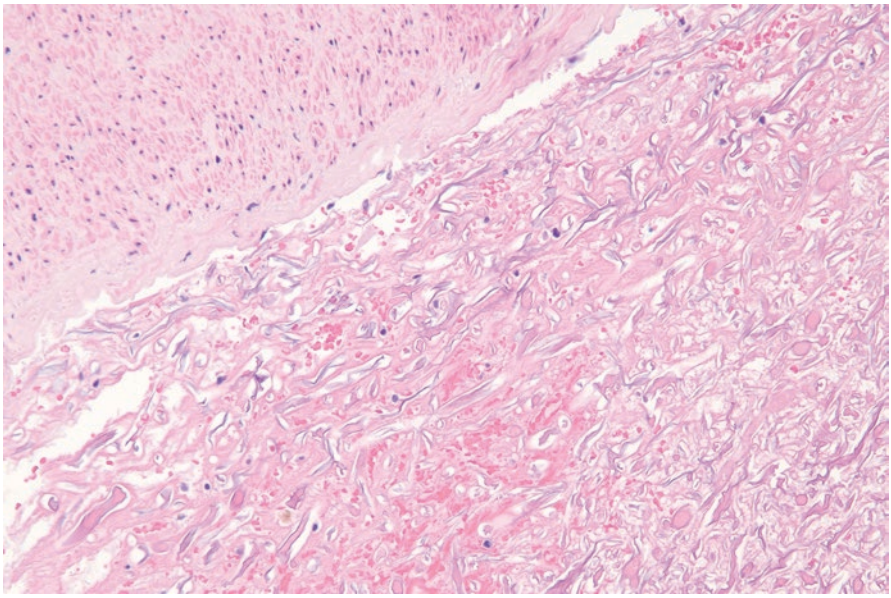

Fig. 11.10 The ribbonlike hyphae fill the vascular lumen of the celiac axis in this case of zygomycosis with *Rhizopus oryzae*

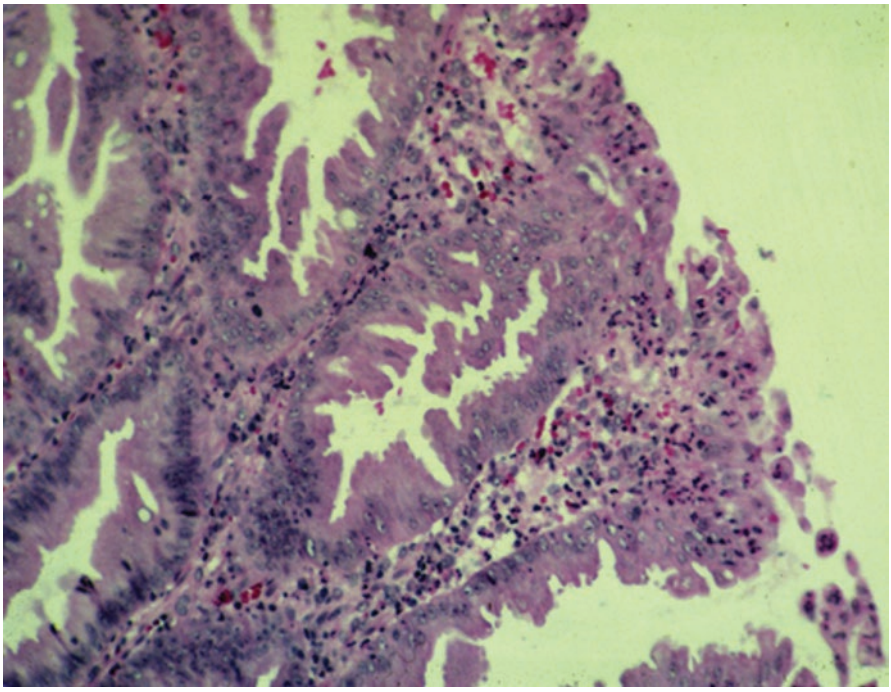

Fig. 11.11 Pseudomembranous colitis with acute inflammation in the lamina propria of the colon, reaching the surface with ulceration. The usual "volcano-like" lesion with expression into the lumen as a pseudomembrane is not visualized here

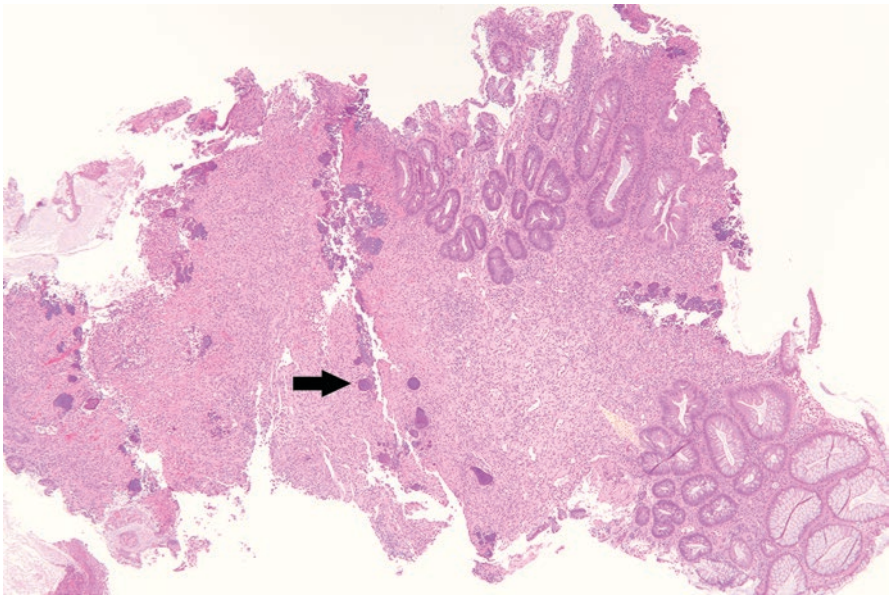

Fig. 11.12 Typhlitis (neutropenic enterocolitis). This is a superficial biopsy with the lamina propria markedly expanded by a neutrophilic infiltrate. Large foci of a pure bacteria colonies are appreciated as basophilic foci (one of which is marked by the arrow)

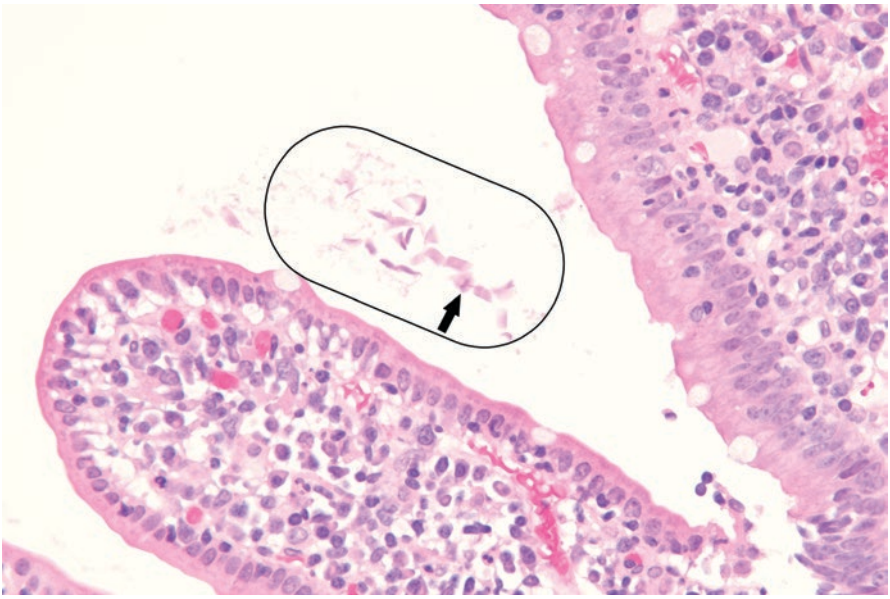

Fig. 11.13 A cluster of *Giardia lamblia* organisms (circled) in the lumen of the duodenum shows a rare form cut tangentially with two (ill-defined) adjacent nuclei pathognomonic of *Giardia* (arrow). Most organisms are cut in cross section appearing as a pile of "leaves"

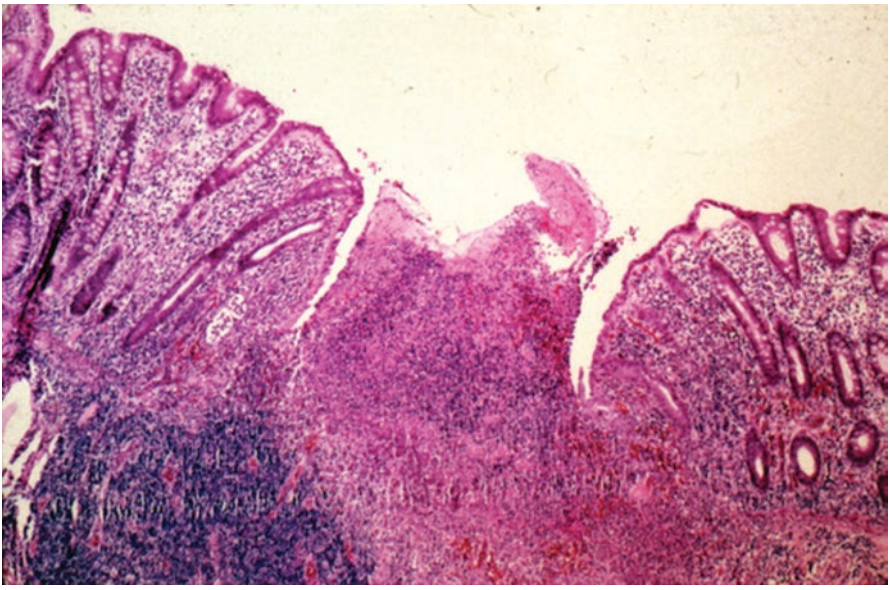

Fig. 11.14 Low power of *Yersinia enterocolitica* ulcer in the terminal ileum

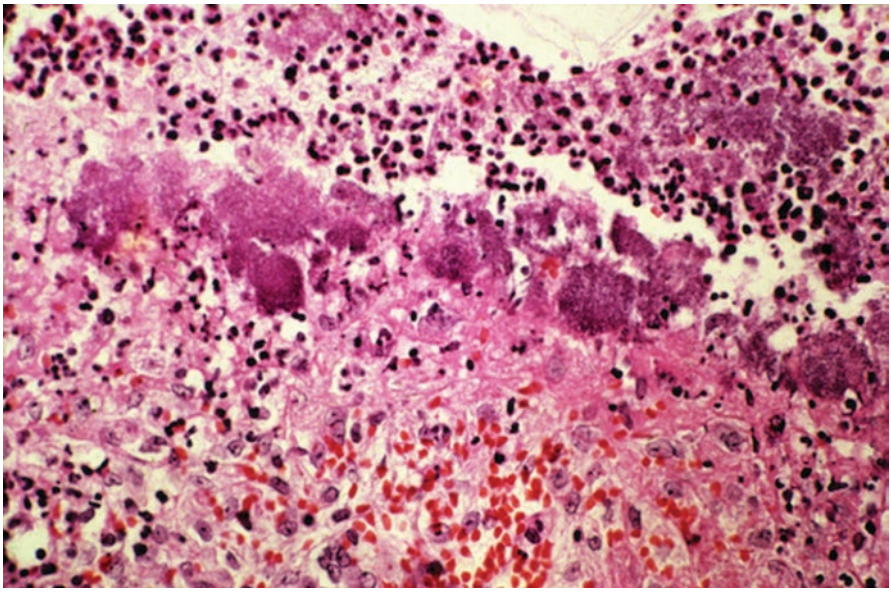

Fig. 11.15 High-power view of the *Yersinia* infection, with large groups of amphophilic bacteria, representing large colonies of the gram-negative rods

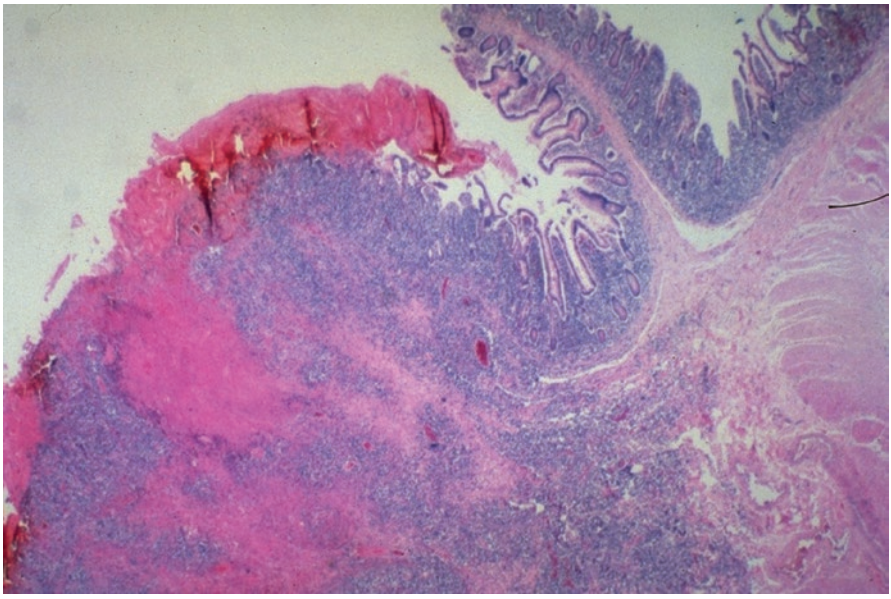

Fig. 11.16 Pseudopolyp with ulceration due to underlying PTLD

PTLD may present as a diffusely thickened gut, as in the case of a 65-year-old female 256 days posttransplant. Whereas the H&E shows a lymphocytic infiltrate, the EBER ISH demonstrates many EBV-infected lymphocytes, easily sufficient to classify as PTLD (Figs. 11.17 and 11.18). PTLD varies in its aggressiveness, with this biopsy classified as a non-destructive PTLD, infectious mononucleosis-like PTLD (IM PTLD). PTLD may be associated with GVHD, but reducing immunosuppression to treat the PTLD may exacerbate GVHD if present [6].

There are other infectious causes of gastroenteritis posttransplant. HSV and VZV may cause gastroenteritis, and both may be detected by plasma PCR as well as by IHC. It should be remembered that the Cowdry type A inclusions of HSV are not morphologically distinguishable from those of VZV. Acyclovir prophylaxis has markedly reduced the incidence of these infections. Interestingly, severe HSV enteritis may not include obvious inclusions, but is reliably detected by IHC. Other causes of gastroenteritis include *Acanthamoeba histolytica*, which usually causes amoebic colitis. *Cryptosporidium* may also occasionally be seen posttransplant. Additional causes of pain and diarrhea include the usual enteritis bacteria and viruses such as *Salmonella* and *Norovirus* [7]. *Norovirus* sometimes exhibits a severe course with extended transmission posttransplant, with duodenal biopsies providing a distinction between *Norovirus* and GVHD [8].

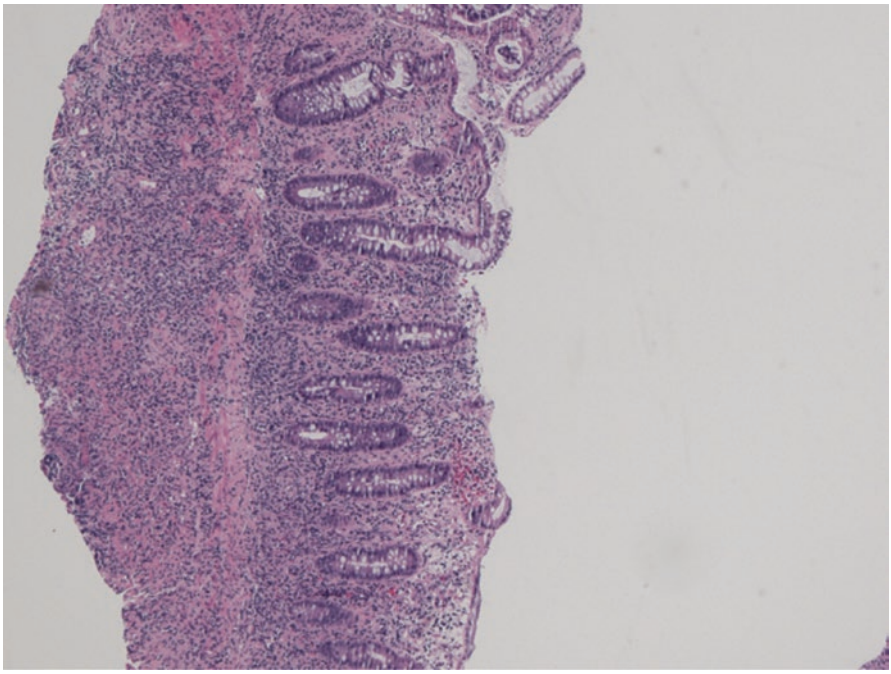

Fig. 11.17 Colon biopsy stained with H&E showing lymphocytes infiltrating and expanding the mucosa and submucosa to the margin of resection

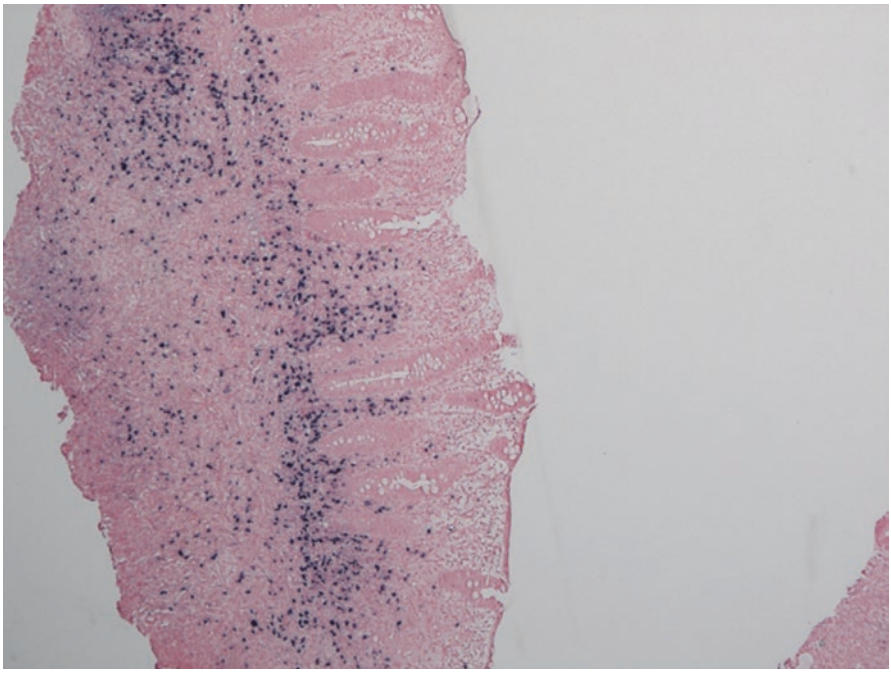

Fig. 11.18 The same section as Fig. 11.17, with EBER in situ hybridization. There are many EBV-infected lymphocytes (blue)

Teaching Points

- Abdominal pain and diarrhea are common in patients with GVHD but may have other causes, frequently infections.
- An endoscopic biopsy may provide information for diagnosis, which may confirm GVHD, or provide morphologic evidence for other causes, such as CMV, adenovirus, HSV, VZV, or PTLD.
- PCR may independently provide etiologic evidence.
- CMV and GVHD often occur together.

Questions

1. True/false: GVHD and CMV are mutually exclusive in the colon.
2. Choose as many as needed: Adenovirus shows what kind of inclusions?
   a. Cowdry type A
   b. "Owl's eye"
   c. "Geographic" inclusions
   d. Cytomegalic inclusions
   e. "Smudge" nuclei

3. Choose as many as needed: GVHD may be distinguished from alternative causes of abdominal pain and diarrhea…
   a. Sometimes by biopsy alone
   b. By PCR of the biopsy because GVHD may be diagnosed by PCR
   c. By plasma PCR because many infectious agents may be diagnosed by PCR
   d. By PCR on the biopsy because many infectious agents may be diagnosed by PCR

Answers

1. Answer: False. CMV and GVHD have long been associated.
2. Answer: a, c, and e. "Owl's eye" inclusions (b) and cytomegalic inclusions (d) are associated with CMV.
3. Answer: a, c, and d. We are not there yet in (b) diagnosing GVHD by PCR.

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Mucosal Chronic GVHD Affecting the Oral Pharyngeal, Esophageal, and Anogenital Regions

12

Howard M. Shulman, David M. Hockenbery, and Cecilia C. S. Yeung

Clinical History

Eight months after Hodgkin lymphoma recurred following an autologous HSCT, this 30-year-old woman received an HLA-matched unrelated donor allogeneic transplant. Hyperacute GVHD developed soon afterward. By day 95, she had developed chronic GVHD (cGVHD) involving multiple sites, including the skin with focal lichenoid skin changes, oral mucositis (Figs. 12.1, 12.2, and 12.3), keratoconjunctivitis sicca syndrome, vaginitis, and pulmonary bronchiolitis obliterans syndrome. She responded well to IS but had persistent oral and

H. M. Shulman (✉) · C. C. S. Yeung
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; cyeung@fredhutch.org

D. M. Hockenbery
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Division of Gastroenterology, University of Washington School of Medicine, Seattle, WA, USA
e-mail: dhockenb@fredhutch.org

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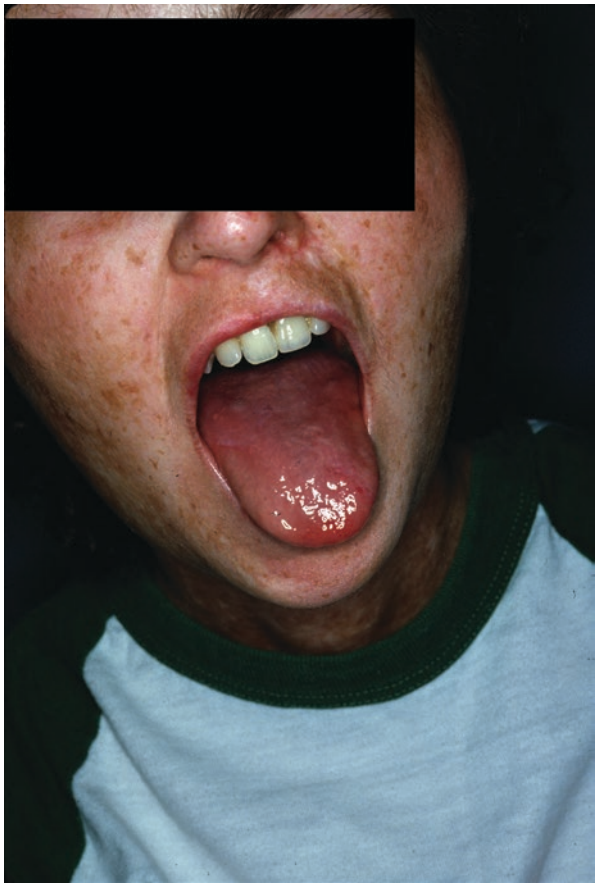

Fig. 12.1 Gross image of oral mucosa 4 years post-transplant. The erythematous extended tongue has atrophy of the papillae, and the facial skin has poikiloderma

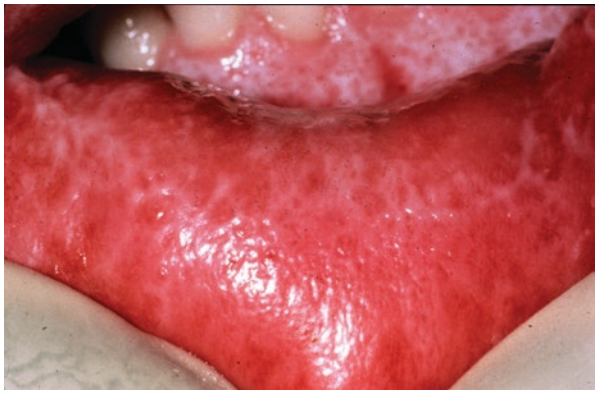

Fig. 12.2 The gums and lip have a reticulated, erythematous appearance

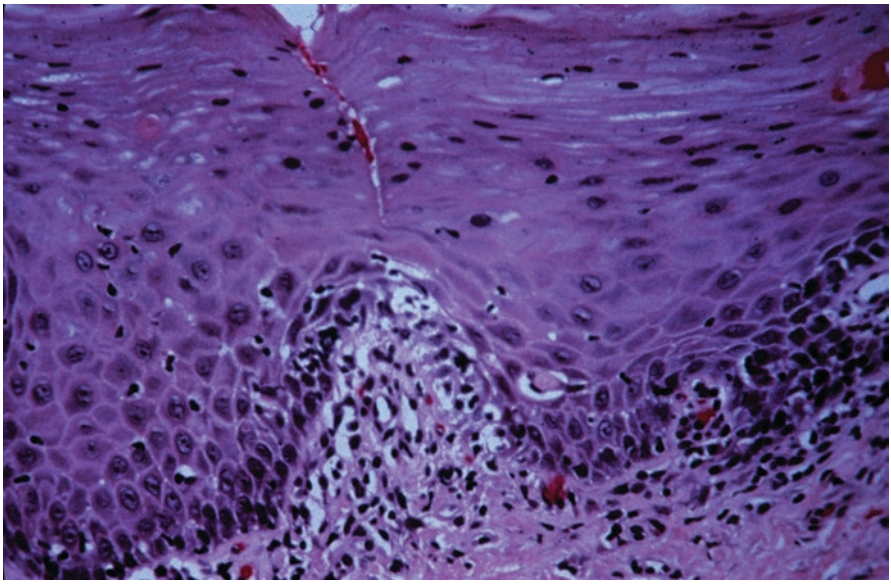

Fig. 12.3 An oral labial biopsy whose squamous mucosa has apoptotic change and chronic inflammation in the underlying submucosa

vaginal involvement (Fig. 12.4). At 15 months, she developed dysphagia with difficulty swallowing pills or dry food, but an upper esophagogastroduodenoscopy (EGD) was negative for GVHD. At 4 years (day 1461), EGD revealed mid-esophageal web formation (Figs. 12.5 and 12.6), and corresponding biopsy of her esophagus and stomach demonstrated GVHD (Fig. 12.7). Treatment with beclomethasone in corn oil alleviated the dysphagia. At 9 years posttransplant, the patient had persistent dysphagia with difficulty swallowing food, oral lichenoid lesions, severe ocular sicca requiring scleral lens, and some vaginal fibrosis. Her pulmonary status was stable but abnormal with mild to moderate dyspnea on exertion with an FEV_1 of 46%.

Diagnosis

Generalized cGVHD of mucosa with chronic inflammation and apoptosis involving (a) the oral cavity along with keratoconjunctivitis sicca; (b) upper esophagus with desquamation, narrowing, and fibrous web formation; and (c) anogenital region with vulvar lichen sclerosis et atrophicus with vaginal fibrosis/stenosis

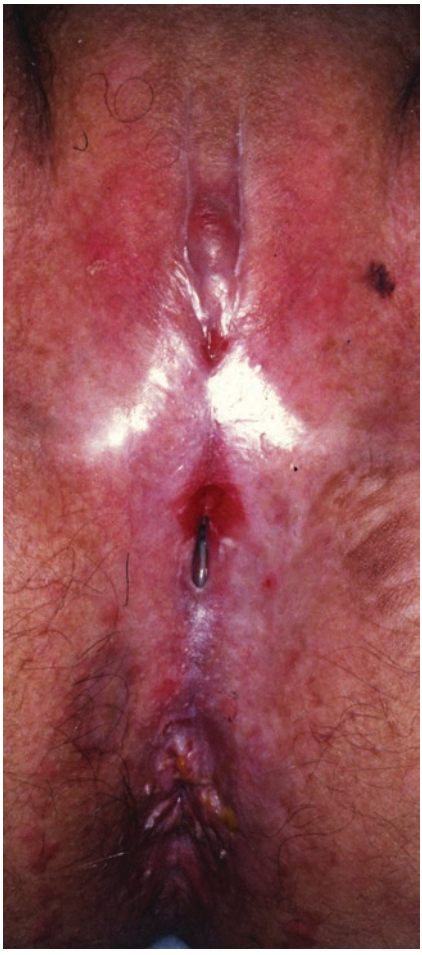

Fig. 12.4 Day 368 of severe cGVHD of the anal-genital region demonstrated by loss of pubic hair; tight, shiny, thin skin; partial agglutination of labia; and narrowed introitus

Differential Diagnosis and Discussion

The reasons for the particular mucosal involvement in cGVHD likely involve an interaction between tissue-specific mucosal endothelial cell vascular adhesion molecules recognized by specific integrins on circulating lymphocytes [1, 2]. A reason that the mucosal changes are not seen in our patient with acute GVHD (aGVHD) may be that the lymphoid subpopulations which develop later in cGVHD, T17 helper cells, T17 cytotoxic (Tc17), follicular helper Th17 cells, and follicular regulatory T (Tfr) cells, differ from those in aGVHD [3]. As a result, their homing affinities for the endothelium in these mucosal sites may differ from the integrins on the T cells involved in aGVHD.

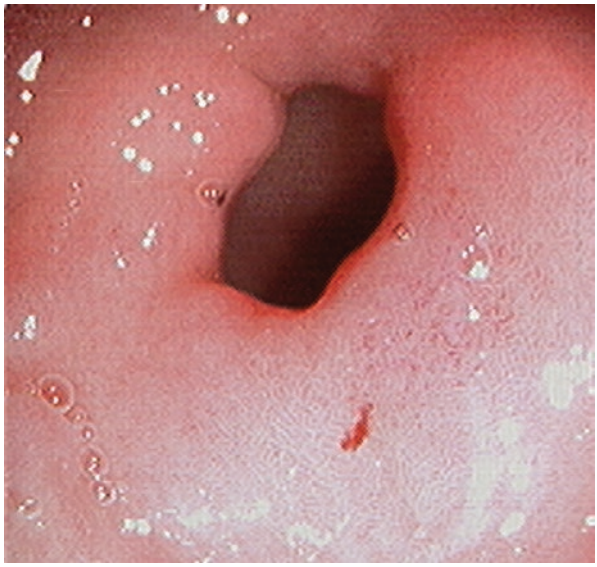

Fig. 12.5 Endoscopies of the upper esophagus show an edematous, friable, crepe-paperlike appearance with endoscopic narrowing in the mid-esophagus

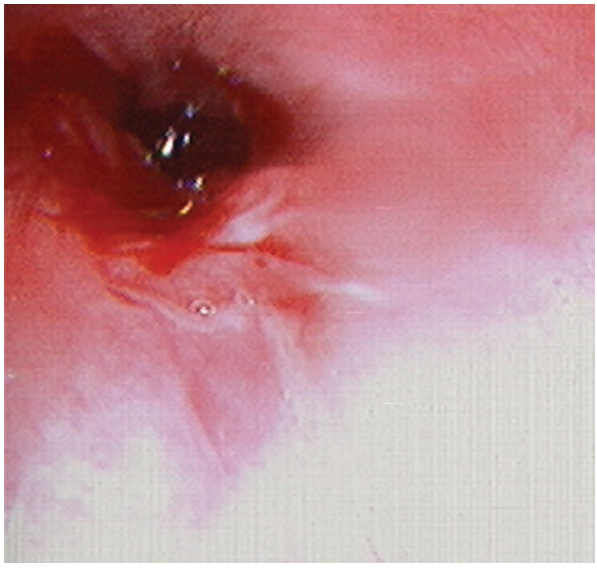

Fig. 12.6 Marked narrowing and erythema in the area of the esophageal web with sloughing of esophageal mucosa

Oral cGVHD

Oral mucositis occurs with aGVHD but lacks the additional classic signs of cGVHD: white hyperkeratotic plaques, reticulation striations, mucosal atrophy, and ulcerations. The buccal mucosa, labial mucosa, and tongue are the most frequently involved sites. The clinical symptoms include sensitivity to certain foods, changes in taste, difficulty chewing or swallowing especially with dry foods, and poor oral hygiene [4]. The histologic findings include hyperkeratosis and/or epithelial atrophy,

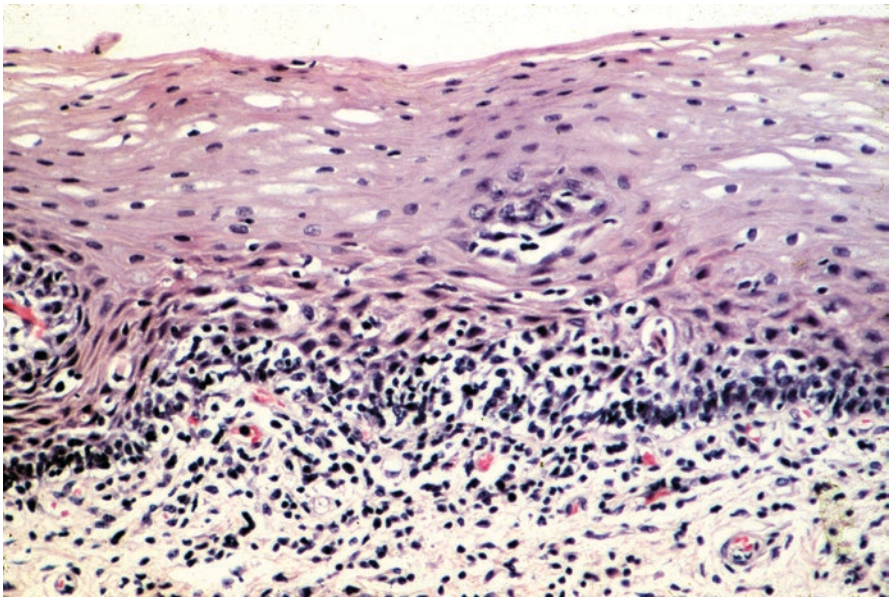

Fig. 12.7 Esophageal biopsy with GVHD showing interface lymphocytic inflammation and apoptosis along the basal layer

and interface lymphocytic inflammation with apoptosis (Fig. 12.7). Sale et al. noted residual mucosal inflammation and salivary sialadenitis after myeloablative conditioning in non-allogeneic recipients. As a result, there are different institutional criteria used to diagnose oral mucosal GVHD, but all rely on the amount of mucosal apoptosis [5, 6]. The NIH consensus chose not to use the histologic oral finding as a major diagnostic criterion for cGVHD relying on the gross oral exam. There has been a decline in the use of oral labial biopsies. The NIH pathology consensus committee urged that in the future minor salivary gland sialadenitis as illustrated in Chap. 18 be considered as a major diagnostic criterion. The differential diagnosis of oral cGVHD includes secondary infections with candida, CMV, and HPV (Figs. 12.8 and 12.9). The differential diagnosis also includes precancerous leukoplakia and squamous carcinoma, as the mouth is the organ with the highest prevalence of secondary malignancies. The frequency of oral cancers is highest in patients transplanted for Fanconi's syndrome as they have a defective DNA repair mechanism [7–10].

Esophageal CGVHD

The upper third of the esophageal mucosa is frequently affected by cGVHD. The location and the association with mucosal inflammation stenosis, anorexia, and weight loss is characteristic enough to be a major diagnostic criterion for cGVHD [11]. Esophageal cGVHD is usually associated with extensive cGVHD and

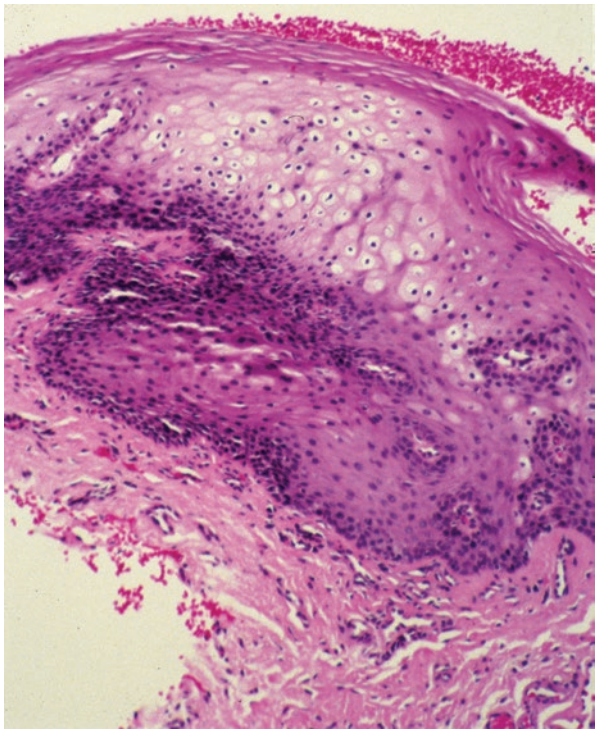

Fig. 12.8 Buccal labial biopsy has irregular thickening of the upper mucosa and keratinization of the mucosal surface

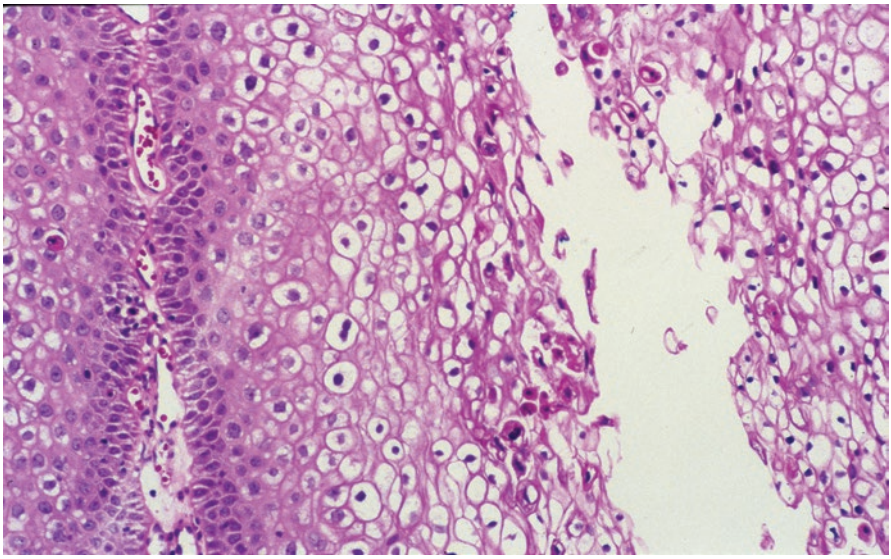

Fig. 12.9 Higher power of the same biopsy as in Fig. 12.8 demonstrates the typical findings of HPV with koilocytotic change

almost always exists with mucosal involvement of the oropharyngeal area. The symptoms of esophageal cGVHD include difficulty swallowing food or pills, odynophagia, retrosternal pain, and weight loss. Esophageal involvement may lead to secondary pulmonary disease with airflow obstruction due to aspiration of gastric contents.

The differential diagnosis of esophageal GVHD includes conditions which cause dysphagia, inflammation, and esophageal luminal narrowing. These include viral infections with HSV (Fig. 12.10) or CMV (Chap. 11) and fungal infections especially with *Candida albicans* (Fig. 12.11). Another differential is eosinophilic esophagitis, which may be a form of allergic response. Grossly, the endoscopist sees white plaques, desquamation, or even ring strictures. Histology shows an increase of eosinophils within the mucosa and the papillae of the mucosa. The criteria for

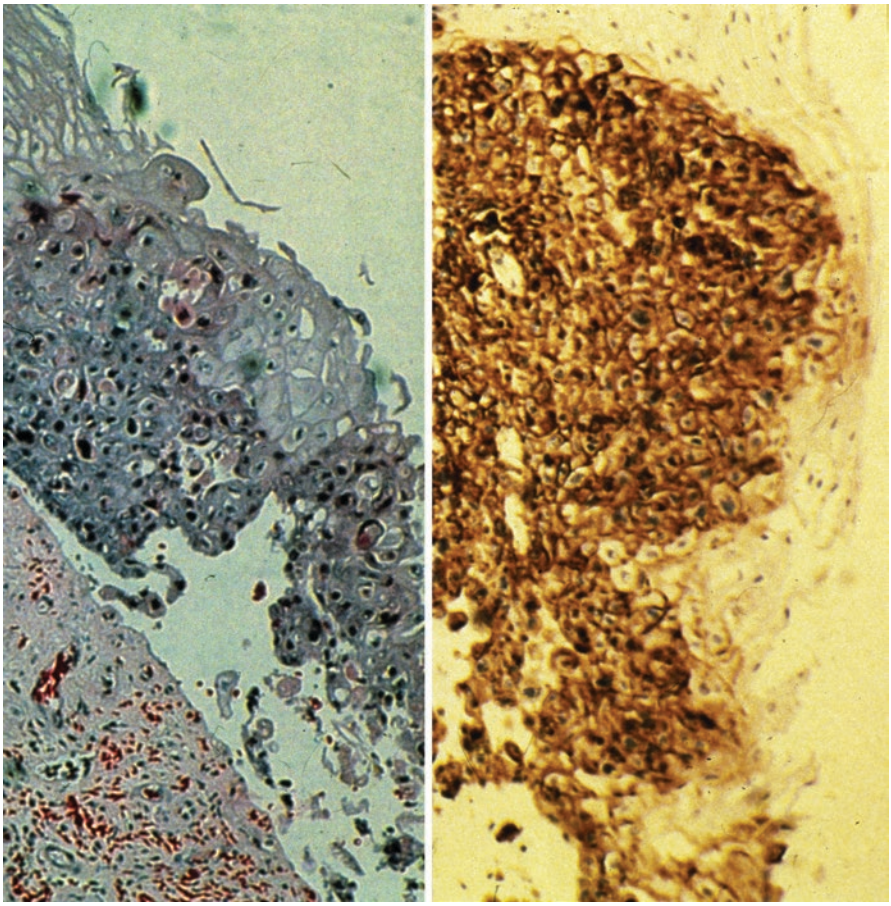

Fig. 12.10 This is a composite of an esophagus with ulceration, cellular disarray, and intranuclear inclusions of the epithelium (left panel). There is strong reactivity with an antibody cocktail targeting HSV I and II (right panel)

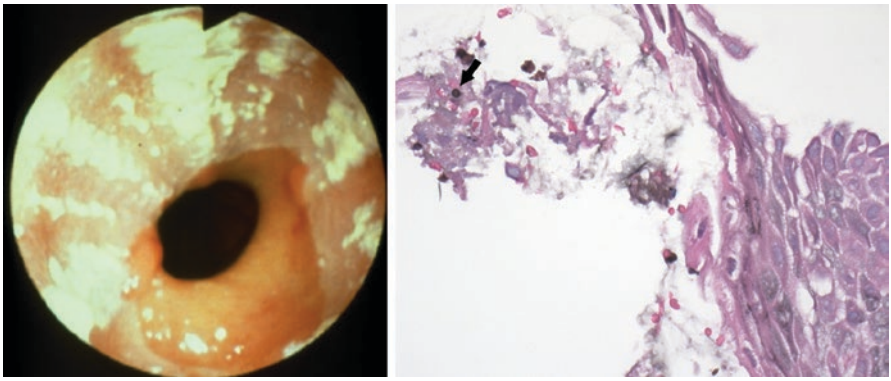

Fig. 12.11 This is a composite of images from a patient with documented candida esophagitis. The left panel is an endoscopic image of the esophagus with white plaques over an erythematous mucosa. The right panel is the high-powered microscopic image of a small portion squamous epithelium, and a rare yeast form (arrow) is seen in the superficial debris as well as in the adjacent ulcer, methenamine silver stain

eosinophilic esophagitis are >20–25 eosinophils per high-powered field or >15 eosinophils in two fields [12]. The differential diagnosis of esophageal GVHD's endoscopic appearance(s) also includes autoimmune diseases such as bullous pemphigoid and dystrophic epidermolysis bullosa.

Gastroesophageal reflux disease (GERD) is caused by the reflux of gastric or duodenal contents into the lower esophagus. Endoscopy shows linear ulcers or erosive or nonerosive changes and strictures. GERD may also cause Barrett's metaplasia with intestinal-type metaplasia. Barrett's esophagus is a well-known precursor of esophageal cancer. The fully developed picture of GERD in the lower esophagus is basal layer hyperplasia exceeding one third of the epithelial thickness, elongation of the rete ridges, papillomatosis, some chronic inflammation, and some eosinophils. It is important to keep in mind that small degrees of mucosal apoptosis may accompany a variety of chronic inflammatory conditions. The 2006 NIH pathology consensus report on cGVHD recommended that the diagnosis of esophageal GVHD be made with caution unless there is the combination of lichenoid interface changes with apoptosis along the basilar portion of the mucosa [13].

In the early era of HSCT, some patients with extensive refractory cGVHD had marked intramural sclerosis of the submucosal layer [14] (see Fig. 10.10). Most patients with esophageal cGVHD today have weight loss and dysphagia, but some are asymptomatic. The diagnosis is made by utilizing both endoscopy and barium contrast x-ray. The endoscopic findings include vesicular bullous change, desquamation, or a reddened friable mucosa (especially in the upper esophagus) that readily peels off the underlying basal epithelial layers. At endoscopy, some patients have a characteristic ringlike web or partial obstruction by delicate bridging synechiae arising from the ulcerated mucosa at the level of the cricopharyngeus (Figs. 12.4 and 12.5). The webs appear to be formed by the juxtaposition and adhesion between

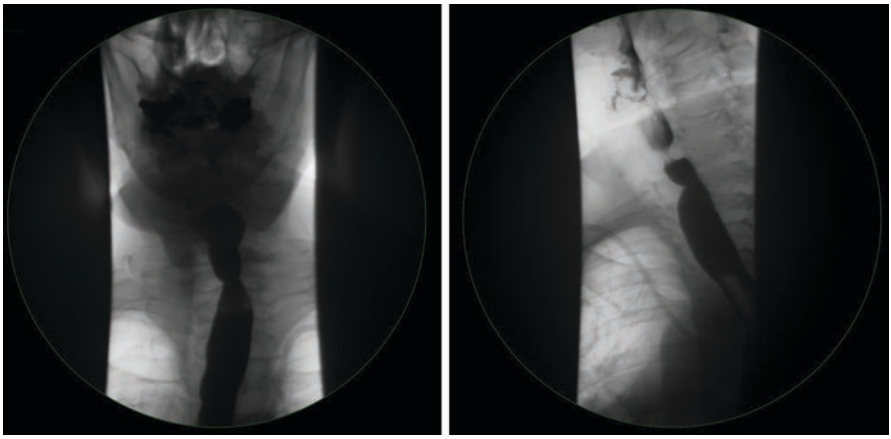

Fig. 12.12 Two views from cine-esophagram of barium swallow demonstrating an esophageal web 10 cm distal to the cricopharyngeus with 40–50% narrowing. The study also demonstrated esophageal dysmotility with an ineffective peristaltic wave with numerous tertiary contractions

adjacent inflamed surfaces. In some patients the fibrotic stenotic narrowing will not allow passage of an endoscope. The workup includes esophageal motility studies, which look for abnormal propagation after swallowing, or aperistalsis [15] (Fig. 12.12).

The prognosis is worse if patients have extensive cGVHD, as opposed to those only involving the oropharyngeal and esophageal mucosa. Esophageal barium swallows do not demonstrate mucosal inflammation but are important for identifying structural abnormalities which would impede the endoscope's advancement and risk perforation (see the index case in Chap. 17). It is important to recognize that many of these findings may be mimicked by esophageal stenosis related to GERD. The consequences of not recognizing or addressing treatment for esophageal cGVHD are greater likelihood of web formation, difficulty with weight loss, and a greater opportunity for gastric aspiration and pulmonary disease.

cGVHD of the Genitalia

GVHD can manifest in the genital regions as well. Up to 20% of men diagnosed with cGVHD report genital symptoms and specific manifestations involving the glans penis including erectile dysfunction, pigment changes, balanoposthitis, lichen sclerosis atrophicus-like changes, severe pain, and ulcerative lesions [16, 17]. Up to 50% of women diagnosed with cGVHD have reported long-term

genital symptoms [18]. The vulvar region may present with erythematous, itchy, painful, very fragile, and thin mucosa. Similar to the development of esophageal webs, chronic inflammation leads to the apposition of labial folds and, within the vaginal vault, synechiae adhesions with eventual introitus narrowing and stenosis of the vaginal vault [16].

Current clinical management strategies have diverted from taking biopsies of the genital area due to poor wound healing capabilities and because the painful procedure usually only shows nonspecific chronic inflammation with or without apoptosis, providing little more information than a thorough clinical exam and follow-up [17, 19–21]. Lichen sclerosis et atrophicus is a common complaint in women who suffer from cGVHD of the genitalia for an extended period of time (months to years). Long-term changes observed in these patients include patchy areas of pigmentary changes, scaling, atrophy, and sclerosis (Fig. 12.4) [22]. Typical microscopic changes include abnormal compact keratinization, sawtooth formation of the rete ridges, and homogenization of the collagen (Fig. 12.13).

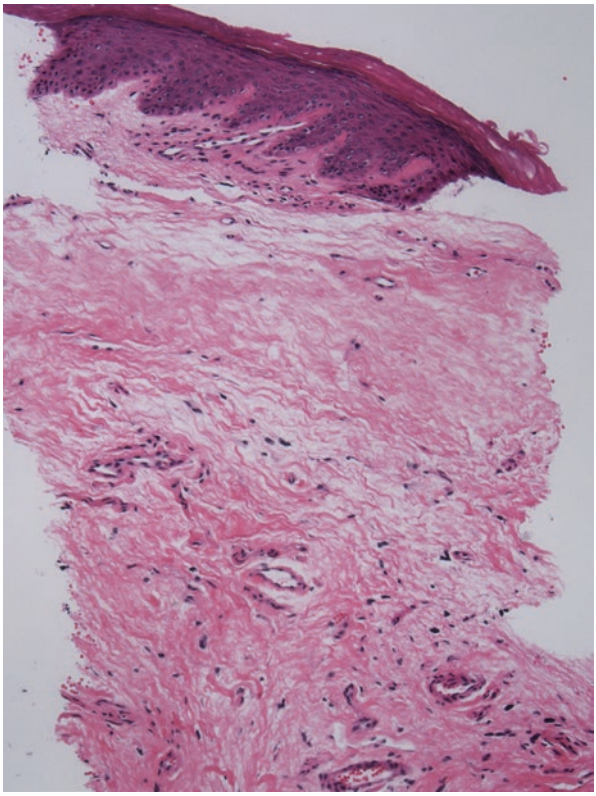

Fig. 12.13 Vulvar biopsy. The squamous epithelium has hyperkeratosis, hypergranulosis, and irregular elongated rete ridges. The underlying dermis has mild nonspecific chronic inflammation and homogenization of the upper dermal collagen

Teaching Points

- The histopathology of cGVHD of the squamous mucosal surfaces of the oral cavity, esophagus, and anogenital regions is similar: chronic inflammation with or without apoptosis. The locations determine the symptoms and complications.
- The physical evaluation of the oral cavity in the workup of cGVHD is a major diagnostic criterion. In the 2015 NIH consensus, oral histology was elected not to be a major diagnostic criterion due to some histologic overlap with changes from residual myeloablative conditioning.
- Differential diagnostic consideration in oral GVHD includes viral and fungal infections and precancerous/cancerous leukoplakia.
- Esophageal cGVHD symptoms of retrosternal pain, dysphagia, and odynophagia can be caused by infections, GVHD, gastroesophageal reflux, or eosinophilic esophagitis.
- Esophageal infections including fungal infection (especially *Candida albicans*) and viral infection (especially HSV) are the usual causes of esophageal symptoms in the earlier post-transplant period.
- Esophageal webs are a diagnostic feature of cGVHD. They are diagnosed by imaging or direct endoscopic visualization.
- Anogenital cGVHD affects up to 20% of male cGVHD patients and up to 50% of female cGVHD patients.
- Chronic inflammation of the female genitalia may resemble lichen sclerosis et atrophicus. Adhesions between the labia folds can result in narrowing of the introitus and vaginal stenosis/fibrosis.

Questions

1. What are the conditions that grossly resemble oral cGVHD?
2. What other conditions besides cGVHD can cause esophageal symptoms such as dysphagia, retrosternal pain, and difficulty swallowing?
3. How is a suspected diagnosis of aGVHD of the esophagus verified?
4. Why is diagnosing GVHD in the esophagus problematic?
5. What is an esophageal web or/and how does it form?
6. What is the common pathology between GVHD-affected oral, esophageal, and genital mucosa?

Answers

1. Answer: HSV, HPV, CMV, and precancerous or cancerous leukoplakia.
2. Answer: Infectious agents include *Candida albicans*, CMV, and HSV. Dysphagia can also be caused by eosinophilic esophagitis, esophageal cancer, and oral sicca leading to difficulty swallowing food.

3. Answer: By direct endoscopic exam with imaging studies. Histologic findings of chronic inflammation with or without apoptosis after excluding reflux and infectious causes with IHC and culture or PCR.
4. Answer: Symptoms associated with GVHD overlap with those of infectious etiology in the GI tract, specifically anorexia, nausea, vomiting, failure to thrive, dysphagia, or odynophagia.
5. Answer: Esophageal webs, which typically occur in the upper third of the esophagus, develop when apposed denuded mucosal surfaces form thin, weblike bridges of squamous epithelia and fibrous tissue. Forceful passage by an endoscope may cause tearing and perforation.
6. All of these tissues have chronic interface inflammation with apoptosis associated with atrophy and fibrosis in the underlying soft tissues.

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13 Pre-transplant Liver Disorders: Post-transplant Impact on Developing Venocclusive Disease/Sinusoidal Obstruction Syndrome and Other Hepatic Problems

Howard M. Shulman

Clinical History

A 48-year-old female had a 3-year history of agnogenic myeloid metaplasia which presented with fatigue and blood pancytophilia. Her marrow cellularity was 100% with myelofibrosis. On admission for hematopoietic stem cell transplantation (HSCT), she had hepatosplenomegaly. Her total serum bilirubin was slightly elevated, 1.6 mg/dL, with normal aminotransferases. The pre-transplant liver biopsy described in Figs. 13.1 and 13.2 showed extramedullary hematopoiesis (EMH) and sinusoidal capillarization (Figs. 13.1 and 13.2). She was conditioned with busulfan and Cytoxan and received a peripheral blood stem cell graft from an HLA-B-mismatched unrelated donor. By day 13, she had developed diagnostic features of hepatic venocclusive disease/sinusoidal obstruction syndrome (VOD/SOS), 10 kg weight gain, renal insufficiency, and total bilirubin of 5.5 mg/dL, which reached a maximum of 14.4 md/dL (Fig. 13.3). On day 18 she began a course of defibrotide; by day 21 her total bilirubin began to decline. She had a difficult post-transplant course with enterococcal pneumonia, biopsy-proven GVHD of the stomach and colon, CMV colitis, and gastric bleeding from GAVE. She was suspected to be developing cGVHD with increased skin pigmentation and an oral exam consistent with GVHD. Her total bilirubin had decreased to 3.7 mg/dL by discharge, but she had anorexia and wasting. She died 20 months post-transplant of renal and respiratory failure.

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

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

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

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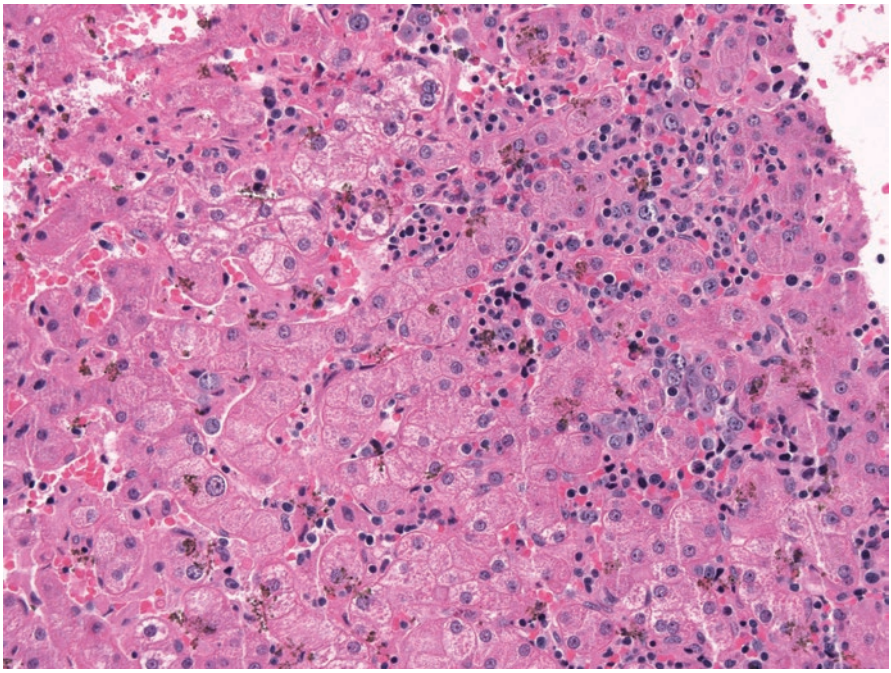

Fig. 13.1 Pre-transplant H&E liver biopsy shows marked extramedullary hematopoiesis filling sinusoids and displacing hepatocytes

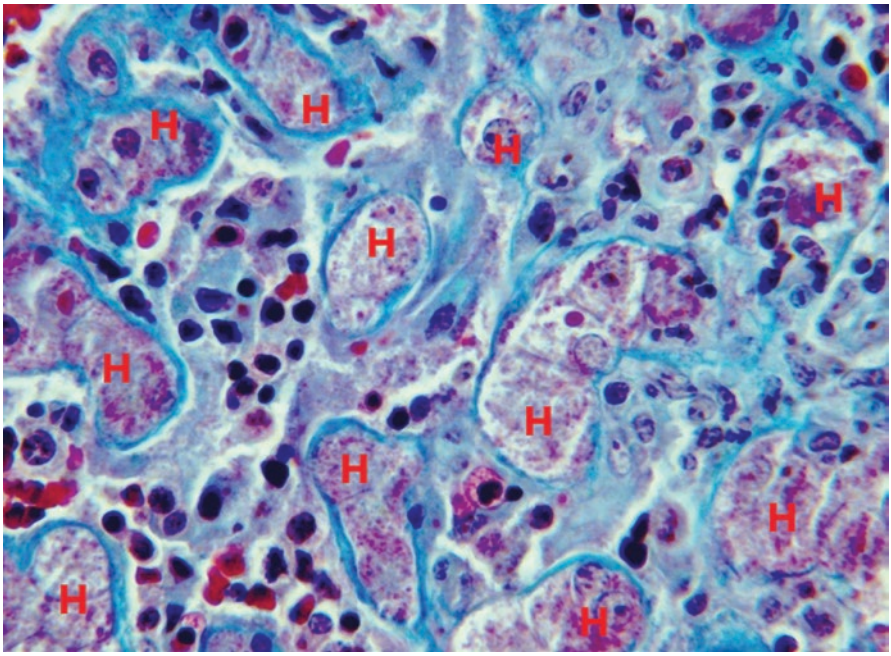

Fig. 13.2 Trichrome stain highlights sinusoidal collagenous capillarization, which surrounds irregularly distributed hepatocyte cords (marked by red H's)

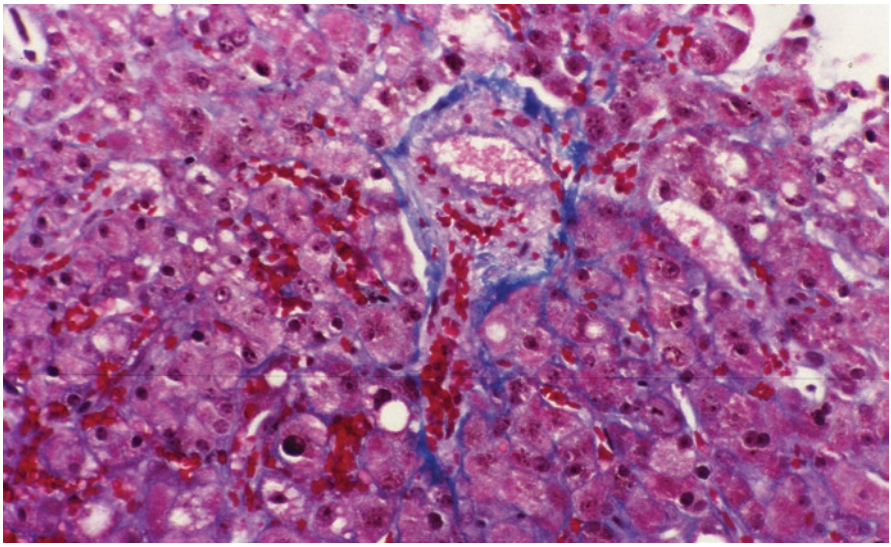

Fig. 13.3 Day 16 transvenous liver biopsy. Changes of VOD/SOS representative of those expected early after onset of symptoms. Trichrome-stained section has a small terminal hepatic venule whose lumen is narrowed by a widened subendothelial zone containing entrapped red cells and loose matrix. The surrounding perivenular (centrilobular) sinusoids are mildly congested, but hemorrhagic necrosis of perivenular hepatocytes is not present

Diagnosis

Pre-transplant liver biopsy from patient with agnogenic myeloid metaplasia with distortion of hepatic sinusoids by EMH and sinusoidal capillarization predisposing to development of post-transplant VOD.

Key Pathology Features

- Pre-transplant liver biopsies are performed to clarify underlying liver disorders, assess suitability for undergoing HSCT including likelihood of developing VOD/SOS, and modify the conditioning regimen if necessary.
- Workup of VOD/SOS relies on the trichrome stain. The reticulin and Verhoeff-van Gieson stains are complementary. Without these latter stains, the terminal hepatic venules (THV) and other smaller sublobular hepatic venules may be difficult to distinguish from small portal spaces. THV are identified by their continuous fibrous adventitia. Serial sections are needed to identify the characteristic histologic changes that may not be present in all sections.
- If the liver biopsy is obtained using a transvenous approach, then a wedged venous pressure gradient between the liver sinusoids and the extrahepatic vena cava above the liver hepatic should be measured. A pressure gradient >10 mm/Hg is highly suggestive of VOD/SOS.

- When transvenous biopsies are obtained using a forceps biopsy, multiple samples should be taken. Some of the small irregular fragments may not contain THV, which are the basis for diagnosing VOD/SOS.
- Changes in zone 3 of the liver acinus (centrilobular) region, especially THV intraluminal narrowing or occlusion, sinusoidal fibrosis, and hemorrhagic necrosis, are associated with the clinical syndrome of VOD/SOS.

Discussion

Detection and Management of Pre-transplant Liver Disorders

A variety of pre-existing liver disorders are more common among HSCT candidates, including malignant infiltration, infections including chronic viral hepatitis B (HBV) and C (HCV), alcoholism, nonalcoholic steatohepatitis (NASH), iron overload related to multiple transfusions, and existing damage from prior exposure to oncology drugs (Figs. 13.4 and 13.5). Post-transplant, these pre-existing conditions can produce clinical and laboratory abnormalities resembling or overlapping with hyperacute GVHD, sepsis syndrome, and congestive heart failure. Failure to recognize pre-existing disorders such as occult cirrhosis or NASH may later lead to hepatic decompensation and death [1]. Pre-transplant clues to an underlying liver

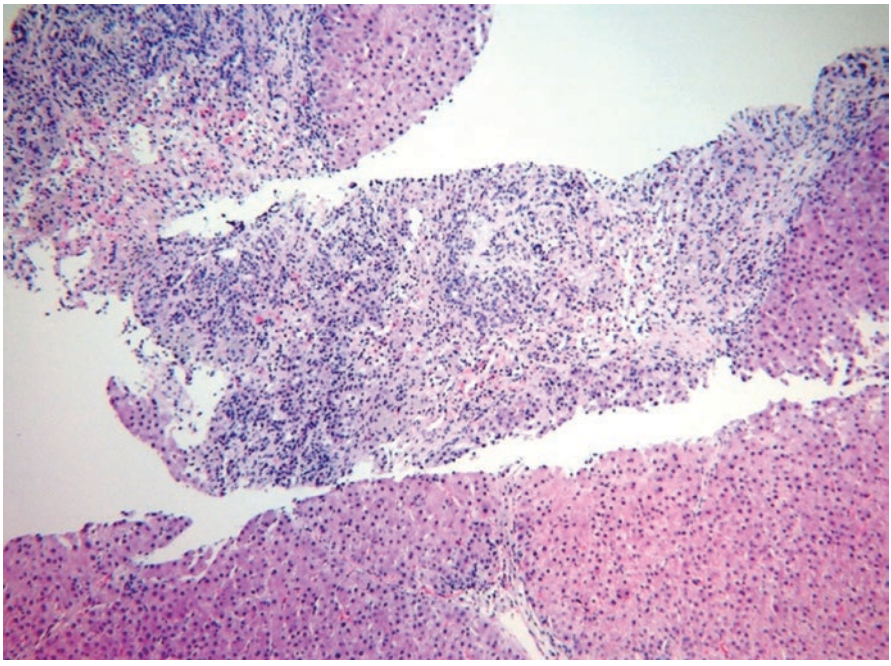

Fig. 13.4 Adverse necroinflammatory drug-related injury after Gleevec was given for CML post-transplant relapse

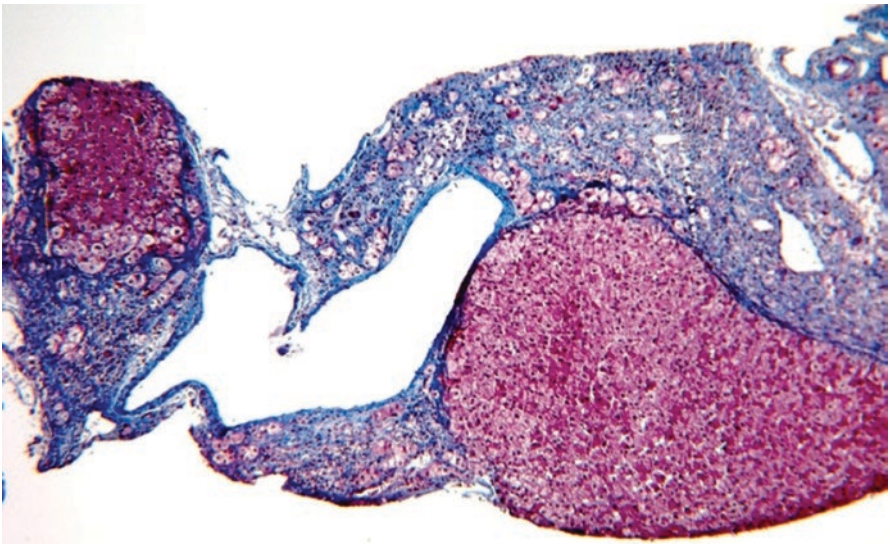

Fig. 13.5 Repeat biopsy 1 year later before second transplant showed irregular fibrous scarring but no ongoing necroinflammatory damage

disorder include the detection of HBV, HCV, and/or increased iron stores in heavily transfused patients, particularly those with thalassemia or myelodysplasia. Increased iron stores are detected by non-ferritin-bound iron, labile plasma iron [2], or surrogate marrow iron >15000ug/g [3]. Other clues are ALT >250 IU/L, jaundice, hepatomegaly, or rising aminotransferases during earlier exposure to an oncology drug. These liver disorders may require pre-transplant iron removal, treatment with the appropriate antiviral agents [4] (Chap. 15), or an alternative conditioning regimen to minimize the chance of liver failure or death (Figs. 13.6 and 13.7). For a more complete discussion on the pre-transplant management of liver diseases in oncology patients, see clinical algorithm in a 2008 review by McDonald and Friese [4].

Pathophysiology of VOD/SOS

VOD/SOS is among the most serious non-GVHD-related post-transplant liver disorders. Its severity can range from mild and reversible to untreatable with progressive multiorgan failure and death. The prevalence, risk factors, clinical features, and pathophysiology of VOD/SOS have been summarized in two reviews [5, 6]. In brief, the pivotal cell type involved in the pathogenesis of VOD/SOS is the perivenular sinusoidal endothelial cells (SEC). Oncology agents which cause direct SEC damage do so by depleting their intracellular glutathione stores responsible for mitigating the toxic metabolites of oncology drugs and/or the effects of irradiation. Pre-transplant conditioning sets up a cascade of injury leading to the rounding up and detachment of SECs, hemorrhage into the space of Disse, and downstream

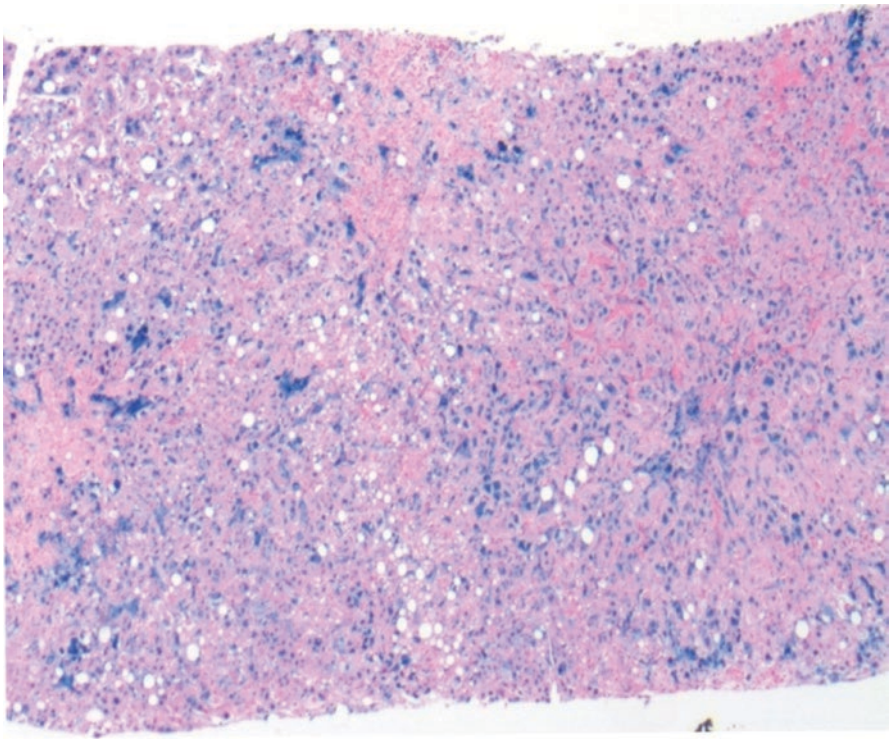

Fig. 13.6 Pre-transplant liver biopsy from a heavily transfused patient with MDS. Prussian blue stain shows markedly increased iron stores

embolization of detached SEC, Kupffer, and stellate cells. This polycellular embolus blocks sinusoidal venous outflow into the THV, thus increasing sinusoidal pressure with subsequent centrilobular hepatocyte ischemia, hemorrhagic necrosis, and later collagenous obstruction of the small venules and sinusoids. To better summarize this process of sinusoid obstruction, VOD's nomenclature was revised to the apropos label "sinusoidal obstruction syndrome" (SOS), hereafter referred to as VOD/SOS since the initial obstruction occurs in the sinusoids with collagenization of venules and later to the sinusoids [7].

Increased risk of developing VOD/SOS is associated with certain conditioning regimens [5, 6], procoagulant, or thrombophilic disorders such as myeloproliferative diseases, liver sinusoidal fibrosis, and underlying inflammatory conditions like chronic hepatitis or nonalcoholic steatohepatitis (NASH). Physiologically, inflammation stimulates the Kupffer cells to release cytokines such as platelet-derived growth factor (PDGF), which activates and promotes proliferation of stellate cells. Activated stellate cells encircle the sinusoids with

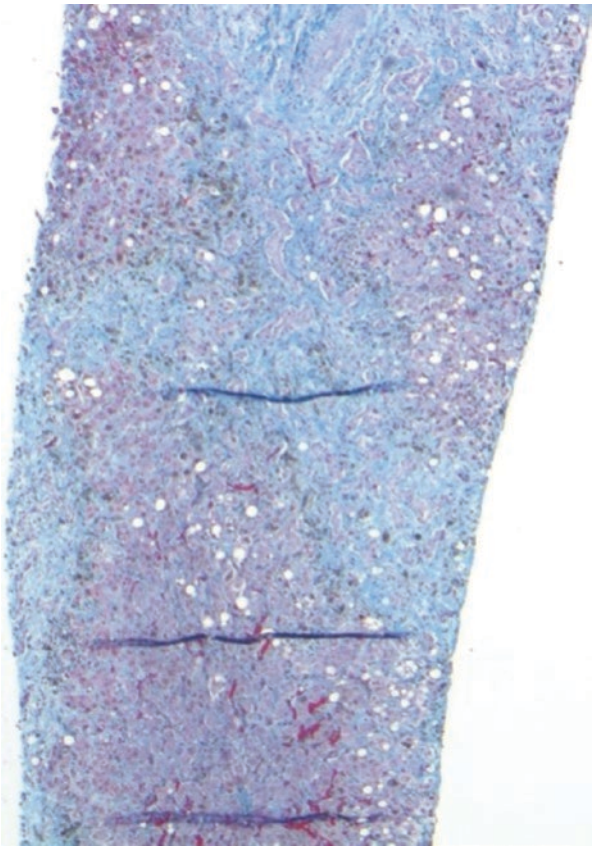

Fig. 13.7 Trichrome stain from the same patient as in Fig. 13.6 shows cirrhosis with broad fibrous bridges between hepatic nodules

contractile extensions and produce fibrosis. These events contribute to elevated intrasinusoidal pressure from embolized sinusoidal cells (Fig. 13.8). Pre-transplant liver disorders with disrupted or obstructed sinusoidal blood flow include chronic HCV hepatitis or cirrhosis (Fig. 13.7), amyloid deposition (Fig. 13.9), and NASH (Fig. 13.10).

The pathogenesis of the VOD/SOS syndrome caused by the drug gemtuzumab ozogamicin (mylotarg) differs from that caused by CY/TBI. Mylotarg is a monoclonal antibody linked to the cytotoxic agent calicheamicin which targets the CD33 receptors expressed on AML cells [8]. The liver injury presumably stems from the non-specific uptake by SEC with damage to SEC, $CD33^{+}$ resident Kupffer cells, and AML. The subsequent release of cytokines, especially PDGF, stimulates the stellate cells to produce collagen and contract around the sinusoids, raising the sinusoidal pressure, ultimately resulting in sinusoidal obstruction [7, 9] (Figs. 13.8 and 13.11).

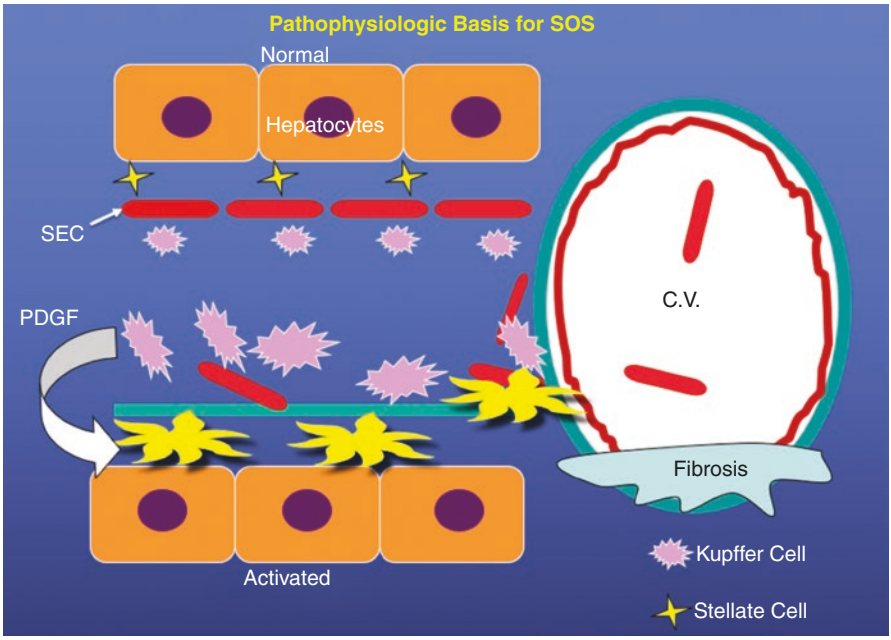

Fig. 13.8 This diagram depicts the structures involved in the genesis of VOD/SOS.Damaged and detached SEC and Kupffer cells activate stellate cell proliferation, leading to deposition of sinusoidal and THV fibrosis

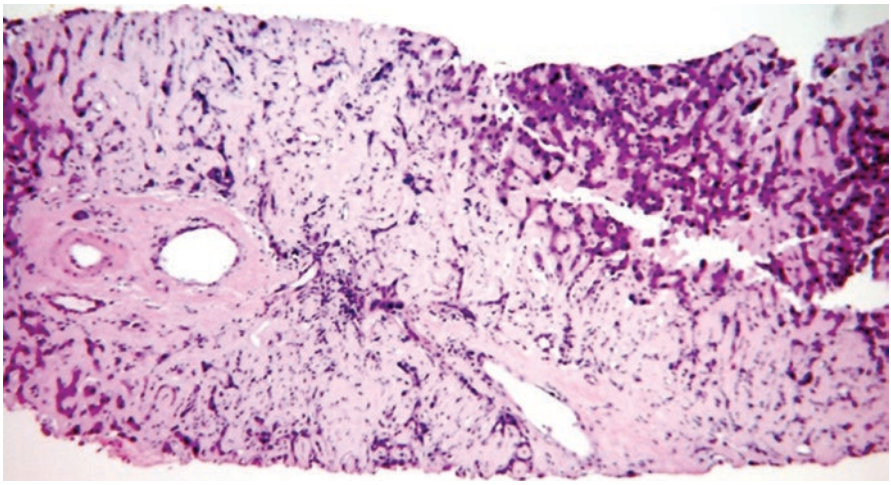

Fig. 13.9 Pre-transplant biopsy with PAS stain has dense sinusoidal amyloid deposition, a contraindication for HSCT in this patient

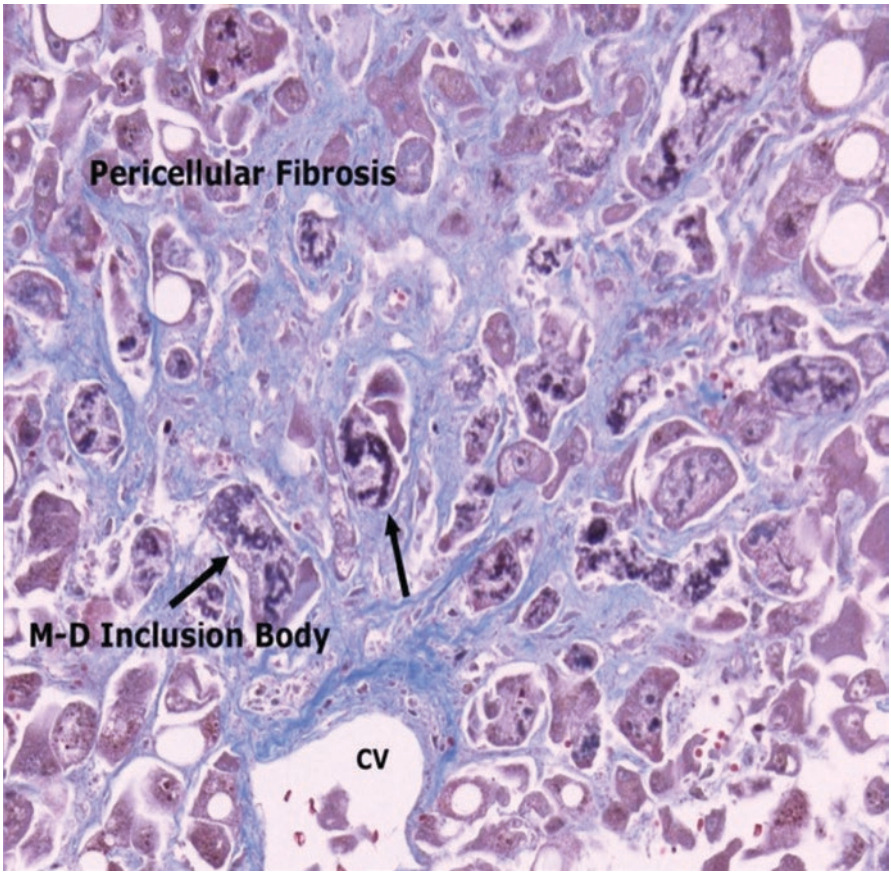

Fig. 13.10 Pre-transplant liver with occult NASH has extensive sinusoidal pericellular fibrosis. The arrows denote Mallory-Denk bodies

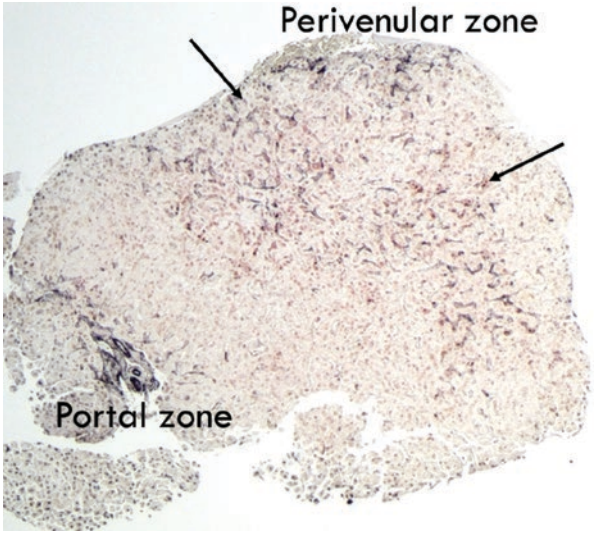

Fig. 13.11 Liver biopsy from patient who experienced mild VOD/SOS symptoms following mylotarg exposure. The IHC stain for smooth muscle actin shows an increase of stellate cells in the perivenular zone

Clinicopathologic Correlation of Histology with Clinical Signs of VOD/SOS

The classic clinical features of VOD/SOS are jaundice, ascites, rapid weight gain, and hepatomegaly. The clinical onset of VOD/SOS may overlap with the development of liver GVHD. However, the histologic features of GVHD are centered on changes around and in the portal zones and their biliary structures and hepatocellular cholestasis (see Chap. 14), whereas the histopathologic features of VOD/SOS are centrilobular (zone 3). The clinical syndrome of VOD/SOS is associated with several centrilobular histologic entities, but no single centrilobular histologic gold standard yet exists [10]. Other alterations are associated with clinical VOD/SOS in addition to occluded central venules, such as eccentric narrowing of the luminal wall (which is presumably a precursor to phlebosclerosis, a thickening of the perivenular adventitia), sinusoidal fibrosis, centrilobular sinusoidal fibrosis, and massive centrilobular hepatocyte necrosis. A 1994 study found that the severity of the VOD/SOS symptoms was correlated to the percentage of affected centrilobular regions as well as the presence of occluded small venules and sinusoidal fibrosis [10]. Cases of long-standing fatal VOD/SOS have a reverse cirrhosis with bridging collapse between centrilobular areas which surround the unaltered portal zones. Intractable ascites is associated with this change (Fig. 13.12). Fortunately, the majority of cases of VOD/SOS are not fatal. In fact, studies of post-HSCT autopsies and liver biopsies have shown that occluded hepatic venules may be asymptomatic [10, 11].

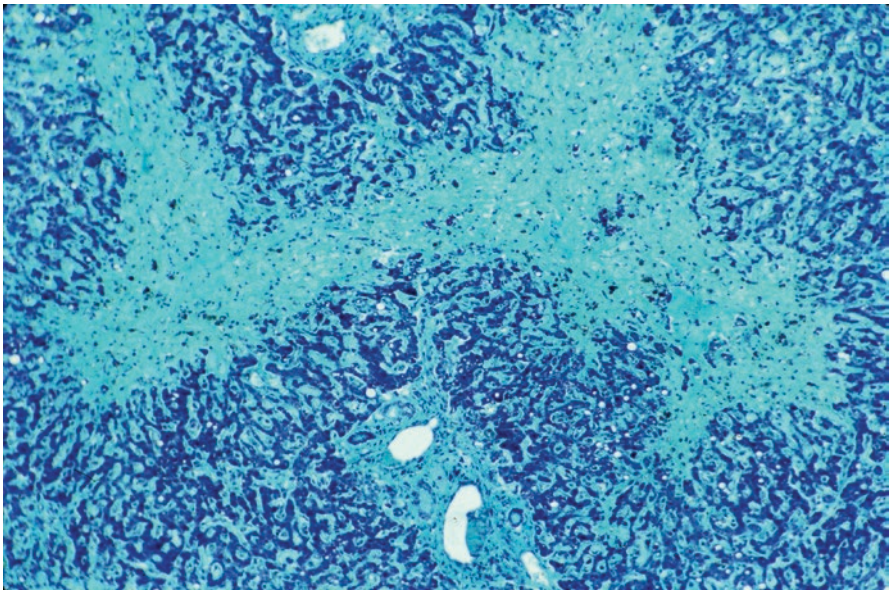

Fig. 13.12 Fatal VOD/SOS had pattern of reverse cirrhosis with fibrous bridging between collapsed centrilobular zone

To wit, 20–30% of autopsied patients who had histologic evidence of venular occlusion were without clinical symptoms [12]. In a retrospective study of liver biopsies, Ma et al. found 78% of biopsies had subtle, and in some subclinical, lesions of terminal hepatic venules [13].

Teaching Points

- Noninvasive procedures used to identify and evaluate pre-transplant liver disorders include liver function tests, measurement of iron stores, and imaging studies to detect hepatomegaly and ascites.
- Pre-transplant liver disorders in the post-transplant period can be confused with hyperacute GVHD, sepsis syndrome, or congestive heart failure.
- Following transplant, occult cirrhosis, NASH, or prior damage from chemotherapy may lead to hepatic decompensation.
- Conditions which increase the risk of VOD/SOS are myeloproliferative disorders, chronic inflammatory states such as chronic viral hepatitis and NASH, thrombophilic and procoagulant blood disorders, and certain high dose myeloablative conditioning regimens.
- There has been a dramatic reduction in the prevalence of VOD/SOS in myeloablative regimens that do not use cyclophosphamide and by adjusting pharmacokinetic doses for busulfan levels.
- The clinical syndrome of VOD/SOS encompasses a number of different histologic findings. There is no single diagnostic histologic gold standard.
- Damaged or occluded THV can be subclinical findings.

Questions

1. What are the most common pre-transplant disorders which have an increased risk of developing VOD/SOS?
2. What stains are needed to diagnose VOD/SOS on a liver biopsy?
3. How do you distinguish GVHD from VOD/SOS?
4. What histologic features are associated clinical VOD/SOS?
5. What are the changes of late fatal VOD/SOS?

Answers

1. Answer: Myeloproliferative diseases, chronic hepatitis, cirrhosis, history of prior liver dysfunction during chemotherapy, NASH, alcoholic liver disease, and cirrhosis from several causes
2. Answer: Trichrome is essential. Reticulin and VVG are complementary but more difficult to interpret. Serial sections should be obtained since the diagnostic histologic changes may not be present in all levels.
3. Answer: The cardinal feature of GVHD is bile duct injury centered around the portal zone (see Chap. 14). VOD/SOS injury is centered around centrilobular venular and hepatocyte alterations.

4. Answer: In the centrilobular zone, occluded venules (though not the diagnostic sine qua non), eccentric subendothelial venular thickening, phlebosclerosis, massive perivenular hepatocyte hemorrhagic necrosis, and centrilobular sinusoidal fibrosis
5. Answer: Fibrotic occlusions of most venules with histology of reverse cirrhosis with central-central fibrous bridging surrounding the unaltered portal zones. Intractable ascites is associated with this change

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The Pathological Spectrum of Hepatic GVHD

14

Keith R. Loeb, David W. Woolston, and Howard M. Shulman

Clinical History

A 29-year-old woman with AML was transplanted in first remission from an allogeneic matched sibling donor. Symptoms of GI GVHD with diarrhea and GI bleeding developed on day 26. Liver test values progressively rose after transplantation until peaking on day 66, with bilirubin peaking at 17.9 mg/dL (Fig. 14.1a), alkaline phosphatase (AP) at 348 IU/L (Fig. 14.1b), and aspartate aminotransferase (AST) at 715 IU/L (Fig. 14.1c). A percutaneous liver biopsy on day 42 demonstrated severe GVHD (Figs. 14.2 and 14.3). She received multiple different IS regimens including extended methotrexate, high-dose corticosteroids, azathioprine, and lastly cyclophosphamide. She passed away on day 71.

Diagnosis

Liver biopsy 42 days post-allograft shows GVHD with hepatocellular disarray, cholestasis, and widespread damaged bile ducts.

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

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

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

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

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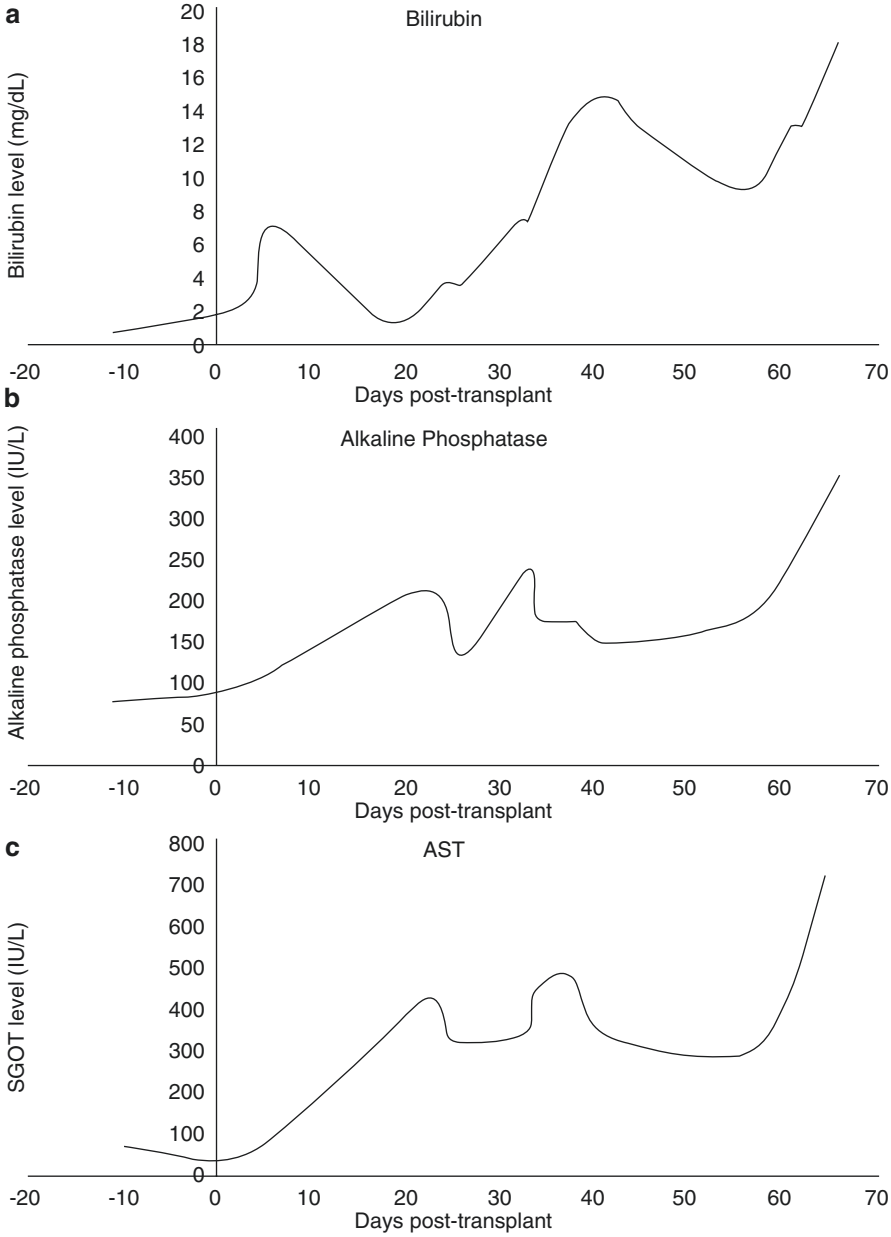

Fig. 14.1 Peak liver test values were observed on day 66 with bilirubin (**a**) at 17.9 mg/dL, alkaline phosphatase (**b**) at 348 IU/L, and SGOT (**c**) at 715 IU/L

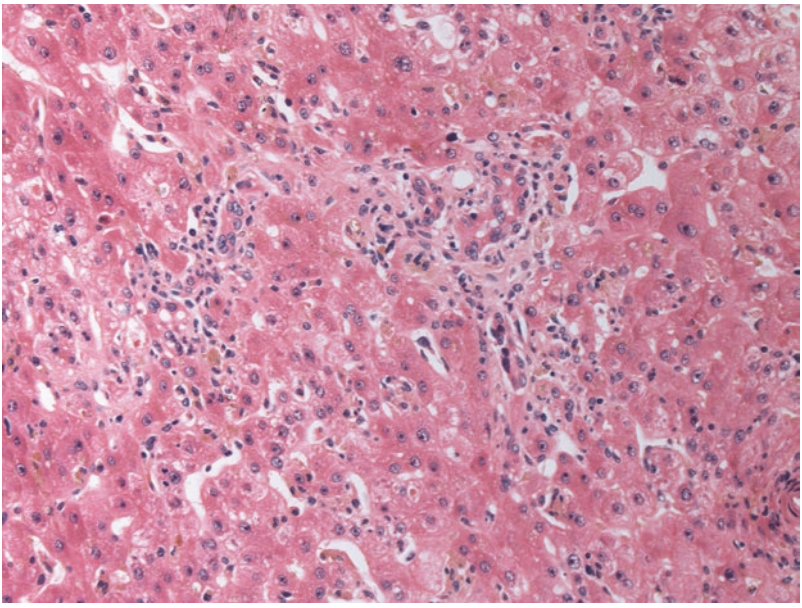

Fig. 14.2 Day 42 liver biopsy: at medium magnification, the liver acinus displays hepatocyte unrest and disarray of the liver cords. There are scattered sinusoidal lymphocytes and some periportal interface inflammation and necrotic hepatocytes

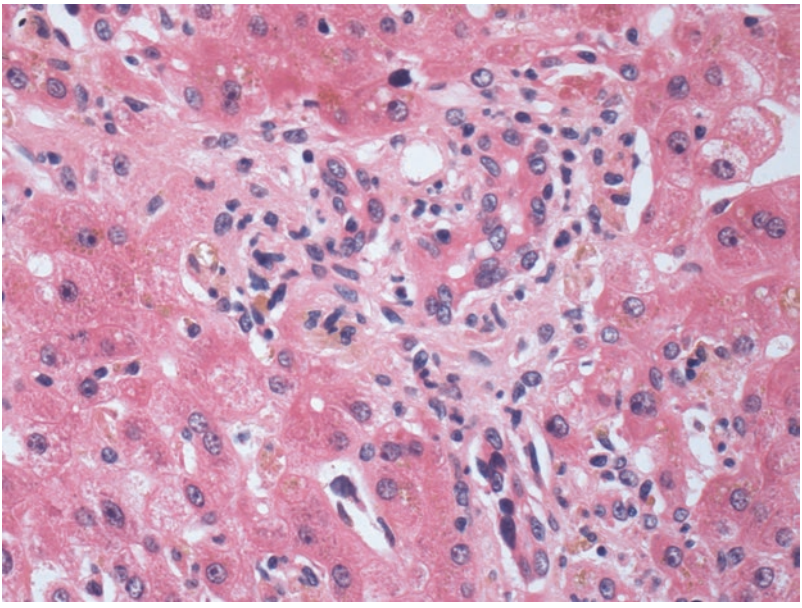

Fig. 14.3 Portal area seen at higher magnification shows markedly distorted bile ducts with anisonucleosis, overlapping nuclei, and segmental nuclear dropout with syncytial like zones of eosinophilic cytoplasm and lymphocytic ductitis with intraductal lymphocytic infiltration. The irregular limiting plate contains proliferating ductules which look cytologically similar to the damaged interlobular bile ducts. There is debate over whether these are also targets of GVHD

Key Pathology Features

- The characteristic features are damaged shrunken small bile ducts with irregular distorted shapes and nuclear atypia with segmental ductal dropout (Figs. 14.4 and 14.5).
- Portal inflammation varies depending on the exposure to IS but is typically paucicellular and lymphocytic (Figs. 14.6 and 14.7).
- The first histologic findings after the clinical onset may be nonspecific hepatocyte unrest and necrotic hepatocytes related to cytokine-induced Fas-Fas Ligand (Fas-FasL) secondary bystander injury. There is a time lag of 7–10 days before recognizable features of GVHD develop.
- The spectrum of cholestatic changes includes hepatocyte unrest, perivenular dropout with ballooning degeneration, and bile stasis with canalicular and periportal cholangiolar bile thrombi (Figs. 14.5, 14.8 and 14.9).
- The consequences of persistent liver GVHD are severe cholestasis (Fig. 14.10) and ductopenia (Fig. 14.11).
- Long-standing liver GVHD may develop portal septate fibrosis, but cirrhosis typically does not occur (Fig. 14.12). Of note, see discussion of chronic alloimmune hepatitis in Chap. 16.

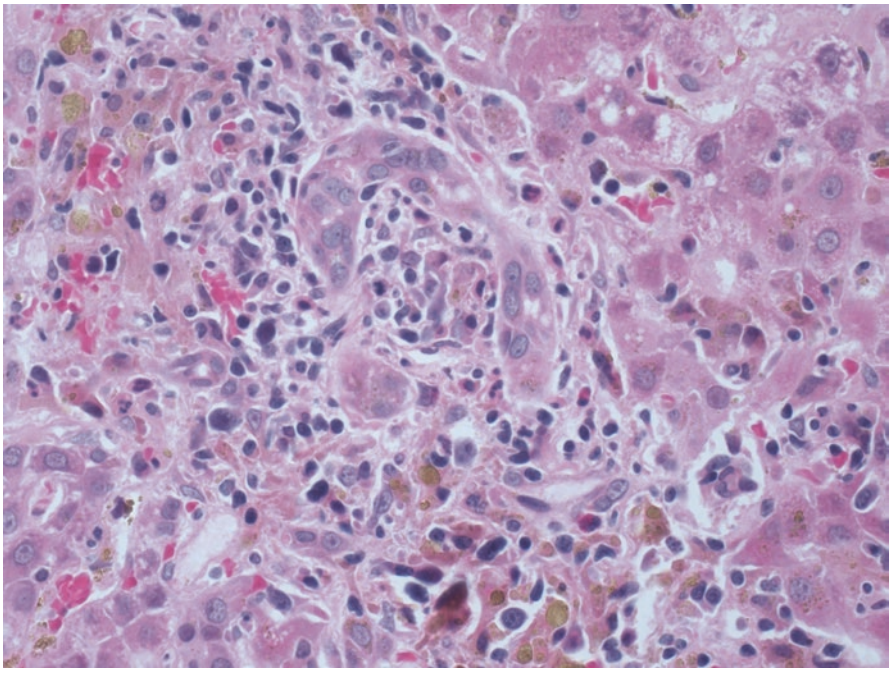

Fig. 14.4 Enlarged portal space contains markedly misshapen bile ducts surrounded by chronic inflammatory cells, which spill over into periportal hepatocytes

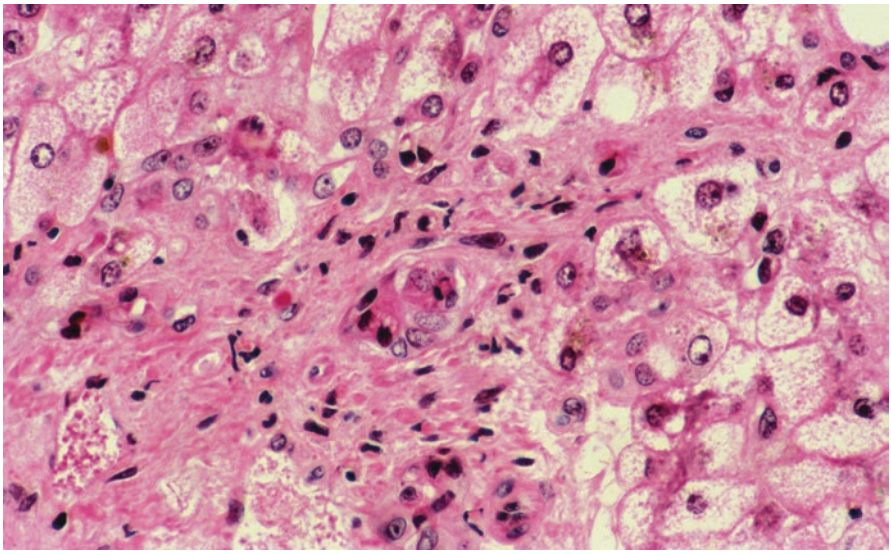

Fig. 14.5 High-powered view of withered bile duct with shrunken eosinophilic cytoplasm. Marked ballooning cholestatic change in periportal hepatocytes

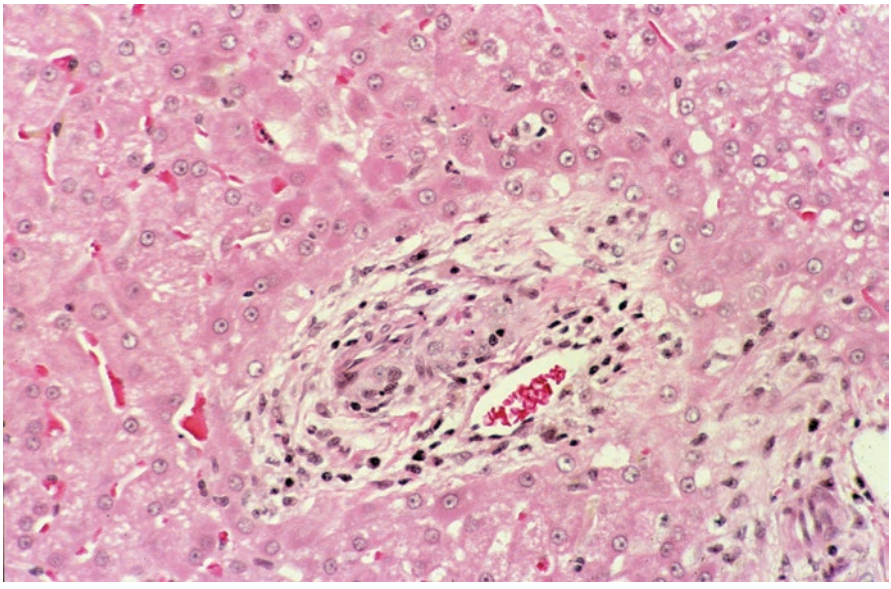

Fig. 14.6 Paucicellular portal space contains a shrunken bile duct with segmental nuclear dropout and lymphocytic ductitis

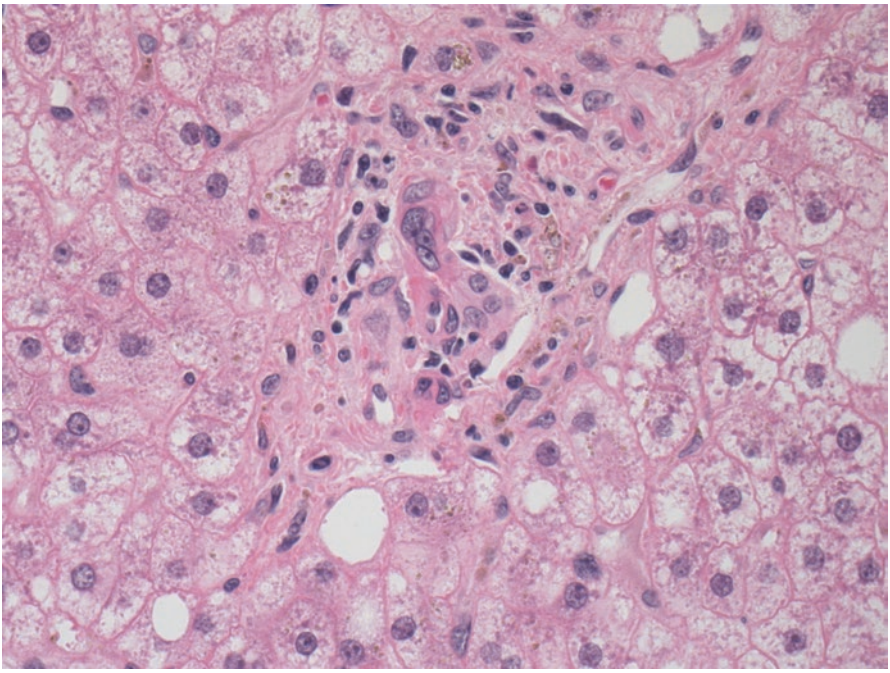

Fig. 14.7 Liver biopsy from day 214: small portal space has a markedly misshapen bile duct with anisonucleosis, overlapping nuclei, and cytoplasmic eosinophilia

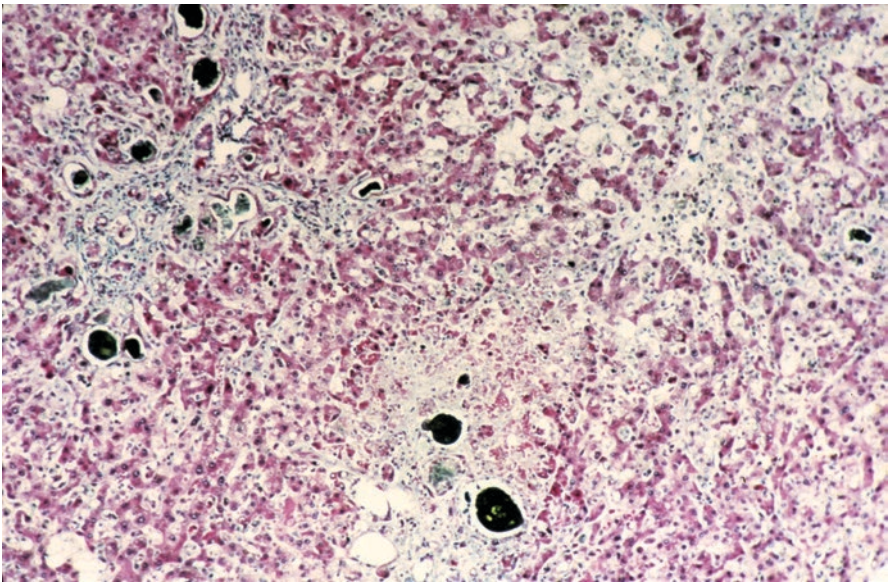

Fig. 14.8 Severe liver GVHD in patient with coexistent gut GVHD. Pronounced periportal cholangiolar cholestasis with prominent periportal cholangiolar bile plugs

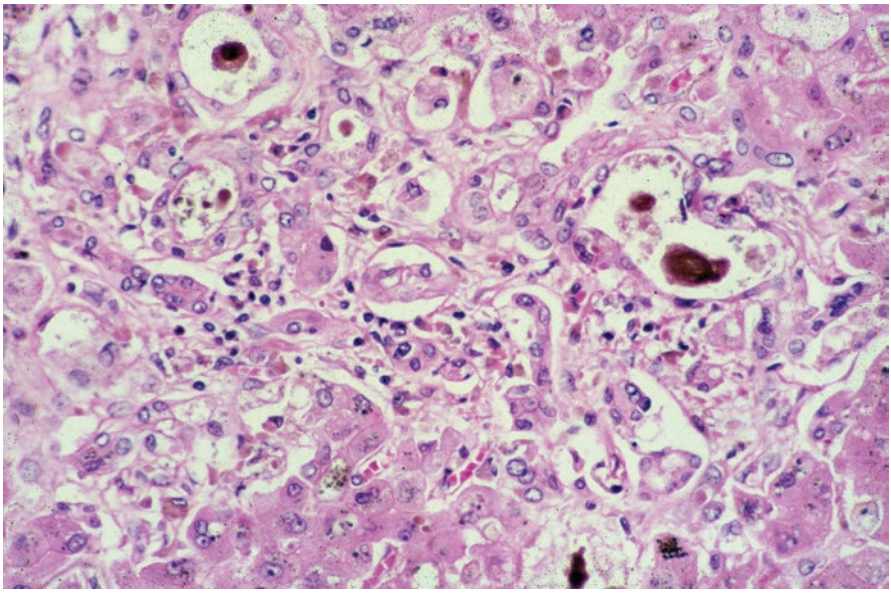

Fig. 14.9 Liver biopsy from day 199: the small, distorted portal space has dysmorphic bile duct with an elongated flattened outline. Surrounding hepatocytes have disarray with ballooning and bile plug formation

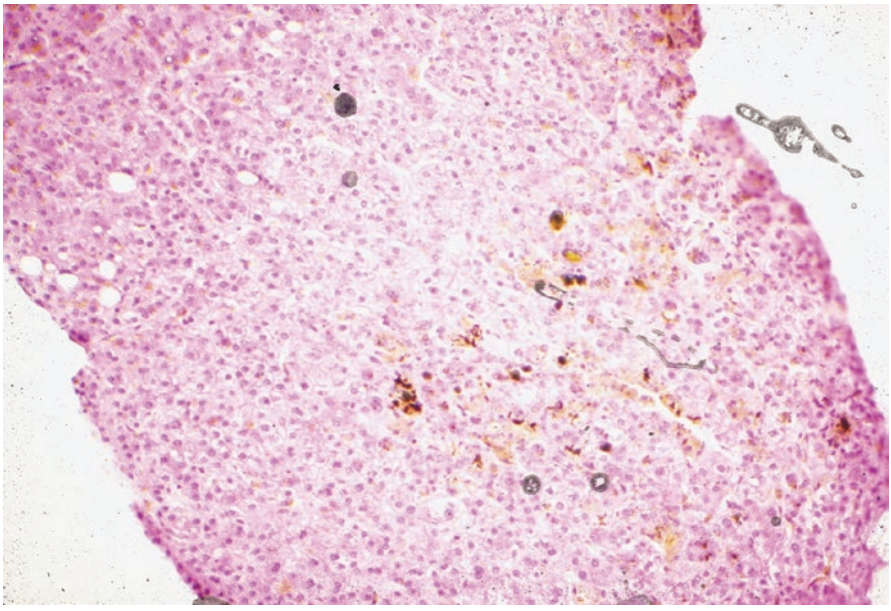

Fig. 14.10 Autopsy from day 458: patient died with hepatic encephalopathy (see case #2 in Chap. 10) and marked jaundice. Periportal bile plugs surround the portal zone, in which no bile ducts were evident

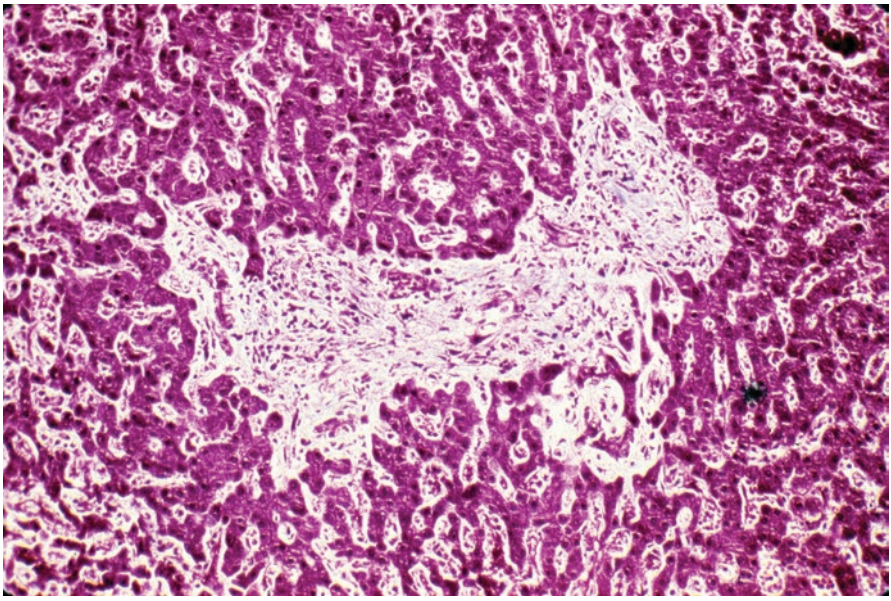

Fig. 14.11 Liver autopsy of a patient 719 days' post-transplant. Patient recovered from earlier severe hepatic GVHD with clearing of jaundice. Autopsy liver histology shows virtually no bile ducts

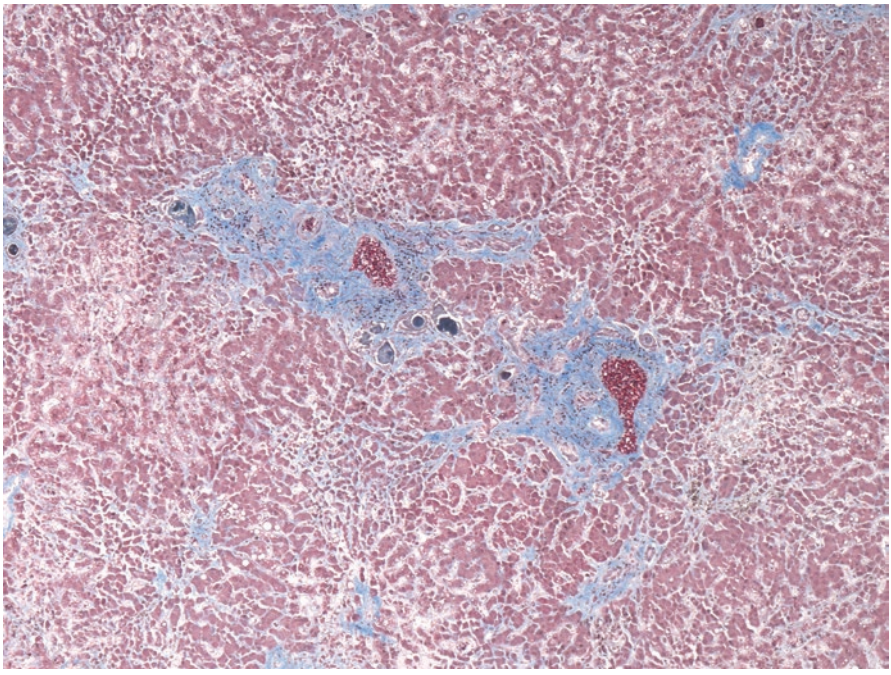

Fig. 14.12 Masson trichrome displaying portal septal fibrosis, periportal bile thrombi, and ductopenia in long standing GVHD

- Multiple stains, especially PAS, PAS-D, and cytokeratins 7 or 19 should be utilized since H&E may not provide clear distinction of bile ducts from other portal structures and adjacent hepatocytes. Trichrome stain is essential to diagnose veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) and nodular regeneration.
- Cytokeratins help quantify bile duct damage. Bile duct loss is determined by locating the hepatic arteriole, which should be within 3 diameters of the bile duct. This can be challenging in the presence of a ductular reaction. Cytokeratin 19 highlights a ductular reaction (Fig. 14.13) whereas CK 7 stains both oval cells and ductules.
- In addition to evaluating for liver GVHD, the PAS stain (with and without Diastase) can be used to characterize Diastase-resistant/Lafora-like bodies and Diastase-sensitive pseudo-ground-glass polyglucosan inclusions [1, 2] (Fig. 14.14).
- Excess iron deposition is very common and needs to be distinguished from lipofuscin by PAS and iron stains. Iron overload results from transfusions and from the anemia of chronic inflammation, leading to increased intestinal iron transport coupled with iron retention in the liver [3].
- Some studies suggest that excessive iron stores can mimic liver GVHD [4]. Others question this dictum, since many patients with marked hepatic hepatocellular iron overload do not have liver tests suggestive of GVHD [5].
- Routine staining for viruses without a relevant clinical situation or suggestive histology is not recommended.

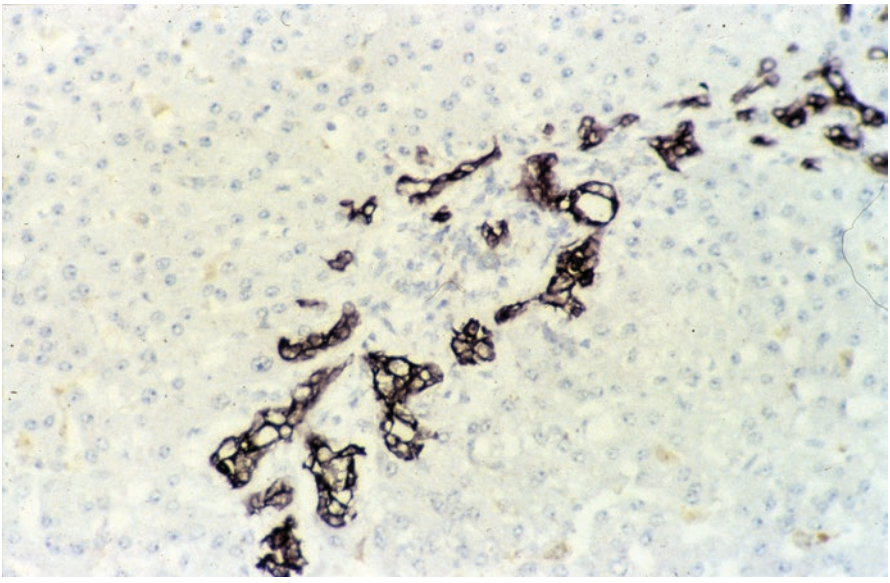

Fig. 14.13 Anti-CK19 immunohistochemistry highlights the ductular reaction surrounding the portal space. It is difficult to identify the artery of the hepatic triad used to locate the bile duct

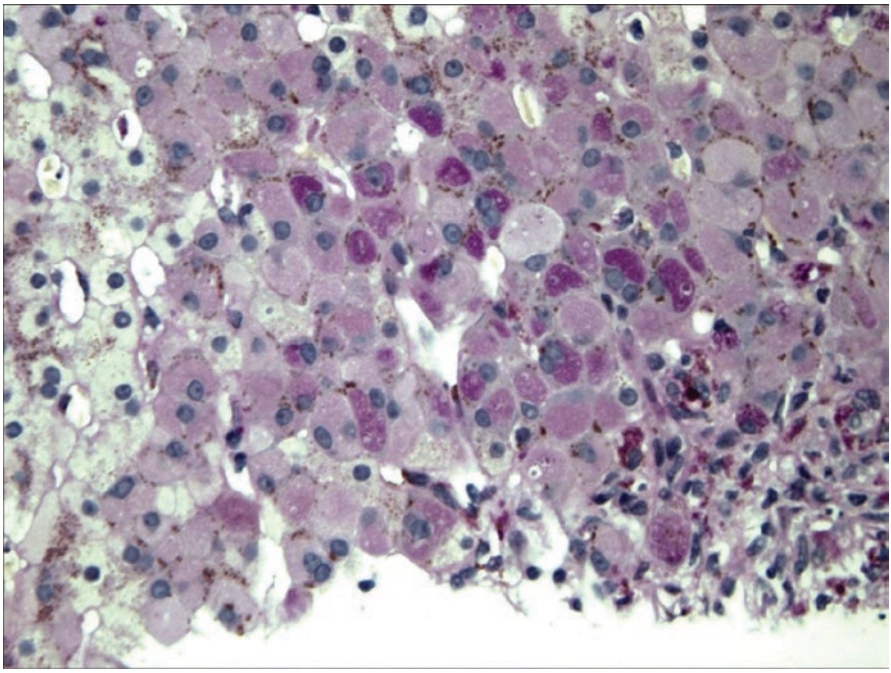

Fig. 14.14 Within the periportal hepatocytes with ground-glass cytoplasmic change are PAS-D-resistant inclusions

Discussion

Hepatic GVHD may have several different clinical presentations [6–8]. In this index case, typical of that during the onset of acute multisystem GVHD, there were mild elevations of aminotransferases and more marked elevations of AP and total bilirubin. The second presentations occurred with prolonged or chronic GVHD. There may be isolated laboratory elevations of slowly rising AP and GGT without jaundice. Alternatively, there may be a cholestatic presentation with slowly rising AP and GGT followed by hyperbilirubinemia reflective of damage to small interlobular bile ducts. Lastly, an acute hepatitis onset presents with very high aminotransferases, initially without hyperbilirubinemia following withdrawal of IS (Chap. 16). A liver biopsy is not necessary in the setting of skin and gut GVHD and abnormal liver tests where the pretest probability of GVHD is high. In contrast, liver biopsy is warranted when abnormal tests exist as an isolated finding or do not improve after immunosuppressive therapy. The hallmark of liver GVHD in all presentations involves damage or loss of small interlobular bile ducts with secondary cholestatic changes. Stueck et al. have proposed an algorithm to predict the diagnosis of GVHD [9]. Nonetheless, the histologic threshold for diagnosing liver GVHD is based on qualitative changes, given the variability in the size and quality of liver biopsies. Initially, the changes of liver GVHD may feature nonuniform involvement of portal

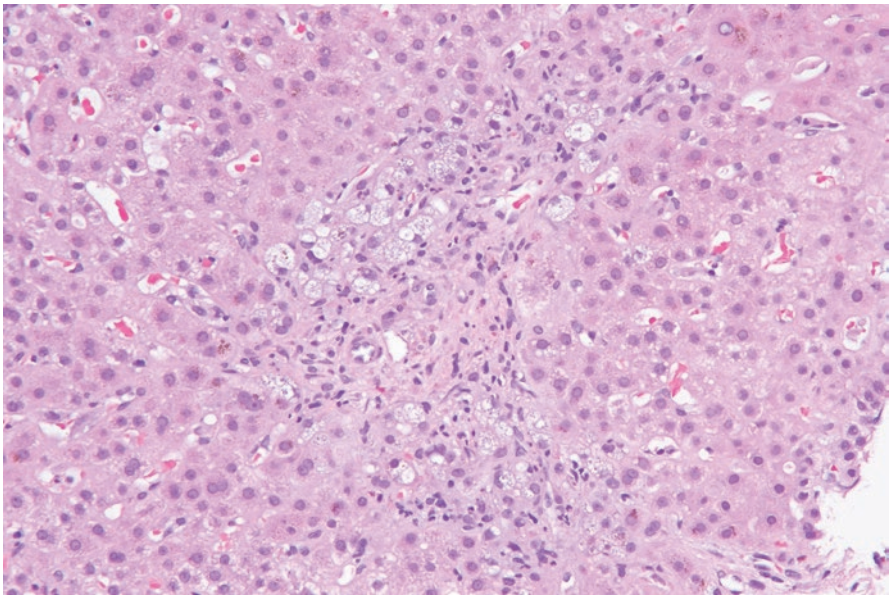

Fig. 14.15 Chronic cholestasis from GVHD of approximately 6 months' duration. Distorted fibrotic portal space has no discernable bile duct. Periportal hepatocytes have swollen bubbly cytoplasms, pseudoxanthomatous change, and granular bile pigment

spaces. Therefore, the minimal diagnostic criteria for liver GVHD are somewhat empiric, being influenced by a number of evaluable portal spaces and after considering any possible drug liver injury or infectious processes. The percentage of affected ducts and the degree of damage varies with the allogeneic disparity, duration of active GVHD, and the effectiveness of any IS intervention. While there is no distinction between an acute or chronic phase of hepatic GVHD, its duration or chronicity impacts the severity of hepatocellular cholestasis (Fig. 14.15), the amount of portal septal fibrosis (Fig. 14.12), and the degree of ductopenia (Fig. 14.11). Nearly all prior reported cases of cirrhosis developing after allogeneic HSCT were related to chronic hepatitis C (HCV) infection [10]. Nonetheless, rare cases of cirrhosis, unassociated with either GVHD or infection, termed "chronic alloimune hepatitis (CAIH)," are discussed in Chap. 16.

The GVHD attack on small bile ducts results in irregular shapes, nuclear dyspolarity, anisonucleosis, and hyperchromatism. With progression, bile ducts appear shrunken with cytoplasmic eosinophilia (Figs. 14.5 and 14.7). Liver biopsies taken early posttransplant or after high-dose IS typically have a paucicellular lymphocytic infiltrate in the portal spaces and within the bile ducts (lymphocytic ductitis), but rarely have apoptosis of the bile duct epithelium. The infiltrate may include a few scattered eosinophils and plasma cells. Hepatocellular changes include unrest, irregularity of liver cords and scattered necrotic individual hepatocytes (councilman bodies). Cholestatic alterations include dropout of perivenular hepatocytes, hepatocyte swelling and ballooning, and canalicular and periportal cholangiolar bile plug

formation. With prolongation of liver GVHD, the shrunken bile ducts become difficult to identify even with the use of cytokeratin immunostains. The presence of ductular reaction (proliferation) secondary to septicemia or concurrent gut GVHD is discussed in Chap. 16 (Fig. 14.13). For unknown reasons, some cases of marked liver GVHD have a pseudo-ground-glass hepatocellular change related to an accumulation of polyglucosan (Fig. 14.16). A related finding is pseudo-Lafora bodies, which are PAS-diastase (PAS-D)-resistant round or crescentic intracytoplasmic bodies [11]. It is unknown why these changes occur in a few patients with severe liver GVHD [1, 2] (Fig. 14.14).

If there is a progressive increase in jaundice or rapidly rising aminotransferases, liver biopsy with IHC for viral antigens and blood PCR studies are highly indicated. Chapters 16 and 17 discuss the differential diagnosis of a number of viral infections.

Drug-Induced Liver Injury Given the widespread exposure of polypharmacy in HSCT, it can be difficult to exclude a drug-induced liver injury (DILI) in the differential diagnosis [6, 12] (Chap. 16). The spectrum of DILI patterns ranges from acute or chronic hepatitis, to acute or chronic necroinflammatory cholestasis, or to some mixtures thereof [12]. Besides describing the pattern of injury, the description should include severity and anatomic regions affected. There are no accurate data on

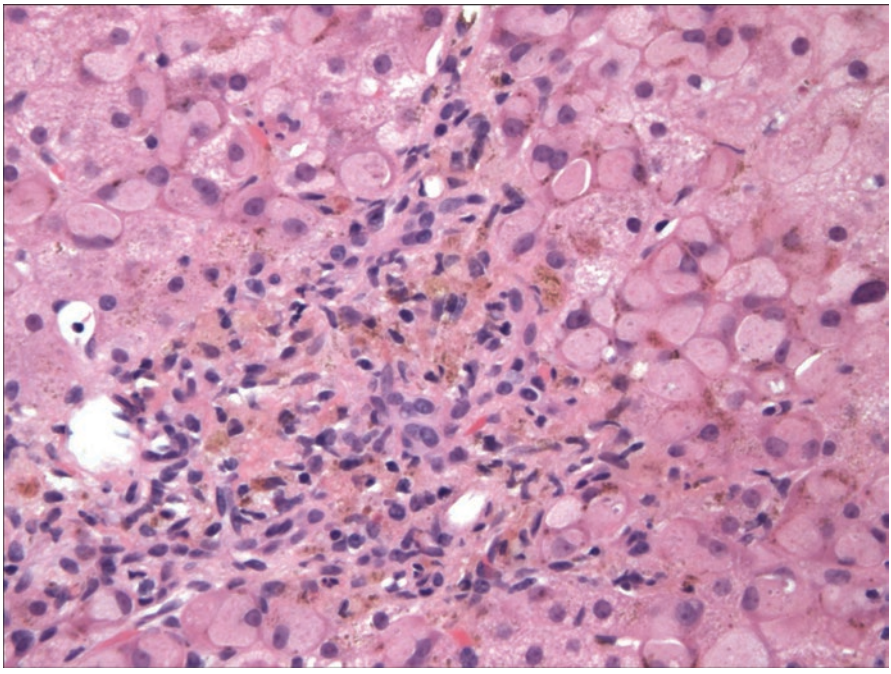

Fig. 14.16 The hepatocytes have a pseudo-ground-glass change related to an abnormal form of glycogen

the frequency of DILI in the HSCT setting, but the most common manifestation appears to be mild cholestasis associated with several azole antibiotics. In our experience, the drug most commonly associated with necroinflammatory injury is trimethoprim-sulfamethoxazole. Some drugs, e.g., azithromycin, can cause bile duct injury. Classical histologic dogma in nontransplant patients states that increase of eosinophil numbers helps differentiate DILI from other etiologies. We have insufficient experience to verify this claim since, when dealing with a possible DILI, the usual course is to simply eliminate the drug rather than doing a liver biopsy. In fact, the presence of small numbers of eosinophils occurs with GVHD, especially cGVHD with the acute hepatitic onset, and should not be taken as evidence of a DILI. Depending on the medications the patient is receiving, the prudent course is to eliminate the putative hepatotoxic drugs.

Interpretation The diagnosis of hepatic GVHD is important because timely IS intervention can prevent the development of ductopenia or fatal types of viral hepatitis. Interpretation must consider the time posttransplant, exposure to IS, potential DILI, and awareness of any preexisting liver conditions, either overt or silent, such as NASH, viral hepatitis, or an underlying myeloproliferative disorder. The pathologist must be aware that the initial findings of liver biopsy taken soon after the clinical onset of elevated aminotransferases may be confined to acidophilic necrosis in scattered hepatocytes secondary to the innocent hepatocyte bystander effect of cytokines. Bile duct damage may not be evident for another 7–10 days [13]. There is no agreed-upon grading scheme for liver GVHD. The overall picture is a snapshot in time reflective of the severity of allogeneic disparity, duration of activity, and effect of IS. There are qualitative and quantitative benchmarks that should be included in the final diagnostic interpretation. Because of the nonspecific findings in the early and mild forms of liver GVHD, and the possibility of another source producing the histologic changes, the pathology report should qualify the level of diagnostic certainty using the NIH diagnostic categories of no GVHD, possible GVHD, and likely GVHD. If there is frank ductopenia and/or other typical features of damaged small bile ducts, we make an unequivocal diagnosis of hepatic GVHD. Additional features mentioned in a comment or the diagnosis include the distribution (focal or widespread) or percentage of portal spaces with damaged ducts, ductopenia if present, cholestatic changes, and if there are increased iron stores, fatty changes, or fibrosis. A liver biopsy taken after a course of IS for persistent liver GVHD can identify or exclude other causes of liver dysfunction, but the trajectory of GVHD cannot be determined by a single liver biopsy. Furthermore, histologic alterations other than a reduction in inflammation may lag behind or persist following IS, even with improvement in the liver laboratory profile. The overall survival and non-relapse mortality of liver GVHD are inversely related to persistent hyperbilirubinemia in a point in time analysis [14]. Patients with cGVHD overlap syndrome have increased mortality associated with hyperbilirubinemia, but not with elevated alkaline phosphatase or aminotransferases [15].

Teaching Points

- The histologic criteria for liver GVHD are empiric, being influenced by the size and quality of the biopsy.
- NIH diagnostic criteria of no GVHD, possible, likely, or diagnostic should be used that consider the period of time post-transplant, the clinical presentation of LFT abnormalities, exposure to other concurrent drugs, infectious processes, and any preexisting liver conditions, e.g. NASH or myeloproliferative disorder.
- Pertinent laboratory values include a gradual rise in bilirubin, alkaline phosphatase, and aminotransferase enzymes AST and ALT.
- Liver GVHD is not classified as acute or chronic. Chronicity or persistence leads to ductopenia and cholestasis.
- Indications for biopsy include isolated persistent abnormal liver tests, progressive jaundice, or rapidly rising aminotransferases. This assumes that early sinusoidal obstruction syndrome has been ruled out.
- A single liver biopsy obtained after prolonged IS treatment for persistent GVHD cannot define the trajectory of the process. Noninflammatory histologic changes may lag or persist for several months despite clinical improvement.
- Persistent hyperbilirubinemia, but not elevated aminotransferases, is significantly associated with non-relapse mortality and reduced overall survival [15].

Questions

1. What are the typical features of hepatic GVHD?
2. What stains are the most useful in defining GVHD-damaged bile ducts?
3. When is a liver biopsy indicated for suspected GVHD?
4. When is liver biopsy not likely to provide clarifying or additional information?
5. What should be included in the final diagnostic report?
6. What other conditions could be excluded by a liver biopsy?

Answers

1. Damaged, atypical small bile ducts and hepatocellular cholestasis.
2. H&E, PAS, PAS-D, cytokeratins 7 and/or 19, and Masson trichrome.
3. Answer: Indications for biopsy include isolated persistent abnormal liver tests, progressive jaundice, and/or rapidly rising aminotransferases.
4. Answer: If a liver biopsy is done shortly after the onset of liver dysfunction caused by GVHD, histology may only consist of necrotic hepatocytes (councilman bodies) caused by cytokine-mediated damages. The bile duct damage may not be visible until 7–10 days after onset of liver dysfunction.

5. Answer: The NIH diagnostic level of certainty (no GVHD, possible GVHD, likely GVHD, or unequivocal GVHD) and a description of the extent and type of small bile duct damages, e.g., focal or widespread ductopenia.
6. Answer: Depends on the time periods. Early post-HSCT, preexisting fibrosing liver disorder, cirrhosis, NASH, HBV, infectious process, later fungal or viral infection, PTLD, nodular regenerative hyperplasia, and hepatitic onset of liver GVHD.

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15 Rapidly Progressing Cholestatic Liver Failure After Allogeneic Stem Cell Transplant from Hepatitis C Virus-Positive Donor (FCHCV)

Keith R. Loeb and Howard M. Shulman

Clinical History

Our patient was a 40-year-old man who received an HLA-matched HSCT from his sibling for treatment of relapsed acute lymphocytic leukemia (ALL) after myeloablative conditioning with cyclophosphamide and total body irradiation (TBI). His GVHD prophylaxis included tacrolimus and mycophenolate mofetil (MMF). Before transplant, the patient was negative for hepatitis B (HBV) and C (HCV) infection with normal LFTs. However, the donor was known to be chronically infected by HCV (viral load of 3.5 million IU/mL). At day 40 posttransplant, the patient developed nausea, vomiting, and elevated serum aminotransferase enzymes (AST 59 U/L, ALT 130 U/L) (Fig. 15.1). On posttransplant day 46, he was found to have hepatitis C viremia with a viral load of 1.2 million IU/ml and both gut and skin GVHD were diagnosed by biopsy. The elevated transaminases were assumed to indicate acute hepatic GVHD, and he was treated with prednisone at 2 mg/kg/d. However, his LFTs continued to rise. On posttransplant day 70, he developed new ascites with worsening liver function (ALT1400U/L). A day 70 liver biopsy showed a lobular inflammation with ballooning of the hepatocytes. The portal spaces were enlarged by chronic inflammation and ductular proliferation. The bile

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

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

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

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https://doi.org/10.1007/978-3-319-42099-8_15

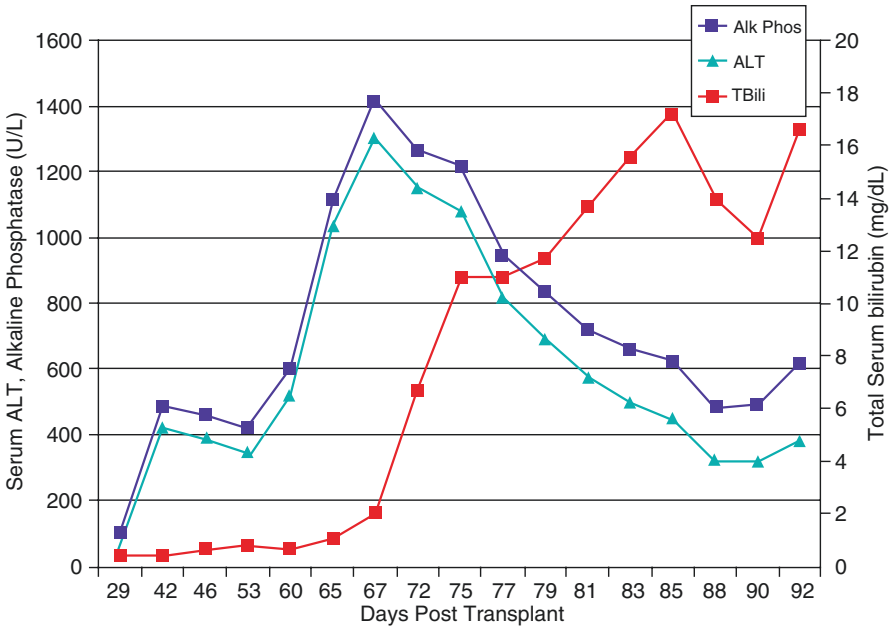

Fig. 15.1 Liver tests of our patient over the post-transplant period

ducts appeared abnormal and consistent with GVHD (Figs. 15.2, 15.3, and 15.4). He was given a dose of ATG for presumed steroid-refractory hepatic GVHD. HCV viral load was found to be 3.5 million IU/mL on day 81. A repeat liver biopsy on day 88 showed progression with marked hepatocellular unrest and cholestasis with ballooning. There were broad serpiginous bands of portal-to-portal bridging collapse containing a marked proliferation of bile ductules admixed with chronic inflammatory cells. The small bile ducts were difficult to identify among the proliferated ductules. On day 89, he developed confusion and hypoxia with pneumonia and evidence of disseminated fungal infection. Before his death on posttransplant day 94, he was deeply jaundiced with elevated LFTs (AST 195 U/L, ALT 291 U/L, AP 134 U/L, total bilirubin 13 mg/dL). Postmortem examination was not performed.

Key Pathology Features

- Broad bands of collapsed hepatocytes.
- Serpiginous portal-to-portal fibrous bridging fibrosis containing marked bile ductular proliferation pattern admixed with lymphocytes.

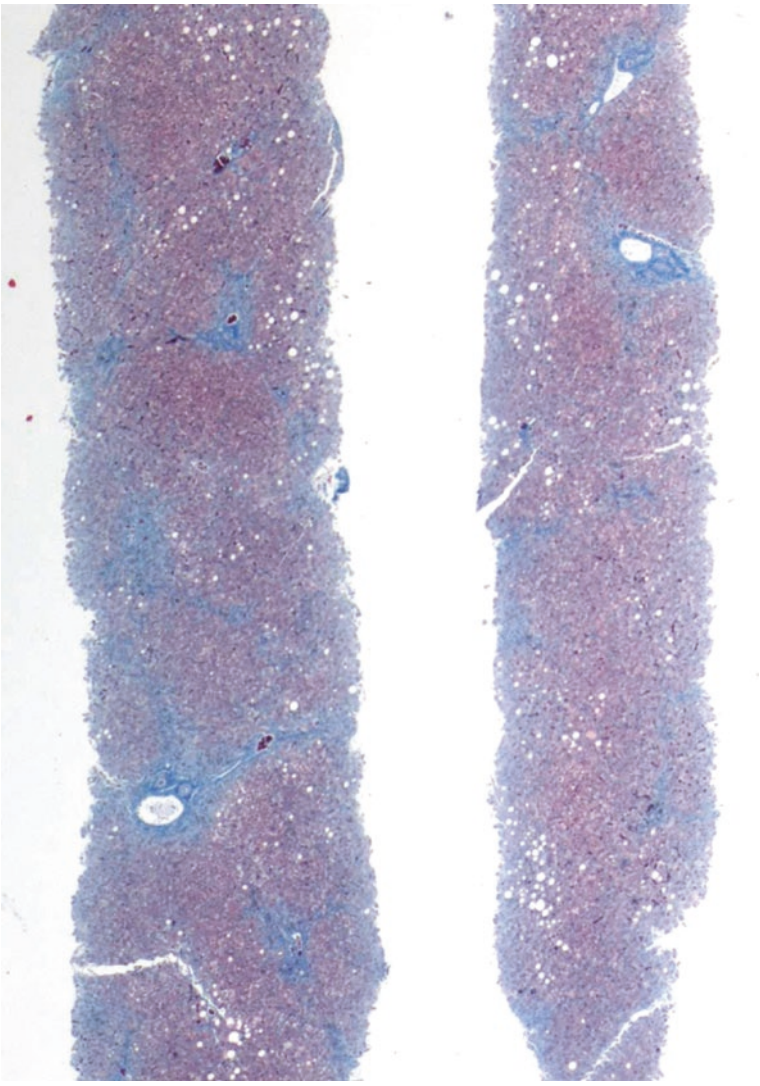

Fig. 15.2 The first biopsy, taken on day 70 (Masson trichrome stain), has mildly enlarged portal spaces containing chronic inflammatory cells and loose fibrous material

- In full-blown FCH, abnormal small bile ducts damaged caused by GVHD are obscured by the fibrosis admixed with ductular proliferation.
- Prominent cholestasis with ballooning degeneration of hepatocytes.
- Sinusoidal lobular inflammation reaction pattern.
- Prominent HCV in-situ hybridization (ISH) detection.

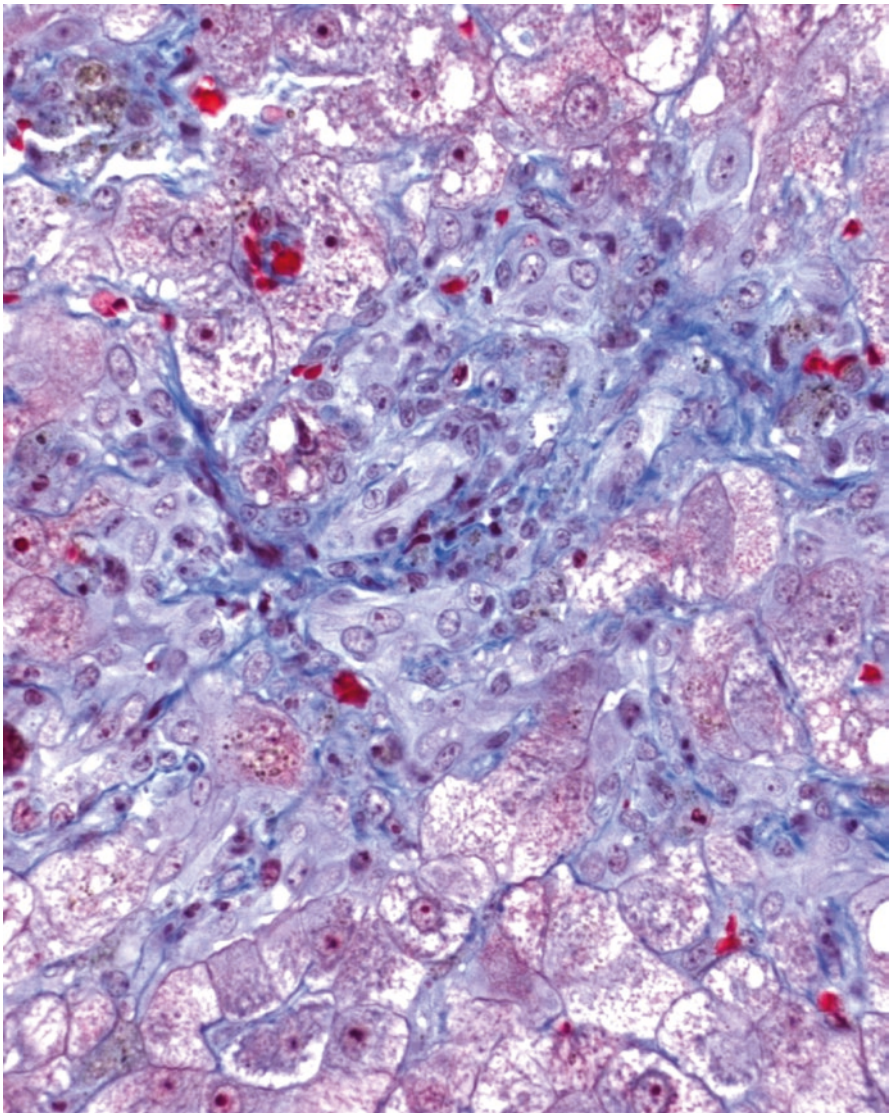

Fig. 15.3 At higher power, the centrally located small bile ducts have features of GVHD, with distorted bile duct outlines and segmental nuclear dropout. There is considerable hepatocellular disarray and unrest

Diagnosis

Fibrosing cholestatic HCV

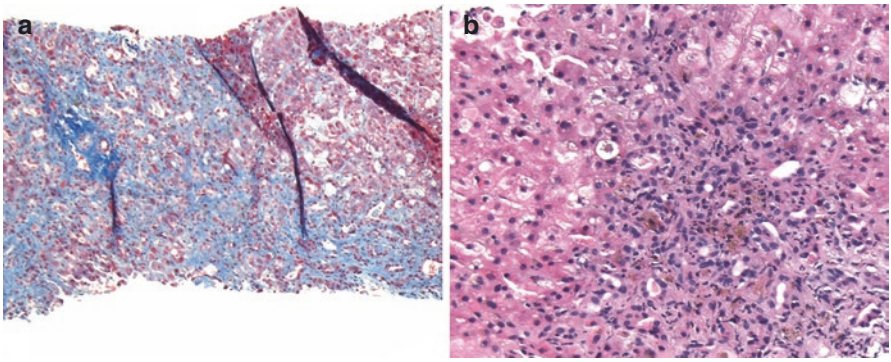

Fig. 15.4 The second biopsy, taken on day 88. Masson trichrome stain (**a**) and H&E stain (**b**) showed a remarkable progression of the inflammatory process with marked bile ductular proliferation associated with serpiginous portal-to-portal bridging collapse/fibrosis. There was also significant lobular inflammation with ballooning hepatocyte and feathery degeneration. These findings, along with the clinical history of HCV in a posttransplant patient with immunosuppression, are consistent with a diagnosis of fibrosing cholestatic HCV

Differential Discussion

Since the inception of HSCT, the hepatotropic viral infections (HCV, HAV, HBV, rarely HDV and HEV) and some non-hepatotropic viral infections have been subjects of concern in the posttransplant setting. They can present similar to GVHD and can be lethal if mistakenly treated with IS or left untreated. The history of pathologists' experience with HCV, the viral hepatitis most often encountered in the American HSCT setting, can be divided in three eras. In the first era, prior to the 1990s when reliable testing via polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) were not available, HCV was simply called "non-A-non-B hepatitis." During this era, a 1987-1988 study by Strasser et al. found 32% of a cohort of 355 Seattle HSCT recipients were HCV positive (HCV+). Nearly all developed chronic hepatitis, and 3.8% of those surviving beyond 20 years had cirrhosis [1]. In a related European study of long-term surviving recipients with chronic HCV that was first acquired post-allogeneic transplant, the cumulative incidence of cirrhosis and/or hepatocellular carcinoma was 24% at 20 years (Fig. 15.5) [2]. This rate of fibrosis progression was more rapid in HCV+ HSCT recipients than in HCV+ patients without cancer. Pre-transplant HCV+ recipients who received cyclophosphamide-based regimens were also at risk of developing veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) [3]. In the second era, systemic screening of blood products greatly reduced the transmission of HCV to <1%. Interferon-based treatment for HCV was only partially successful and associated with adverse side effects. Since 2009, the third era, the development of effective combination therapies with direct-acting antiviral (DAA) agents has led to clearance and/or cure of most HCV.

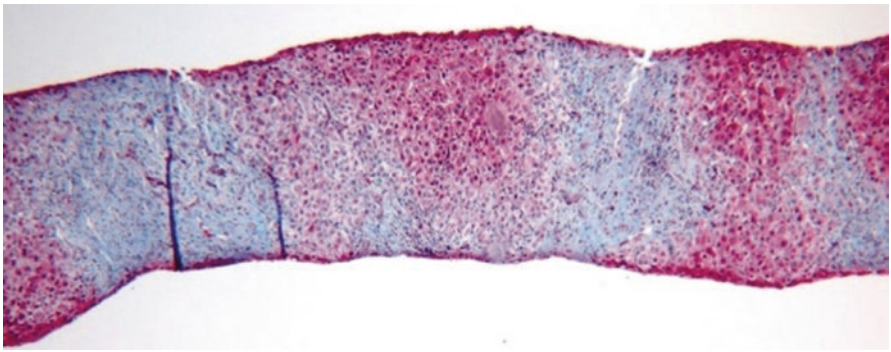

Fig. 15.5 Cirrhosis in a long-lived HSCT with chronic HCV hepatitis. A trichrome-stained biopsy displays broad bands of dense fibrous tissue surrounding irregular hepatocyte clusters

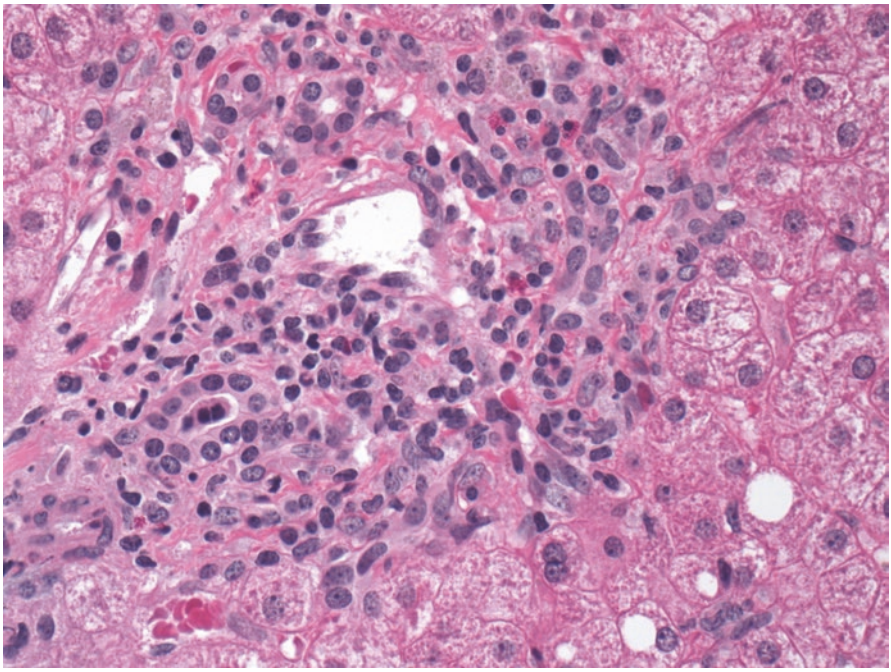

Fig. 15.6 Chronic HCV hepatitis typically presents mildly expanded portal lymphoid infiltrates and some interface inflammation. Hepatocytes display unrest and mild steatosis

Since our patient was transplanted in 2007, pre-transplant DAA treatment for the HCV+ donor was not yet available. The collective experience of the typical posttransplant course in HCV+ recipients is mild elevations of ALT (5–10× normal) [4, 5]. Histologically, persistent HCV post-HSCT has mild portal inflammation, sometimes with lymphoid aggregates and mild steatosis (Fig. 15.6). In both HSCT and

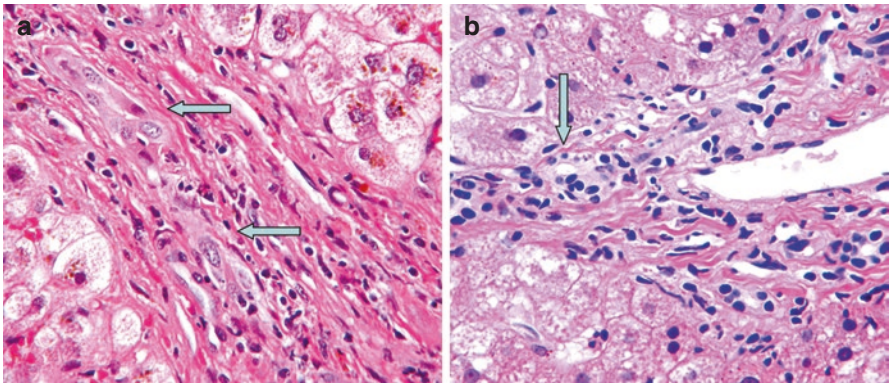

Fig. 15.7 Comparison of bile duct damage (arrows) between severe GVHD (**a**) and from a case of non-transplanted patient with chronic HBV (**b**)

non-HSCT settings, bile duct alterations can occur in up to >30% of chronic HCV and ~10% of chronic HBV cases [6] (Fig. 15.7). On day 46, our patient had multisystem GVHD established by clinical criteria for gut involvement and by biopsies of the skin and gut. The clinical course did not behave like typical hepatic GVHD. Despite treatment with high-dose IS, levels of aminotransferase rose to >1200 IU/L, and ascites developed. The day 70 biopsy had more portal inflammation than would be expected after treatment with high-dose IS but did have bile duct epithelial damage most consistent with GVHD (Figs. 15.2 and 15.3). The second biopsy from day 88 showed rapid progression with fibrosing bridging collapse, ductular proliferation, and marked cholestasis. These abnormalities developed concurrently with the rise in HCV viral copy number (Fig. 15.4).

The term "fibrosing cholestatic hepatitis" (FCH) was first coined in 1991 to describe a rapid fulminant cholestatic hepatitis with severe jaundice, coagulopathy, and encephalopathy with recurrent HBV in liver allograft recipients (Fig. 15.8). Subsequently FCH associated with HCV was reported after liver and kidney organ transplantation and when there is coinfection with HIV. Despite our center's vast prior experience with HCV positivity in HSCT recipients, the first report of FCH with HCV was in 2015 and included this patient. The suspected common thread in the three patients was the use of the immunosuppressant MMF, as this complication had not been recognized before its use in HCV+ recipients. A less severe form of posttransplant chronic HCV reactivation is defined as an otherwise unexplained 3× rise in ALT and a rise in HCV RNA of at least 1 $\log_{10}$ from baseline [7]. The guidelines for using posttransplant DAA treatment for chronic HCV have not been established since there may be considerable drug-drug interactions with agents commonly used in HSCT patients [8]. In 2017, Oliver et al. reported on the first successful use of DAA treatment in a patient with the early onset of HCV FCH before day 30 [7]. Likewise, there has been a marked reduction in the frequency of posttransplant FCH from HBV with the use of antivirals entecavir and lamivudine [8].

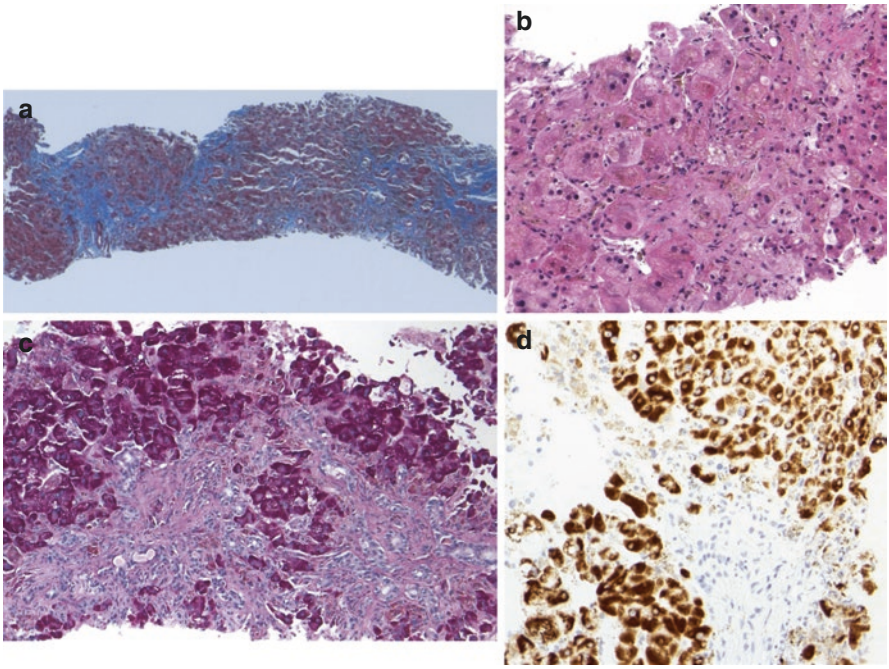

Fig. 15.8 Liver biopsy from a patient 27 days' post-HSCT with chronic active HBV hepatitis developing fibrosing cholestatic hepatitis. Montage depicts serpiginous fibrosis (trichrome) (**a**), marked cholestasis (H&E) (**b**), ductular proliferation (PAS) (**c**), and diffuse hepatocellular IHC staining for HBV surface antigen (HBVsAg) (**d**)

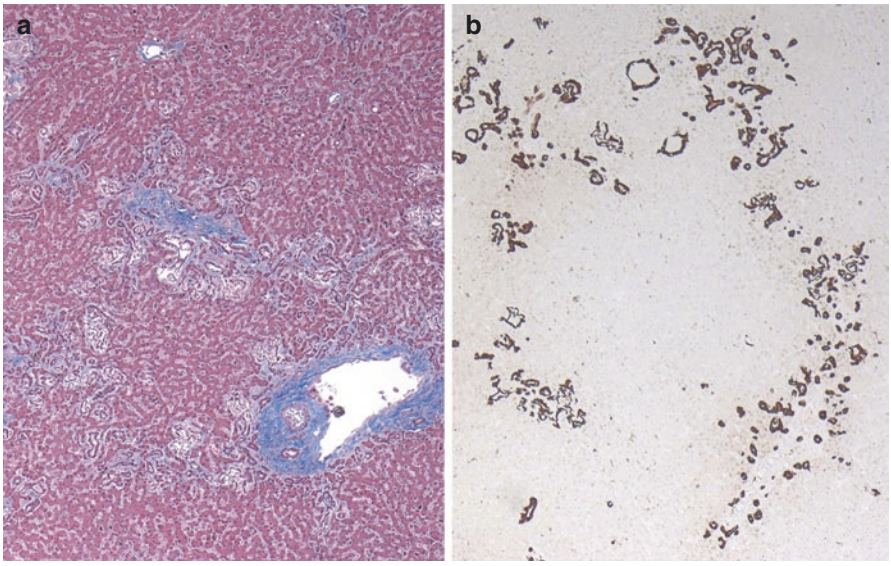

Fig. 15.9 A liver biopsy 27 days' post-autograft shows cholangitis lenta caused by vancomycin-resistant *Enterococcus*, visualized by Masson trichrome stain (**a**) and CK19 IHC stain (**b**), demonstrating marked ductular reactions

The differential diagnosis of jaundice depends on the level of elevated LFTs and the time posttransplant. In practice, any ALT > 10× with jaundice should warrant immediate investigation with consideration for isolating viral DNA in sera (Chap. 16).

The differential diagnosis of cholestatic hepatitis following HSCT includes sepsis-associated cholestasis: cholangitis lenta. The typical clinical presentation is usually associated with severe sepsis with fever, jaundice, and abnormal LFTs in the first 30 days posttransplant. The histologic findings include a prominent ductular reaction (proliferation) pattern with lymphocytic cholangitis and canalicular bile plugs (Fig. 15.9). The second biopsy in our patient, taken on day 88, showed a remarkable inflammatory progression with marked bile ductular proliferation associated with portal-to-portal bridging collapse/fibrosis (Fig. 15.4). There was also significant lobular inflammation with ballooning hepatocytes and feathery degeneration. These findings, coupled with the clinical history of HCV in a posttransplant patient with IS, are consistent with a diagnosis of fibrosing cholestatic hepatitis C virus (FCHCV). A more frequent cause of ductular proliferation occurs when there is concomitant gut GVHD [9]. Gut GVHD causes breakdown of the mucosal barrier, and subsequent bacterial translocation of endotoxin. Endotoxin showers the portal blood and in turn activates cytokines that stimulate ductular proliferation. Cholangitis lenta differs from the ductular proliferation caused by biliary obstruction, whose features include portal edema and more marked acute inflammation. In FCH, the marked bridging fibrosis with ductular proliferation is related to the marked necroinflammatory process.

Many of the drugs used in HSCT patients can develop hepatotoxic side effects, so the differential diagnosis of elevated LFT often includes drug-induced liver injury (DILI) [10]. There is a long list of potential hepatotoxic drugs [11]. The most common histologic patterns of drug injury are necroinflammatory, cholestatic, or a mixture thereof. In our experience, the drug most commonly associated with necroinflammatory injury is trimethoprim-sulfamethoxazole. Cholestatic injury is most often associated with azole antibiotics. The classical histologic dogma in non-transplant patients states that increased numbers of tissue eosinophils help differentiate DILI from other etiologies including the acute hepatitic onset of GVHD (Chap. 16). Unfortunately, it is difficult to verify this dictum, derived from the non-HSCT setting, that tissue eosinophilia implies DILI. It should be noted that a large study

Teaching Points

1. FCH is a rapidly progressive hepatitis with hepatic failure that occurs in immunosuppressed patients with solid organ allografts or HSCT recipients with high viral loads from HBV or HCV.
2. Clinical features include cholestasis with increasing hyperbilirubinemia and markedly elevated serum transaminases (1000 IU/L), coagulopathy, and encephalopathy.
3. Rapid progression with extensive portal inflammation with broad serpiginous portal-to-portal bridging collapse admixed with marked ductular proliferation.

4. The usual posttransplant clinical course in an HCV+ recipient is a low-grade elevation of aminotransferases with liver histology showing mild portal inflammation and little or no fibrosis.
5. Prior to the development of DAA therapies, 20% of HCV+ HSCT recipients developed cirrhosis or hepatocarcinoma at 20 years of follow-up.
6. The development of FCH occurs in severely immunosuppressed patients with HCV and HBV. In rare cases of HCV/FCH, prophylactic MMF was the suspected cause.
7. With the advent of effective treatment of patients or their donors for HCV+ with DAA and/or of HBV+ with entecavir and lamivudine, the occurrence of FCH is expected to become rare.
8. The differential diagnosis of rapidly rising ALT and jaundice depends on the time of onset and related clinical details. Early posttransplant VOD/SOS and ischemic hepatopathy are the two main causes. Later onset includes several different viral infections including HEV, acute hepatitic onset of GVHD, and DILI.

cohort of 977 patients with cGVHD found that 16% had eosinophilia >400/μL [12]. When dealing with a possible DILI, the usual course is to simply eliminate the drug rather than taking a liver biopsy [11].

In summary, this case illustrates the difficulty in distinguishing HCV-associated FCH from hepatic GVHD. There is histologic overlap between the features of hepatic GVHD and FCH with extensive ductular proliferation. In Chap. 16, we present another cause of rapidly rising ALT and jaundice following discontinuation of IS, the acute hepatitic onset of hepatic GVHD.

Questions

1. What is the common thread in cases of FCH after allogeneic transplant?
2. What is the differential diagnosis in a patient with rapidly rising aminotransferase levels > 10× with jaundice?
3. What features distinguish FCH from GVHD?

Answers

1. Answer: Immunosuppression given posttransplant in HCV and HBV + patients.
2. Answer: Viral hepatitis HAV, HCV, HBV, HEV, VZV, adenovirus, DILI, and acute hepatitis onset of GVHD.
3. Answer: FCH progresses rapidly. FCH histology shows fibrous portal-to-portal bridging collapse composed of proliferated ductules and marked hepatocellular cholestasis. At this stage, however, one cannot easily discern FCH from GVHD.

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Acute Hepatitic Onset of Liver GVHD Occurring 9 Months Post-transplant

16

Howard M. Shulman and Keith R. Loeb

Clinical History

A 40-year-old woman received an allogeneic-matched sibling transplant. The only post-transplant problem consisted of mild skin GVHD which responded to prednisone. At 8.5 months post-transplant, she finished a 2-week course of famciclovir for zoster. Two weeks later her liver function tests (LFTs) (Fig. 16.1) revealed normal bilirubin with mild elevations of alkaline phosphatase (AP) 405 IU/L, SGOT 112 IU/L, and SGPT 133 IU/L. Within 2 weeks SGOT had markedly risen to 2086 IU/L, SGPT 1641 IU/L, and AP 347 IU/L. The first liver biopsy was performed 26 days after the onset of the liver elevations at 10 months post-transplant (Fig 16.2). Foscarnet was given preemptively for treatment of presumed zoster hepatitis. Tests for hepatitis A, B, and C were negative, as were CMV studies by PCR. There was no potential exposure to hepatotoxic drugs to cause her symptoms. In the ensuing month, transaminases and AP levels declined; however, her SGOT again rose to 475 IU/L, and her bilirubin continued to rise to a peak of 30 mg 33 days later when a second liver biopsy was done at 11 months post-transplant (Fig. 16.3). Follow-up treatment with high-dose steroids and cyclosporin led to rapid improvement in liver tests with normalization of bilirubin in 2 months.

H. M. Shulman (✉) · K. R. Loeb
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; kloeb@seattlecca.org

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Diagnosis

Nine months post HSCT solitary onset of liver GVHD clinically presenting as an acute hepatitis after cessation of IS.

Key Pathology Features

1. Presentation as a lobular hepatitis with markedly elevated transaminases >2000 iu.
2. Marked lobular inflammation with numerous acidophilic bodies (necrotic hepatocytes).
3. Enlarged portal spaces containing a mixture of lymphocyte plasma cells and scattered eosinophils.
4. Interface inflammation.

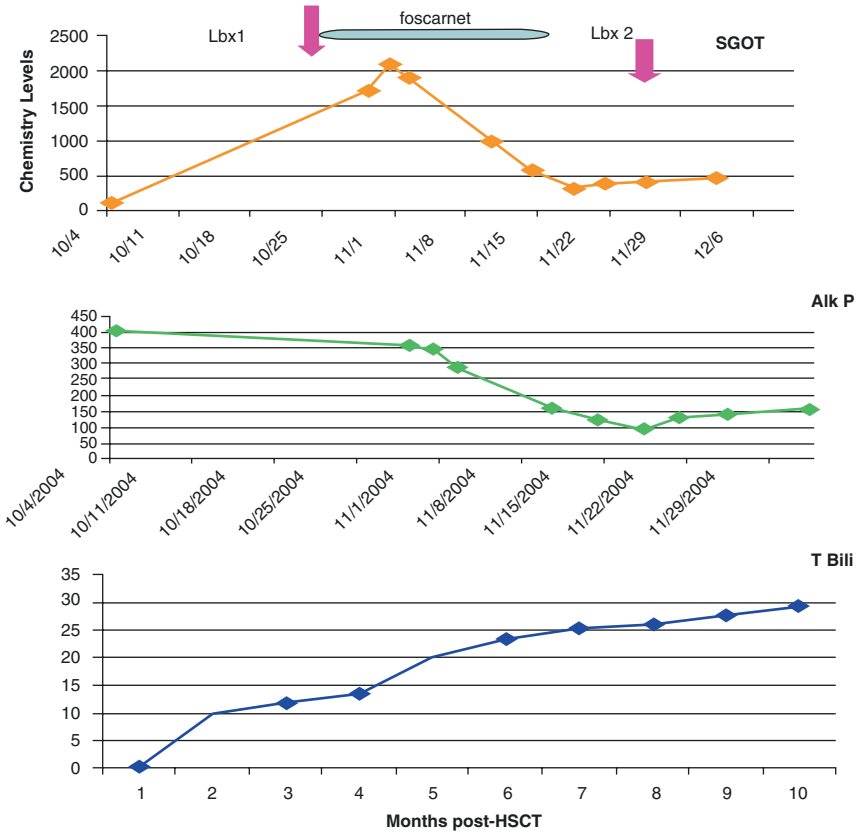

Fig. 16.1 Timeline of liver test abnormalities

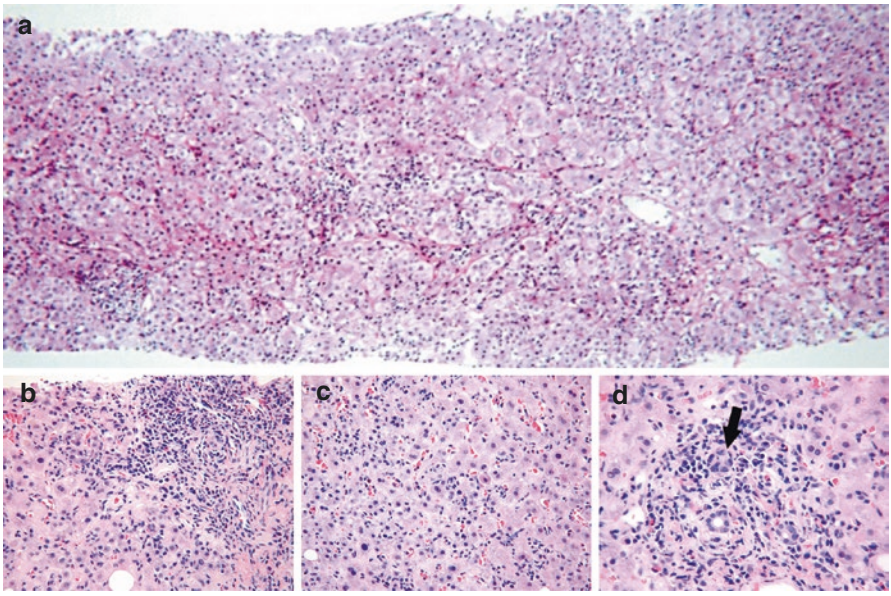

Fig. 16.2 The first liver biopsy was performed at 1 month from the initial onset of LFT elevations. It had a striking lobular hepatitis with disarray of the hepatocyte cords, hepatocyte unrest with many sinusoidal lymphocytes, prominence of Kupffer cells (**a**), and many scattered acidophilic hepatocytes (**b**). The expanded portal spaces contained a mixed infiltrate of lymphocytes and macrophages, as well as a few eosinophils and plasma cells, spilling over into the surrounding periportal plate (interface inflammation) (**c**). The larger bile ducts were unremarkable; however, some of the smaller bile ducts (one is indicated by the arrow) had swollen cytoplasms with nuclear dyspolarity, anisonucleosis, or segmental loss of nuclei and contained a scattering of intraepithelial lymphocytes (**d**)

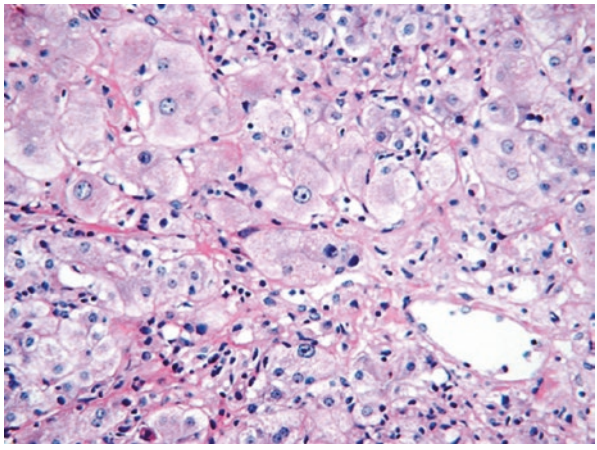

Fig. 16.3 The second liver biopsy, performed at 11 months, displayed pronounced cholestatic changes in the hepatocytes with canalicular bile plugs and marked swelling of pigment-laden hepatocytes in zone 3. This was associated with focal hepatocytolysis, perivenular lymphocytic inflammation, and sinusoidal fibrosis

5. Bile ducts obscured by inflammatory cells. Bilirubin rise occurs after spike in transaminases.
6. Follow-up biopsy taken has more pronounced bile duct damage and hepatocellular cholestasis including perivenular hepatocytolysis.
7. Initiation of steroid treatment before bile duct destruction led to complete clinical resolution.

Discussion

The typical presentation of liver GVHD in long-term survivors with concomitant cGVHD in other organs is an indolent or slowly progressive cholestatic liver disease. In contrast, we have a patient recently off immunosuppression (IS), with no other signs of GVHD, with an explosive onset of a hepatitis-like clinical picture with extremely high transaminases [1]. Her bilirubin which was initially normal became markedly elevated. The initial elevation of transaminases was related to the effect of cytokines on the Fas and Fas Ligand (Fas-FasL) interaction causing necrosis of hepatocytes preceding the actual damage to the bile ducts. It was interesting that in this case the initial biopsy mainly showed the hepatitic features, while small bile duct changes were less pronounced (Fig. 16.4). The pronounced portal inflammation contained a mixture of inflammatory cells which included eosinophils and many plasma cells. The second biopsy showed obvious bile duct damage, hepatocellular cholestasis, and perivenular hepatocytolysis [1]. It is important to recognize this acute hepatitic presentation of GVHD since delay of treatment can lead to severe loss of bile

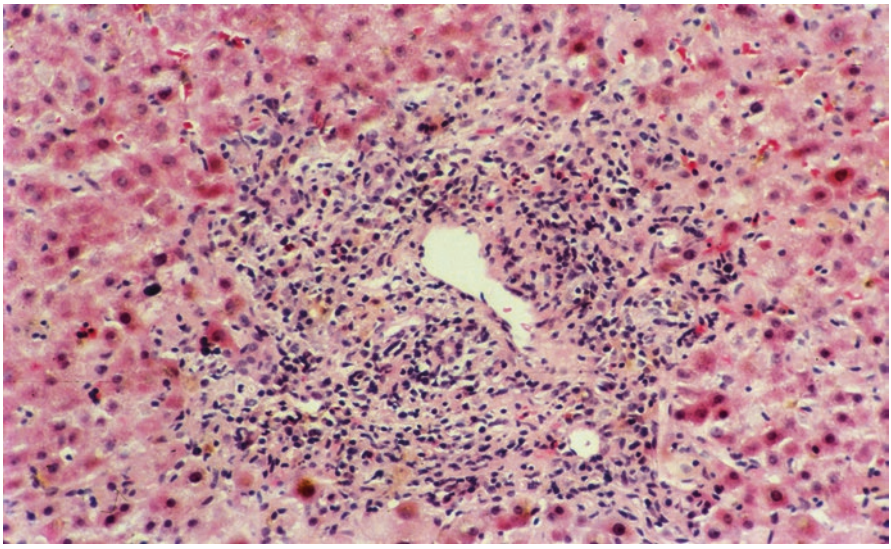

Fig. 16.4 A separate case of acute hepatitic GVHD with marked portal inflammation with plasma cells and some eosinophils with interface inflammation. Small bile ducts are not overtly damaged early after onset, consistent with the concept that the early changes are mostly related to cytokines and later to cellular-mediated damage

ducts and irreparable liver damage. Antiviral therapy should be started as a precautionary measure even before the diagnosis is established. Similar acute hepatitic presentations of GVHD have been reported by other studies [2, 3], especially following donor lymphocyte infusions given in an attempt to stimulate a graft-versus-leukemia response, though the transaminase elevations were less pronounced [4].

A rare, slowly progressing inflammatory and fibrosing non-infectious hepatitis resembling autoimmune-like hepatitis (AIH) has been reported [5, 6]. It has been described in recent literature as a histopathological overlap of primary biliary cirrhosis (PBC) and progressive systemic sclerosis (SSc): "PBC/SSc overlap syndrome" [17]. In our clinic, some of these cases developed many years post-allogeneic HSCT, in the absence of either protracted aGVHD or stigmata associated with cGVHD. We propose that the name for this form of hepatitis be called "chronic alloimmune hepatitis," abbreviated to "CAIH," as the immune system in cases described in the literature is derived from fully allogeneic donor hematopoietic cells. The corresponding histology displays expanded portal spaces filled with lymphoplasmacytic inflammation, damage to and/or destruction of small bile ducts, interface inflammation. and portal and lobular fibrosis tending toward cirrhosis. Some cases had high titers of autoantibodies to LKM type 2 [7] (personal communication: GB McDonald). Some cases had autoantibodies of non-organ specificity (ANA and ASMA) that overlapped with some of the same sera antibodies as those in cGVHD [8]. Sporadic cases of cirrhosis that occur 5-15 years post-HSCT and are attributed to GVHD isolated to the liver may well be a late manifestation of CAIH [15, 16]. To summarize, CAIH may be viewed as an autoimmune-like manifestation, similar to those in Chap. 20, whose genesis develops in the milieuof an altered immune reconstitution after allogeneic HSCT.

In the early era of HSCT, liver GVHD was posited to be pathogenetically related to PBC. These early studies reported positive anti-mitochondrial antibodies (AMA), a marker specific for PBC, in 5–81% of patients with cGVHD. In 1999, Quaranta et al. evaluated sera from 89 cGVHD patients for AMA using more precise analytes and methodology. None of the 89 patients had positive AMA, but they did have a variety of other non-disease-specific autoantibodies. Finally, immunohistochemistry (IHC) in GVHD-affected liver biopsies for PDC-E2 by the PBC-specific monoclonal antibodies against the epithelial luminal antigens of PBC bile ducts was negative in all 89 cGVHD patients. In summary, hepatic GVHD is not a model for PBC because of the rarity of cirrhosis, absence of granulomata, sparing of large bile ducts, and absence of specific IHC staining against the epithelial luminal mitochondrial target of PBC [8].

The remaining clinical differential possibilities are the same as those discussed in Chap. 15 including viral hepatitis caused by HAV, HCV, HBV, and that more recently ascribed to HEV [9, 10]. Rapid diagnosis using DNA/RNA PCR on serum and tissue IHC staining is needed to avoid a fatal outcome since these infections have effective antiviral treatments. Additional viral infections, though rare, include hepatitis from several herpes group viruses. Herpes simplex hepatitis causes massive hepatic necrosis (Figs. 16.5 and 16.6). Varicella-zoster hepatitis may present with severe abdominal pain from visceral involvement without skin lesions. Zoster hepatitis produces random foci of hepatocellular necrosis and involvement of bile ducts (Fig. 16.7). EBV lymphoproliferative syndrome causes massive infiltration of the portal spaces by plasmacytoid cells (Figs. 16.8 and 16.9). EBV is readily identifiable by finding elevated plasma PCR DNA levels and histologically by EBER in situ hybridization

studies. HHV6B hepatitis has been described after liver allograft. Though HHV6 viruses are ubiquitous in transplant recipients, there is only a single case report of HHV6B hepatitis after HSCT documented by PCR and in situ hybridization of the liver which histologically had periportal necrosis [11]. CMV infection in the liver is usually part of disseminated CMV, especially with concomitant gut involvement. Nonetheless, it rarely results in significant liver dysfunction [12, 13]. Therefore, performing IHC for CMV with clinical acute hepatitic presentation is unnecessary and should not be regarded as the explanation [14]. Adenovirus hepatitis produces random punched-out foci of necrosis with the diagnostic virally infected cells along the periphery. In such cases, the ALT elevations are not as great as those caused by other viral infections (Figs. 16.10 and 16.11). If there is coexisting chronic HCV or HBV infection, fibrosing cholestatic hepatitis should be considered (Chap. 15). In addition to any infectious etiologies, any known hepatotoxic drugs should be discontinued.

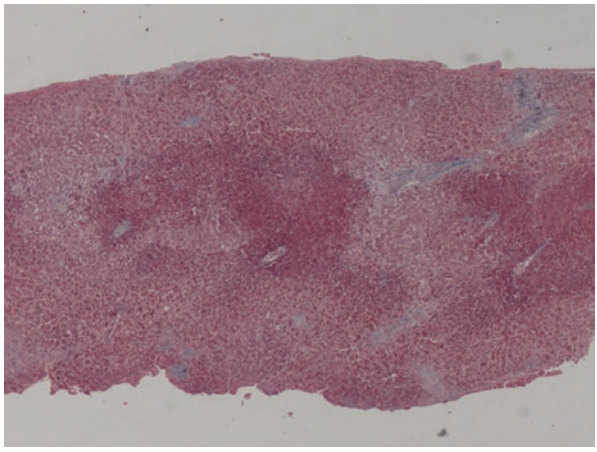

Fig. 16.5 Herpes simplex hepatitis at low magnification shows regular foci of hemorrhagic necrosis

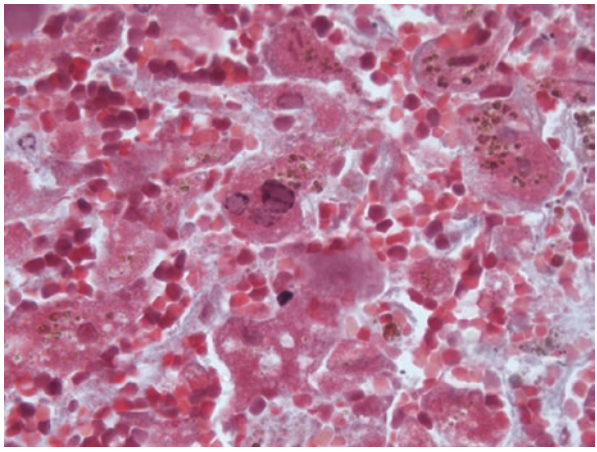

Fig. 16.6 High magnification shows degenerating hepatocytes containing smudgy nuclear inclusions of Herpes Simplex Virus

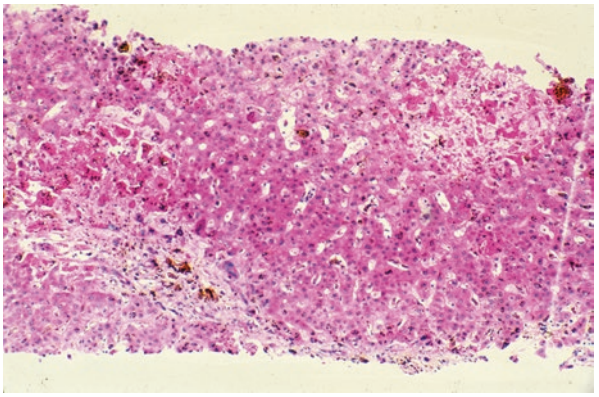

Fig. 16.7 Random foci of hepatocellular necrosis and involvement of bile ducts in a patient with viceral herpes zoster

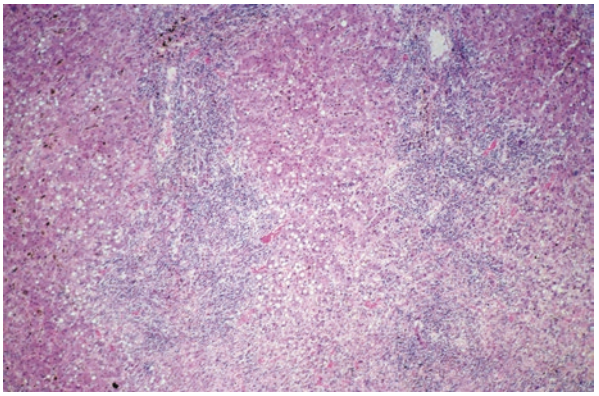

Fig. 16.8 EBV-driven post-transplant lymphoproliferative disease in the liver arising after T-cell depletion. Lower power view shows massively infiltrated portal spaces

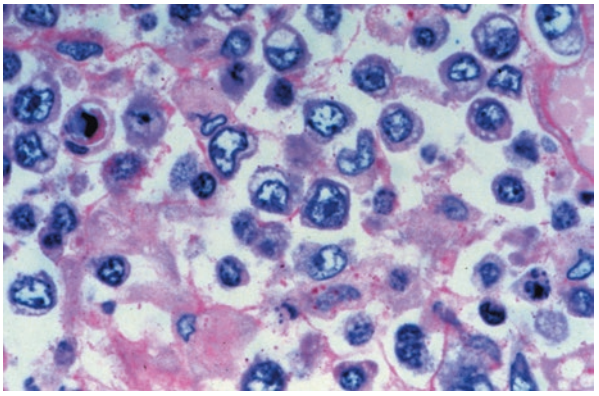

Fig. 16.9 High power view of the same biopsy as Fig. 16.8 shows the neoplastic cells with a plasmacytoid appearance

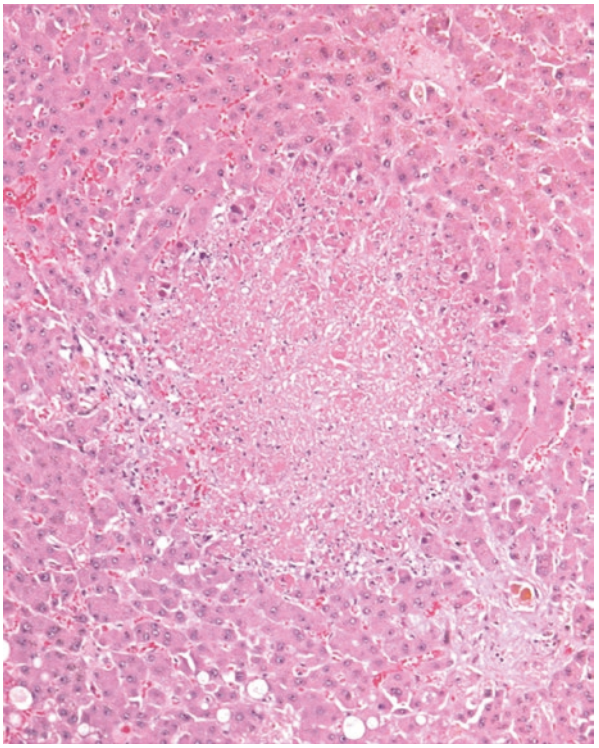

Fig. 16.10 Randomly punched-out necrotic focus from adenovirus in the liver

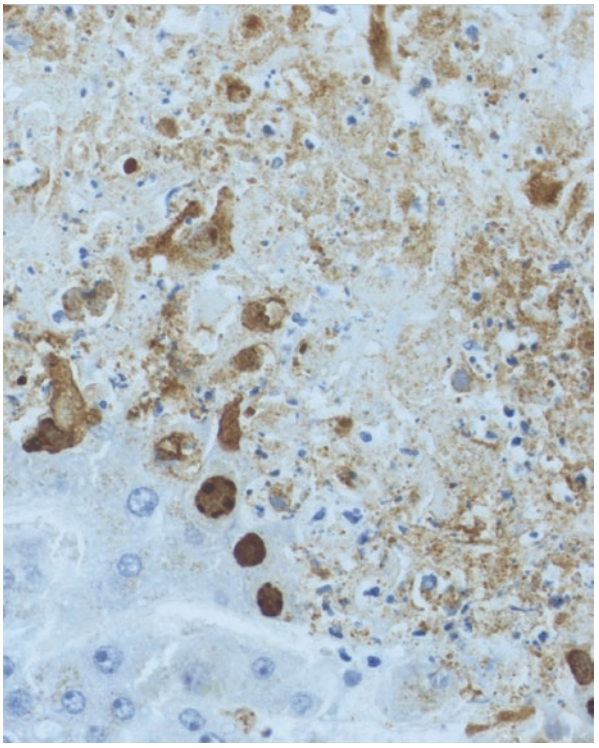

Fig. 16.11 IHC for adenovirus of the punched-out lesion shows many infected cells along the periphery

Teaching Points

- The acute hepatitic onset of liver GVHD presents with high aminotransferases following cessation or tapering of IS.
- The bilirubin rise may lag behind the marked transaminases. This is related to hepatocyte acidophilic body formation secondary to cytokine activation.
- Acute hepatitic onset may follow infusion of donor lymphocytes given to promote a graft-versus-leukemia effect.
- Histology of acute hepatitis onset shows a lobular hepatitis with interface inflammation with a necroinflammatory infiltrate that may include eosinophils and many plasma cells in the portal areas. Damaged bile duct changes may be more evident later, but portal inflammation may be reduced if IS treatment has been initiated.
- Differential diagnosis includes viral infections which must be ruled out. Antiviral treatment should be started even before there is positive confirmation.

Questions

1. What is the usual setting when the acute hepatitic onset of liver GVHD develops?
2. What is the differential diagnosis?
3. Why does the marked rise in aminotransferases occur before the appearance of histologic signs of bile duct damage and histologic changes?

Answers

1. Answer: Following tapering or cessation of IS
2. Answer: Viral infection caused by HBV, HCV, HSV, VZV, EBV, and adenovirus. Any potential hepatotoxic drugs should be stopped.
3. Answer: The initial rise in liver enzymes reflects the effect of IL-6 and IL-2 cytokines' nonspecific FAS-FASL interactions with hepatocytes, resulting in hepatocyte death and subsequent release of aminotransferases (the innocent bystander effect). The actual immunologic targets (the bile ducts) are later injured due to cellular attack.

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GVHD Manifesting as Sicca Syndrome

17

Cecilia C. S. Yeung and Howard M. Shulman

Clinical History

The patient was a 29-year-old man with a history of aplastic anemia who received an allogeneic BMT from his HLA-matched sibling donor. There was no acute GVHD (aGVHD), but the post-transplant course was complicated by *Pneumocystis jirovecii* pneumonia on day 65. On day 108, he was diagnosed with skin GVHD with a rash that initially developed as a sun burn. Physical exam on day 136 showed lichenoid skin changes, oral mucositis, and dry eyes with keratitis, indicating involvement of the oral mucosa and lacrimal glands. Schirmer's test had no tear formation which necessitated use of artificial tears. He also had anicteric liver dysfunction, with an elevated alkaline phosphatase (6–10x higher than normal). A liver biopsy on day 138 showed GVHD. He was treated with a variety of IS regimens including high-dose steroid therapy and several different regimens including combination of cyclophosphamide and azathioprine, with steroids. On day 186, 10 days following treatment with ATG, the skin biopsy showed destruction of the pilosebaceous glands and low-level residual GVHD activity in the follicular shaft. Imaging

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

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

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

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studies of the esophagus were obtained because of weight loss and difficulty in swallowing, which demonstrated a partially obstructive web in the upper third of his esophagus (see Chap. 12). An esophageal perforation occurred during the dilation procedure which was surgically repaired. Post-op he continued to suffer between bouts of infections requiring tapering of immunosuppression and flares of GVHD necessitating reinstitution of immunosuppression for skin, lacrimal gland, and liver GVHD. At his 1 year anniversary exam, the patient's skin had poikiloderma with atrophy and alternating hypo- and hyperpigmentation, loss of subcutaneous fat, and alopecia. By day 382, there was clinical improvement of his skin and liver GVHD (Fig. 17.1). A series of punch and elliptical skin biopsies demonstrated skin cGVHD with dermal sclerosis causing reduced range of motion including limited opening of his mouth. Confounding the cGVHD skin rashes, there were two different skin eruptions linked to drug reactions: first a vesicular eruption linked to Bactrim which was stopped after discontinuation of the drug and second at day 470 with a severe erythematous rash on the back shortly after erythromycin was given. He continued to have skin ulceration on his back and severe oral mucositis with marked discomfort and cachexia requiring hyperalimentation. Because of continued cGVHD-associated absolute xerophthalmia with exposure keratitis, he required wet contact lenses. The patient developed hearing loss and renal failure (creatinine 4.1), presumed to be related to use of topical nephrotoxic antibiotics. However, the urinary sediment contained cellular, granular, and red cell casts suspected to be from an immune complex disorder related to his cGVHD (see Chap. 19). He died suddenly from a septic shock on day 726. At autopsy, disseminated infection confirmed the cause of death. The ulcerated upper third of the esophagus was covered by a bacterial pseudomembrane. Small colonic ulcers were also overladen with bacteria. The

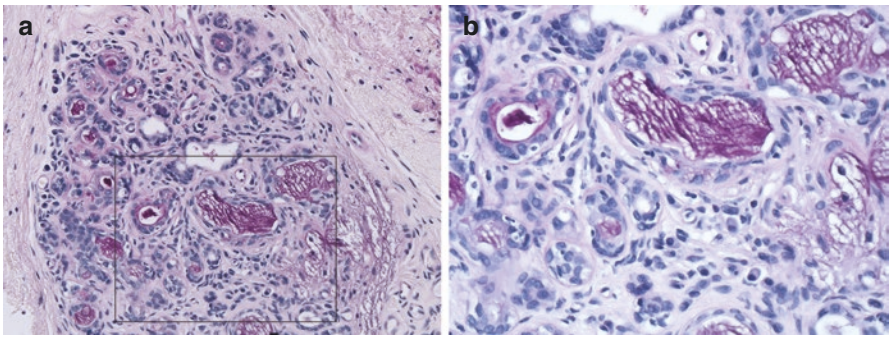

Fig. 17.1 Minor salivary gland from an oral labial biopsy on day 600. Panel A shows overall architectural disturbance with degeneration of acini and increased interstitial fibrous tissue. Panel B is a high-power view of the outlined region in panel A, demonstrating inflammatory infiltrate primarily characterized by lymphocytes and plasma cells, invading the dilated, irregularly shaped intralobular duct. A rare apoptotic cell within the wall of the duct indicates ongoing GVHD activity

lungs had bronchopheumonia and residual pneumocystis. The submucous glands of the large airways were fibrotic. In the liver 50% of the portal spaces lacked bile ducts. The major salivary glands were unremarkable.

Diagnosis

Extensive chronic GVHD with sicca syndrome.

Key Pathology Features

- Architectural disturbance with degeneration of acini
- Increased intervening fibrosis disrupting acini architecture
- Lymphoplasmacytic infiltration of the glands and ducts
- Dilated irregular-shaped intralobular ducts

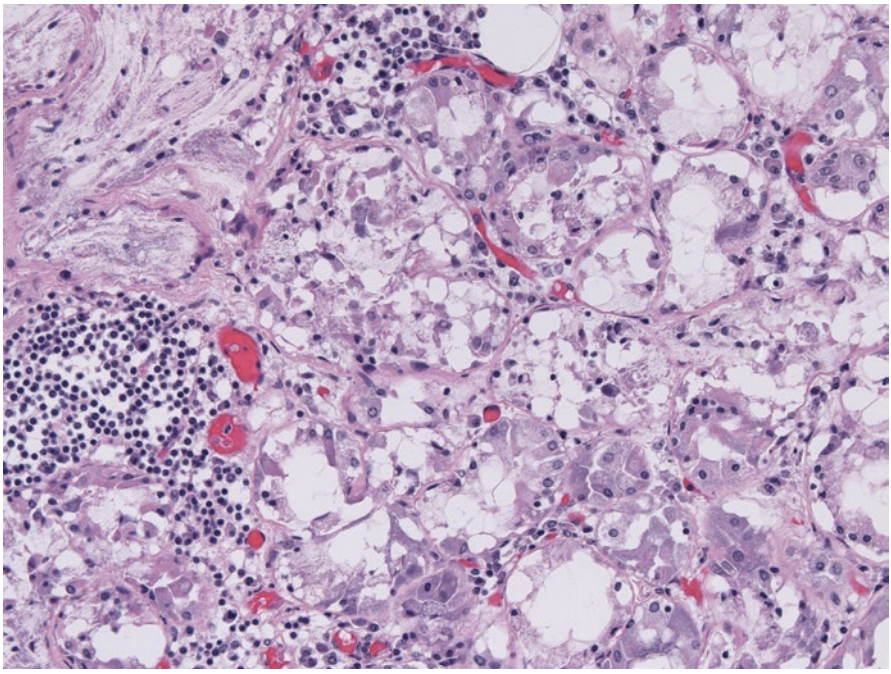

Fig. 17.2 This is the lip/salivary gland tissue from the autopsy of the same patient after immunosuppressive treatment. While there are still some inflammatory collections of lymphocytes and plasma cells at the periphery of the glands, there is regression of fibrous tissue and near-normal acini architecture without infiltrating lymphocytes or apoptotic cells

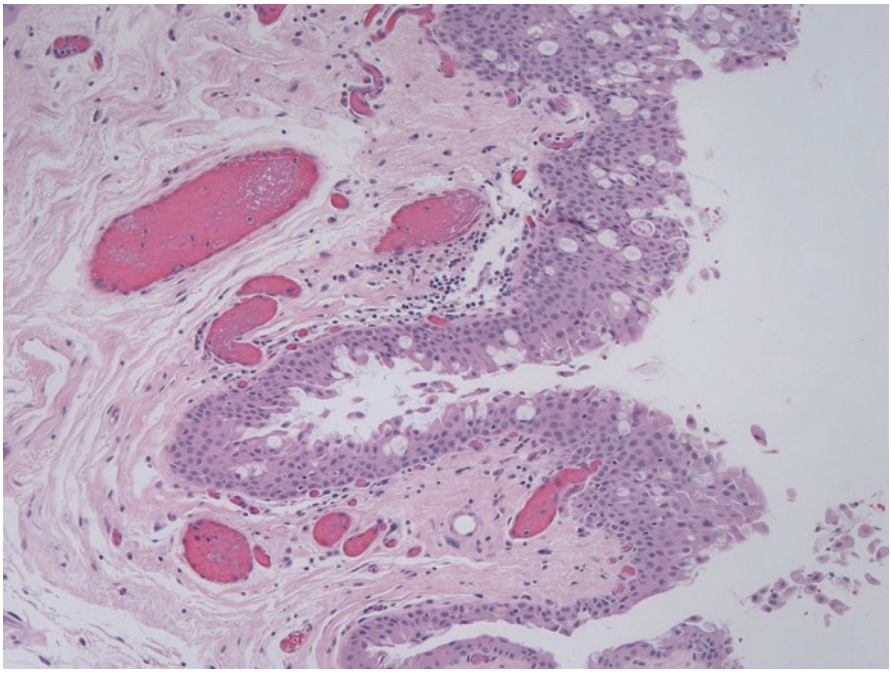

Fig. 17.3 This is a high-power view of the conjunctiva epithelium showing an area with preserved mucus cells on the left side of the image, adjacent to the right side of the image where there is loss of mucous cells and atrophy. Note the lymphocytic inflammation in association with the epithelium with preserved mucus cells and the reactive epithelium

Differential Discussion

The development of sicca syndrome post-transplant is a common occurrence with cGVHD. Sicca syndrome describes a condition where there is progressive immune attack on the tubuloalveolar glands in the salivary and lacrimal glands creating a clinical syndrome characterized by xeropthalmia (dry eyes) and xerostomia xeropthalmia (dry oral mucosa). CGVHD targets tubuloalveolar glands including those in the lacrimal glands, meibomian glands, salivary glands, peritracheal glands, and peribronchial glands.

Salivary Glands

Xerostomia has been reported in 40–70% of patients with cGVHD [1, 2]. When GVHD affects the salivary glands, typical clinical symptoms include reduction in saliva flow and alteration to the saliva composition. These changes result in compromised dental health, speech, taste, mastication, and swallowing, leading to increased

dental caries, infection, and malnutrition [3, 4]. Xerostomia commonly occurs concurrently with xerophthalmia, previously reported in 77% of patients with xerophthalmia [3].

Diagnosis of sicca syndrome may include oral labial biopsy of the minor salivary glands. Care should be taken to include enough individual glands (current NIH consensus recommendations are ≥10 [15]) for sufficient analysis of ducts or glandular acini in order to differentiate between previously damaged glandular tissue and active disease: active GVHD can have variable distribution or show only focal minimal changes. Atrophy of the glands, fibrosis, and an inflammatory infiltrate predominated by lymphocytes and plasma cells are associated with salivary gland dysfunction in patients with oral cGVHD [3] (Figs. 17.4 and 17.5).

Alborghetti et al. conducted a study evaluating serial biopsies of the minor salivary gland of patients with cGVHD. They demonstrated that persistent xerostomia after cGVHD therapy is very common (all patients at the end of their study) [5]. They further related the persistence of the disease to continued intense lymphocytic inflammation and more fibrosis with absence of recovery or destruction of minor salivary secretory units [5]. Prior studies have shown that when cGVHD involved the minor salivary glands, salivary dysfunction did not correlate with GVHD involvement of the oral mucosa [3]. Quantitative proteomic analysis by tandem mass spectrophotometry of saliva has demonstrated altered protein expression profiles in patients with active oral chronic GVHD [6]. Saliva protein expression studies via ELISA immunoassays for decreased IL-1 antagonist receptor and cystatin B

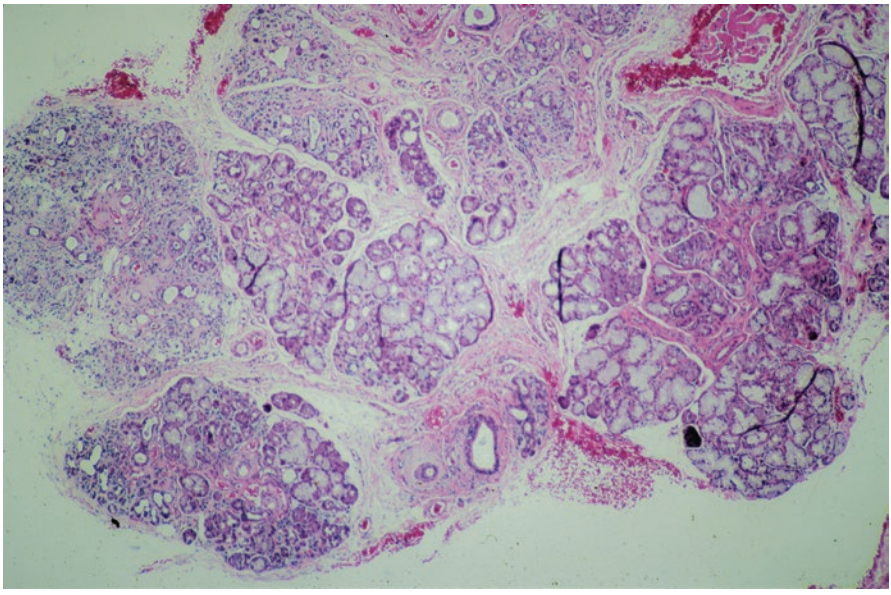

Fig. 17.4 Low power image of the minor salivary glands in a lip biopsy demonstrating overall architectural disturbance local destrucion of acinar lobules with increased intervening fibrosis and patchy areas with increased lymphocytes

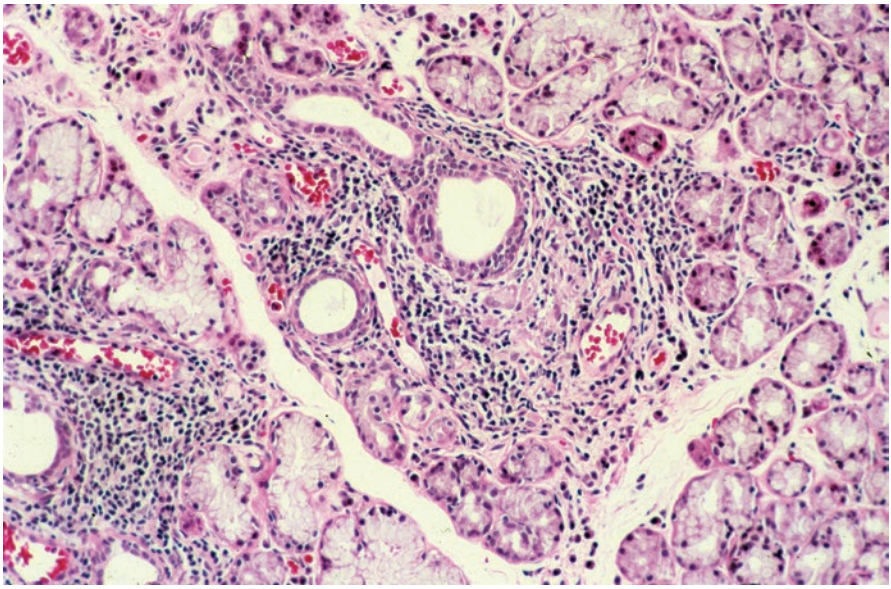

Fig. 17.5 High power image demonstrating lymphocytic infiltration of glands and ducts with mild fibrosis

have been used to distinguish patients with active oral cGVHD with a reported sensitivity of 85% and specificity of 73% [6].

Conjunctival and Lacrimal Glands

Xerophthalmia is seen in 30–85% of patients with cGVHD [7]. There is reduced tear production and flow, which can be confirmed by a Schirmer test. The Schirmer test measures basic tear production by placing a piece of filter paper into the conjunctival fold for 5 minutes and measuring the length of wetting of the paper strip (>10mm is considered normal). Keratoconjunctivitis secondary to xerophthalmia includes a number of differentials including steroid therapy, severe infections, toxicity from preparative regimens, and chemotherapy in addition to GVHD.

Histological findings of ocular GVHD of the conjunctiva and lacrimal glands include prominent fibrosis, infiltration of lymphocytes and plasma cells around medium-sized ducts, and loss of cells in the lobules [8–12]. Alterations in the lacrimal gland acinar tissue resemble those in minor salivary glands with fibrosis, glandular atrophy, and inflammation. However lacrimal gland biopsy is invasive and may impair function. In contrast, a conjunctival biopsy may be obtained with little risk. In cases where the diagnosis of ocular GVHD is in question, evaluation of conjunctiva histology may aid in the diagnosis and management of ocular GVHD in symptomatic patients with conjunctival disease who have normal or unchanged Schirmer's test with or without GVHD of other organs [10, 13, 14]. Histological features of conjunctival GVHD include vacuolization of the basal epithelium,

lymphocyte predominant inflammation, satellitosis, and apoptosis of epithelial cells [10, 13, 14]. Nonspecific features that can be seen but are not sufficient for the diagnosis of ocular GVHD include epithelial attenuation and goblet cell depletion [12] (Fig. 17.5). When indicated, conjunctival specimens may also be tested for viral involvement.

Teaching Points

- Xerophthalmia and xerostomia are common in patients with cGVHD.
- Xerostomia is not specific for oral mucosal GVHD. Other etiolgies include chemoirradiation, inflammation, and infection.
- Atrophy of the glands, fibrosis, and an inflammatory infiltrate predominated by lymphocytes and plasma cells are features of salivary gland and lacrimal gland chronic GVHD.
- A Schirmer test measures basic tear production and can be a screen for sicca syndrome.
- Conjunctiva biopsy may be more accessible than a lacrimal gland biopsy.

Questions

1. True or false: Sicca syndrome is uncommon in patients who have received an allogeneic HSCT.
2. Histologic features of conjunctival GVHD would not include which of the following:
   A. Vacuolization of the basal epithelium
   B. Lymphocyte predominant inflammation with satellitosis
   C. Apoptotsis of epithelial cells
   D. Goblet cell hyperplasia
   E. None of the above
3. A 65-year-old patient with long-standing cGVHD from an allo-HSCT 4 years prior complains of severe dry mouth. Which of the following features on microscopic examination will help confirm the diagnosis?
   A. A small biopsy with five evaluable glands featuring acute inflammation
   B. A biopsy demonstrating atrophy of the glands, fibrosis, and an inflammatory infiltrate predominated by lymphocytes and plasma cells
   C. A biopsy demonstrating obliteration of glands by a lymphoplasmacytic infiltration and fibrosis, occasional eosinophils, and elevated IgG4
   D. A biopsy demonstrating obliteration of the glands by a prominent lymphoid proliferation

Answers

1. Answer true or false: False. Sicca syndrome is common in allogeneic HSCT patients. Up to 70% of patients with cGVHD report xerostomia, and up to 85% of patients with cGVHD report xerophthalmia.

2. Answer: D. Conjunctival GVHD will result in loss of goblet cells.
3. Answer: B. A describes a biopsy that is too small to adequately evaluate for GVHD in the salivary gland, C describes a case of chronic sclerosing sialadenitis, and D describes a case of MALT lymphoma involving the salivary gland.

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Noninfectious Pulmonary Manifestation of GVHD: Bronchiolitis Obliterans Syndrome

18

Cecilia C. S. Yeung, Sahl Ali, and Howard M. Shulman

Clinical History

A 63-year-old man received a PBSCT from an HLA-matched sibling donor for acute myelogenous leukemia (FAB M2) in persistent relapse. Approximately 2 months post-transplant, biopsies confirmed GVHD in the skin and upper GI. The GVHD was treated with cyclosporine and high-dose prednisone which was tapered in 2 months. Approximately 3.5 months post-transplant, a lower endoscopy with biopsy noted zygomycete infection of the colon. Subsequent workup also noted splenic abscesses believed to have been caused by the fungal infection. The patient was treated with itraconazole and amphotericin B and placed on TPN for chronic malnutrition and hypoalbuminemia. Due to increased dyspnea upon exertion, at day 140 chest CT was performed which noted bilateral

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

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

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

S. Ali
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

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pulmonary opacities as well as pleural effusions. Blood cultures remained negative throughout. Pulmonary function testing (PFT) performed on day 144 showed a restrictive pattern with decreasing DLCO. Subsequent chest CTs showed increased opacities, pleural effusions, lymphadenopathy, and atelectasis. BAL contained no infectious organisms or other abnormalities. Video-assisted thoracoscopic surgery (VATS) was performed on day 169 with a lung biopsy confirming the diagnosis of cryptogenic organizing pneumonia (COP), formerly called bronchiolitis obliterans with organizing pneumonia (BOOP). This pathologic finding combined with the restrictive pattern PFT leads a clinicopathologic diagnosis to be consistent with pulmonary GVHD. He was treated with Solu-Medrol 2 mg/kg and an array of Levaquin, Zosyn, and Vancomycin while remaining on amphotericin B antifungal medication. On day 170 he developed acute renal failure and hyperbilirubinemia, so the amphotericin B was held. The patient also developed fever and septic shock in association to the infection and was intubated on day 172 for increased respiratory rate and decreased oxygenation ability. The patient passed away on day 173 from pulmonary decompensation. An autopsy was performed.

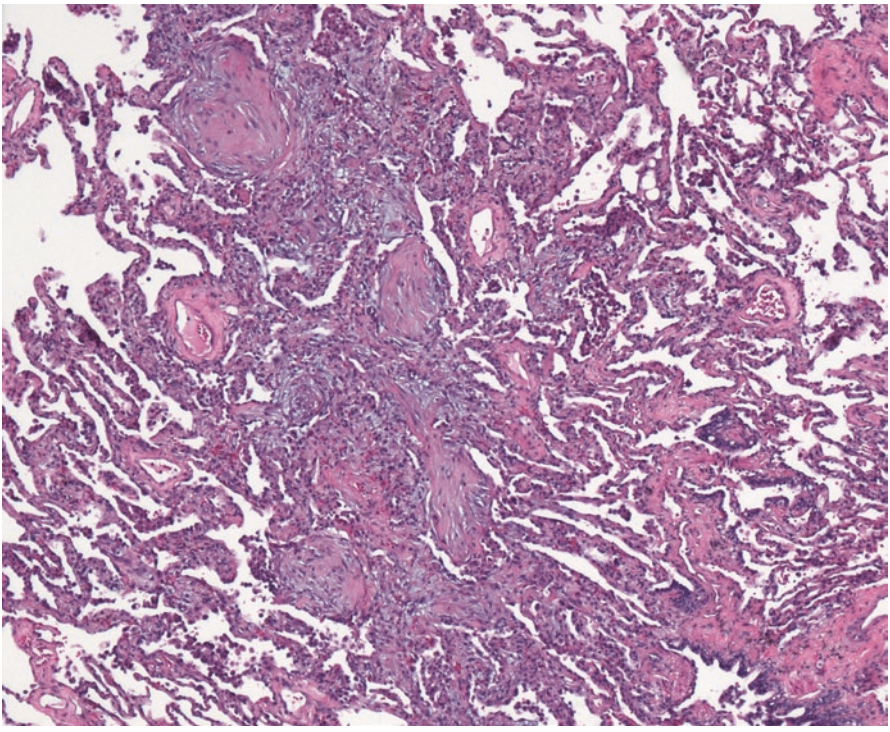

Fig. 18.1 Lung biopsy from day 169 demonstrating a segment of lung with a focal area of consolidation, in which there are numerous fibrous, onion skin-like Mason bodies obstructing the bronchioles and alveolar ducts

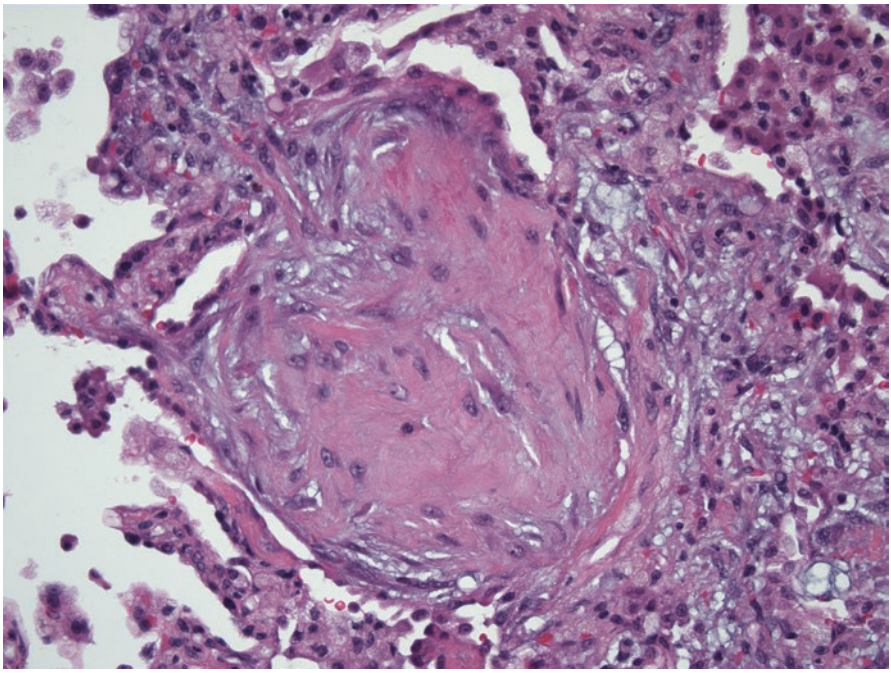

Fig. 18.2 A higher-power image of the same day 169 lung biopsy as in Fig. 18.1 shows a fibrotic foci obliterating a small airway

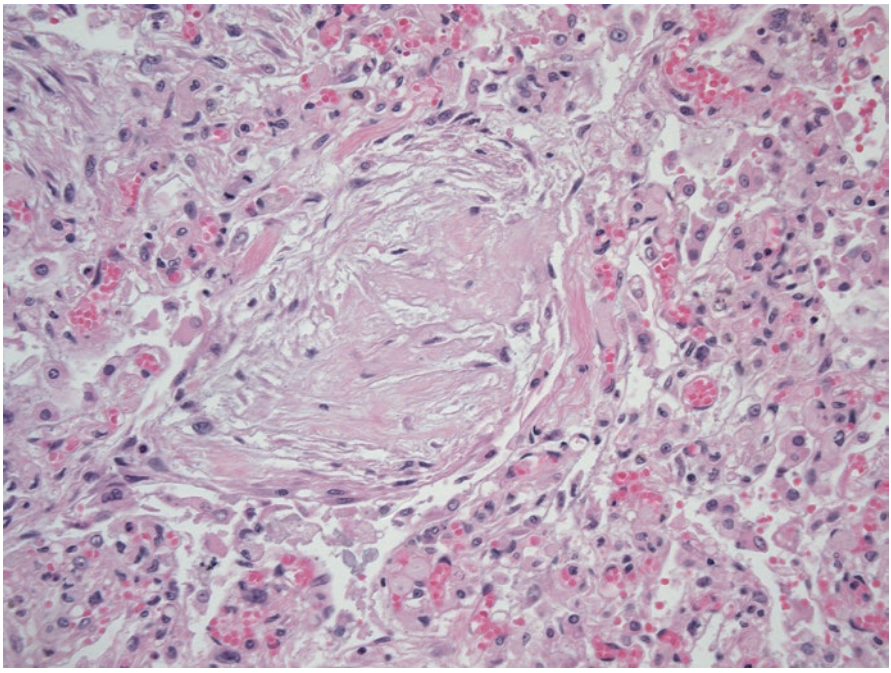

Fig. 18.3 This is a high-powered image of an obliterated small airway within a background of acute pneumonia of our patient at autopsy

Diagnosis

Cryptogenic organizing pneumonia (COP) with restrictive PFTs consistent with pulmonary graft-versus-host disease in the lung biopsy with acute organizing pneumonia superimposed at autopsy

Key Pathology Features

- The distribution of and degree of changes in obstructed bronchioles are correlated with the pulmonary function studies (PFTs).
- COP is characterized by patchy nodular consolidation which may be adjacent to uninvolved lung parenchyma. The consolidation foci consist of granulation tissue plugs that fill the lumens of the distal airways in a patchy distribution, extending into the alveolar ducts and alveolar sacs, and are associated with chronic interstitial inflammation.
- Lymphocytic bronchiolitis (LLB) describes chronic inflammation surrounding and infiltrating small bronchi and bronchioles.
- Constructive bronchiolitis obliterans (CBO) is characterized by dense fibrosis within the lumen of small bronchioles.

Differential Discussion

Our patient had a complex set of pulmonary findings including PFTs which showed a restrictive pattern and biopsy that showed COP, with CT studies which showed opacities, pleural effusions, and other findings concerning for an evolving infection. At autopsy, additional pulmonary findings were acute organizing pneumonia with zygomycete infection. We felt this case was a good example of complex borderline histopathology; when taken into consideration with the clinical context and additional PFTs, the diagnosis of pulmonary GVHD could be made. Pulmonary complications following HSCT can generally be categorized as infectious, noninfectious, or some combination of both etiologies. Bronchiolitis obliterans syndrome (BOS) encompasses the noninfectious clinical manifestations of pulmonary cGVHD which develop within several months to 2 years post-HSCT. In the NIH 2015 revised histopathologic diagnosis of GVHD, pathologic features of both LLB and CBO satisfy the criteria for diagnosis of BOS [1]. The 2015 NIH clinical criteria define BOS as an obstructive pulmonary disorder defined by PFTs of an FEV1/VC of <70% [2]. Based on the NIH clinical criteria, BOS is noted in 14% of patients with cGVHD. The mortality rates for BOS range from 25 to 50% (Table 18.1).

The histopathologic findings in CBO are classified as major diagnostic feature of cGVHD. The findings resemble those after rejection of a lung allograft. Other entities resembling CBO are systemic Castleman's disease, post-infectious scarring, chronic severe esophageal reflux, and toxic fume exposure. The characteristic findings in CBO

Table 18.1 Terms, acronyms, and definitions of the spectrum of histologic legions associated with pulmonary manifestations of acute and chronic GVHD

Terminology	Definition
Bronchiolitis obliterans syndrome (BOS)	The clinical manifestation from transplantation (lung or HSCT) where there is CBO or LLB.
Constrictive bronchiolitis obliterans (CBO)	A fibroproliferative process where there is progressive narrowing and eventual fibrous obliteration with loss of small airways. CBO is the late stage of BOS.
Lymphocytic bronchiolitis (LLB)	An early process in BOS where small airways are inflamed by a lymphocyte predominant infiltrate. It is a chronic inflammatory process that surround and infiltrate small bronchi and bronchioles. LLB can also be caused by a viral infection.
Pulmonary veno-occlusive disease (PVOD)	A rare manifestation of GVHD where intimal fibrosis narrows and occludes pulmonary veins of various sizes. Diagnosis requires combined clinical and radiographic evidence.
Restrictive lung disease (RLD)	A group of diseases characterized by increasing fibrosis on imaging studies and pleural pulmonary fibrosis. These are not currently considered diagnostic or distinctive of pulmonary cGVHD, although they encompass entities such as cryptogenic organizing pneumonia (COP) that may be associated with pulmonary GVHD.
Cryptogenic organizing pneumonia (COP)	Formerly known as bronchiolitis obliterans with organizing pneumonia (BOOP), this is an inflammatory process of the alveolar ducts, interstitium, and small bronchioles characterized by fibroblastic proliferation in the lumen of small airways. This process has been associated bacterial pneumonia, but often the inciting event is unknown.

are chronic inflammation with eventual fibrous obliteration of small bronchioles (Figs. 18.3 and 18.4). Special connective tissue stains, VVG, and trichrome allow distinction from pulmonary arteries and highlight the smooth muscle layer which surrounds bronchioles. Later secondary changes include distal mucostasis, aggregates of macrophages, and the late development of bronchiectasis. Early changes of BOS include LLB, whose major histologic features include small airways with subepithelial fibroproliferation and varying degrees of lymphocytic inflammation (Fig. 18.5). LLB has a multifactorial etiology including viral infections and hypersensitivity pneumonitis.

Workup of pulmonary dysfunction in the early post-transplant period must include microbiology studies, PFTs, X-rays, and CTs, which are necessary for identifying the presence of lobar, multilobar, or diffuse pulmonary infiltrates. A lung biopsy is only necessary when there are pulmonary symptoms suspicious for BOS and no other evidence of cGVHD can be diagnosed at other sites. A new diagnostic technique for BOS is currently under investigation, termed parametric response mapping. This technique involves a high-resolution (helical) CT of inspiration with a CT of expiration encouraged. This technique permits visual representation of the lung affected by BOS versus lung tissue with normal aeration or restrictive disease and may be a valuable noninvasive diagnostic tool in the future [3].

Williams describes the management of BOS and the variations in PFT profiles [4]. The pathologic spectrum of lesions encompassed within BOS is shown in the images from our index case and discussion images (Figs. 18.4, 18.5, and 18.6).

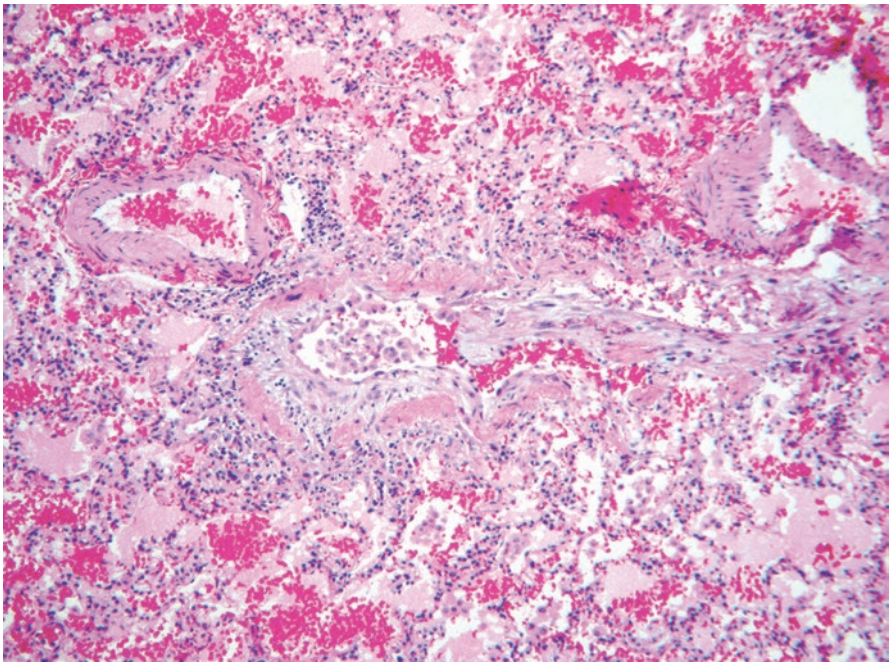

Fig. 18.4 This image demonstrates an evolving stage of BOS with incomplete obliteration of the airway. There is edema and lymphocytic infiltration beneath the ulcerated bronchiole epithelium. Image courtesy of Dr. Robert Hackman

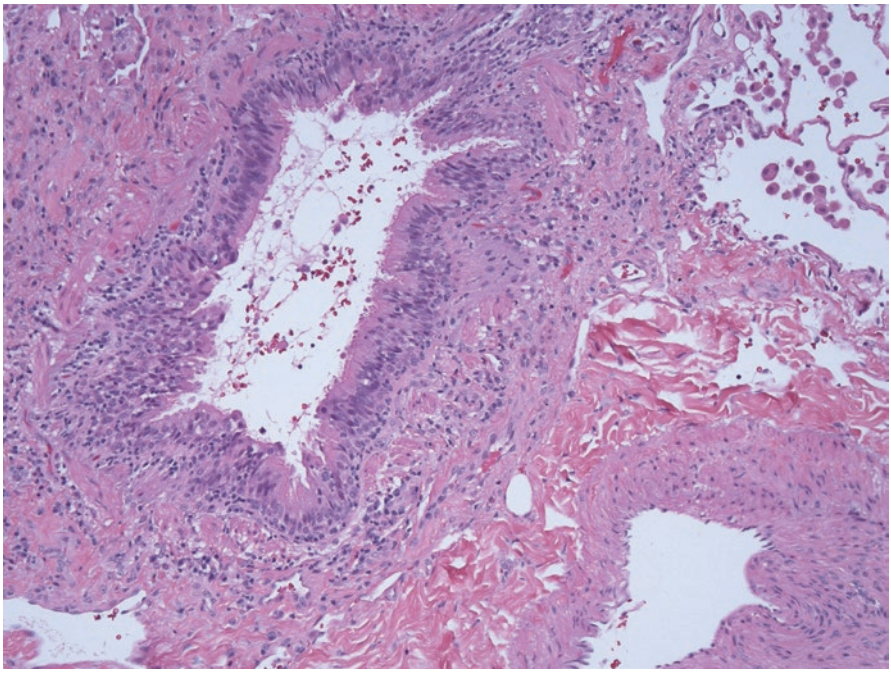

Fig. 18.5 This is an image of lymphocytic bronchiolitis featuring an airway with lymphocytic infiltration surrounding and infiltrating the bronchiole wall

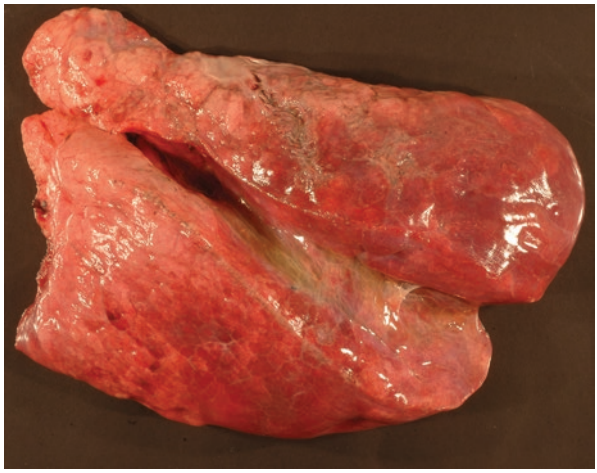

Fig. 18.6 This is a gross photo of a lung from an 18-year-old patient post-HLA-matched allo-HSCT with surface adhesions, widespread fibrosis, and inflammation who died of acute bronchopneumonia

They reflect the immune-mediated injury to small airways leading to fibrotic occlusion and obliteration. Both Chien et al. and Hildebrant et al. have found that genetic variation in the innate immune pathway influences the risk of developing CBO [5, 6]. Three studies have found that clinical syndrome of BOS included both LLB and CBO, which follow a final common pathway in the development ofsmall airway obstruction [7–10]. Gazourian has proposed that the unifying features may be PFT airflow disturbances [11]. The distinction between these entities has clinical relevance; patients with LLB had improved survival and response to treatment in comparison to patients with CBO [7].

The relationship of prior large airway inflammation, lymphocytic bronchitis (LB), to CBO has been studied. Greenland et al. evaluated endoscopic bronchial biopsies in lung allografts. Their findings suggested that LB in larger airways can predict the subsequent development of CBO (BOS) [12]. A 1978 study by Beschorner et al. done in the early era of HSCT found that LB was associated with the onset of aGVHD which in turn led to the development of bronchopneumonia caused by damage to the bronchial mucociliary apparatus [13]. O'Brien et al. conducted a large canine study of LB in allografted, autografted, and non-transplanted control dogs. They did not find any association between LB, aGVHD, or acute pneumonia and concluded that LB represents a nonspecific inflammation rather than a manifestation of pulmonary GVHD [14]. In summary, there is no clear evidence that LB is a step in the final common pathway leading to CBO.

Tracheobronchomalacia describes a rare clinical disease characterized by weakness of the trachea and bronchi due to softened supporting cartilage and hypotonic myoelastic fibers of the trachea and bronchus. It has been described after allo-HSCT as a potential mimic of BOS with similar abnormal PFTs to suggest obstructive airway disease [15].

Progressive restrictive lung disease (RLD) is manifested by increasing fibrosis on imaging studies and pleural pulmonary fibrosis. Though not currently considered diagnostic or distinctive of lung cGVHD, they are a topic of active investigation. The PFTs show a decrease in forced vital capacity (FVC) in conjunction with a lesser decline in FEV_1, reduction in total lung capacity, and decrease in diffusion

capacity for carbon monoxide. The most common RLD is COP. Clinical features of the disease include dyspnea, dry cough, shortness of breath, and rales. Imaging studies show diffuse peripheral fluffy infiltrates consistent with airspace consolidation. Histologically, COP displays patchy nodular consolidation with inflammation of bronchioles and surrounding lung tissue. A characteristic feature is plugs of fibroblasts filling alveolar ducts with an onion skin-like appearance, so-called Mason bodies (Figs. 18.1 and 18.7). COP has a strong statistical association with acute and chronic GVHD [16]. Key diagnostic features of COP include patchy fibrosis, granulation tissue within alveolar spaces, alveolar ducts, respiratory bronchioles, and absence of infectious organisms. However, in recent years due to better treatments and alternative testing strategies, the use of lung biopsies in the post-transplant setting is declining [17]. Differentiation between obstructive and restrictive lung diseases is important as CBO and COP differ in response to therapy. COP is quite responsive to corticosteroids and can resolve spontaneously, whereas CBO will not. Another differentiating characteristic is that RLD often has an earlier onset within the first 3 months, whereas obstructive lung disease will have later onset in 3–12 months.

Pulmonary veno-occlusive disease (PVOD) is a rare manifestation of GVHD featuring intimal fibrosis that narrows and occludes pulmonary veins of various sizes. Clinical diagnosis of PVOD requires radiographic evidence of pulmonary

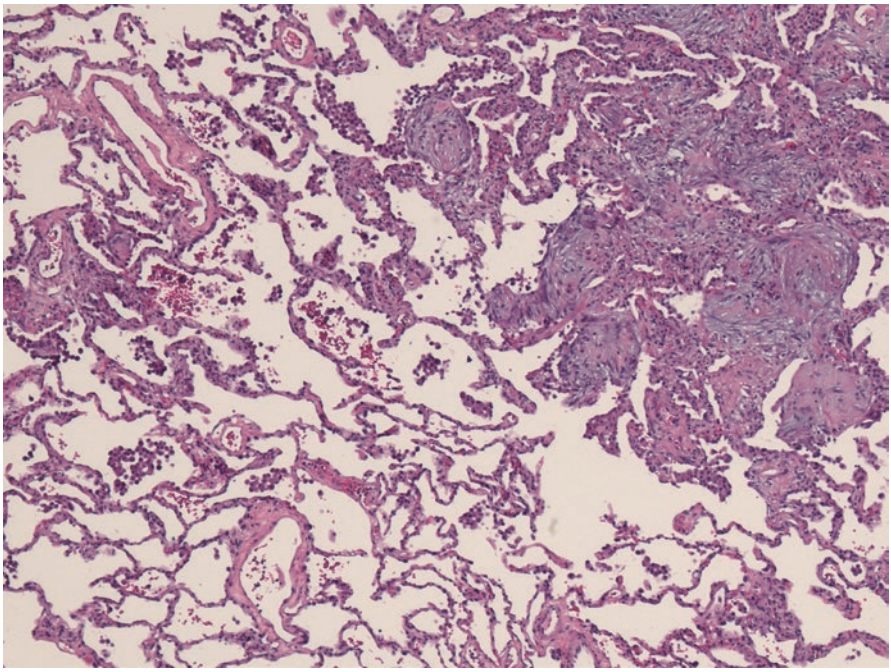

Fig. 18.7 This is an image of COP at low power. Note the patchy distribution with relatively normal alveoli adjacent to a segment of inflamed bronchioles with thickened walls

edema and a normal pulmonary artery wedge pressure. A study by Gazourian et al. found a spectrum of pulmonary pathologies in the lungs from 35 patients who survived at least 1 year after HSCT (80% had cGVHD). BOS was seen in 10 patients (including some who were asymptomatic), but PVOD was seen in 12 patients indicating PVOD may be more under-recognized in post-HSCT patients than previously anticipated [9].

Infections are a particular concern for immunocompromised patients in the HSCT setting who are often neutropenic and/or on IS treatment for GVHD, making these patients especially susceptible to a wide variety of infections. Bronchoscopy with bronchoalveolar lavage (BAL) fluid analysis can identify many infectious processes when complemented by a full panel of diagnostic testing such as bacterial and fungal cytologic stains, PCR, shell vial cultures, etc. The galactomannan test on serum or BAL fluid detects a heteropolysaccharide antigen that suggests or strongly points to invasive aspergillus [18] and may also detect other fungi. The panel of microbiology studies should include opportunistic organisms as well as unusual pathogens as immunocompromised patients are at increased risk for a wide variety of infectious agents [19–23]. Histopathology examination of the BAL cellular and background composition also provides critical information for other differentials commonly considered in post-HSCT patients including diffuse alveolar hemorrhage, pulmonary alveolar proteinosis, hypersensitivity pneumonitis, aspiration pneumonia, and involvement by relapse/persistent disease. Pulmonary alveolar proteinosis has a characteristic "milky" appearance in the BAL fluid due to high concentrations of surfactant, proteins, and lipids [24]. Microscopic examination of cytospin preparations will reveal many proteinaceous acellular fragments of densely PAS-positive material which is diastase resistant [25] (Fig. 18.8). Hypersensitivity pneumonitis is characterized by increased numbers of small lymphocytes in the BAL cellular composition, although mast cells, plasma cells, eosinophils, neutrophils, and rarely granulomas may also be seen [26].

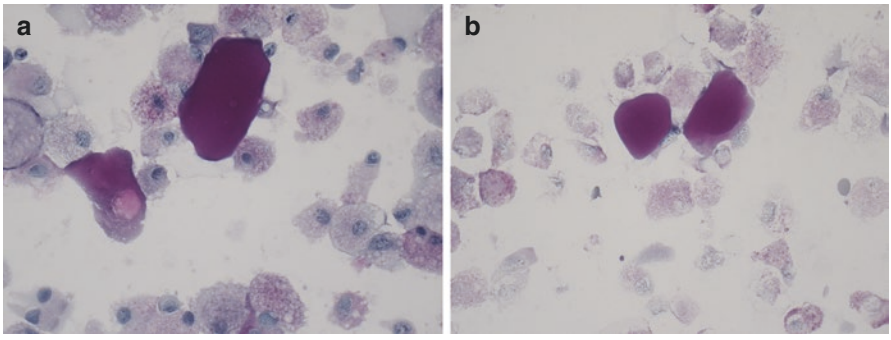

Fig. 18.8 Cytospin preparations are from a bronchoalveolar lavage from a patient with pulmonary alveolar proteinosis. Slide A contains two PAS-positive dense acellular lipoproteinaceous concretions. In slide B, similar dense concretions stained with PAS-diastase, have retained their positive staining

Teaching Points

- Bronchiolitis obliterans syndromes encompass LLB and CBO.
- LLB is considered a precursor lesion to CBO though the same changes may occur after viral bronchiolitis.
- CBO, the end stage of BOS, is characterized by dense fibrosis within the lumina of small bronchioles. These changes are best identified with VVG and trichrome stains which identify the outer smooth muscle layer surrounding the obstructed bronchiole.
- Lung biopsy is only indicated when there are abnormal obstructive pulmonary PFTs without other evidence of cGVHD in other sites.
- The diagnosis of BOS is based on PFT and imaging studies in a patient with other stigmata of cGVHD.
- Bronchoscopy with BAL is used to rule out infection.
- Differences in the PFTs, histology, and response to therapy distinguish obstructive from restrictive lung disorders. Restrictive lung disease presents earlier, often within the first 3 months, whereas obstructive lung disease presents later, between 3 months to two years post-transplant. COP is responsive to corticosteroids and can resolve sponteneously, whereas CBO is variable in its responsiveness to treatment [4].

Questions

1. Which entities produce symptoms and PFTs resembling BOS?
   A. Viral bronchiolitis
   B. Post-infectious scaring
   C. Tracheobronchomalacia
   D. Inhalation injury from toxic fumes
   E. All of the above
2. What is the main goal of treatment for lung injury in BOS?
   A. Kill infectious agent
   B. Reverse damages to obliterated airways
   C. Preserve lung function, decrease immune attack
   D. Prevent pulmonary hemorrhage
3. What tests should be performed on lung biopsy tissues?
   A. Cultures for bacteria on fresh tissue
   B. PCR for viruses including metapneumovirus in special media
   C. Special stains for bacteria including legionella with a modified Gimenez stain
   D. Special stains for VVF and trichrome stains to highlight the small bronchioles and any intraluminal fibrosis
   E. All of the above

Answers

1. Answer: E
2. Answer: C, since the changes caused by disease are often irreversible, the goal of treatment is to preserve whatever lung function that patient has left by decreasing the immune response and providing oxygen support.
3. Answer: E

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Kidney Involvement in GVHD

19

Abbie Ruth Bauer, Laura S. Finn,
and Sangeeta R. Hingorani

Clinical History

The patient is an 11-year-old boy with a past medical history of AML s/p COG AAML1031 protocol which included treatment with sorafenib, cytarabine, and etoposide. He underwent peripheral blood stem cell transplant due to relapse of his primary disease. His post-transplant course was complicated by an episode of acute kidney injury (AKI), mucositis, gut and skin GVHD, and multiple infections including aspergillosis, *Clostridium difficile*, and adenovirus viremia. He recovered

A. R. Bauer
Division of Nephrology, Seattle Children's Hospital, Seattle, WA, USA

Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA
e-mail: abbie.bauer@seattlechildrens.org

L. S. Finn
Department of Laboratories, Seattle Children's Hospital, Seattle, WA, USA

Department of Pathology, University of Washington School of Medicine,
Seattle, WA, USA
e-mail: laura.finn@seattlechildrens.org

S. R. Hingorani (✉)
Division of Nephrology, Seattle Children's Hospital, Seattle, WA, USA

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

Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
e-mail: sangeeta.hingorani@seattlechildrens.org

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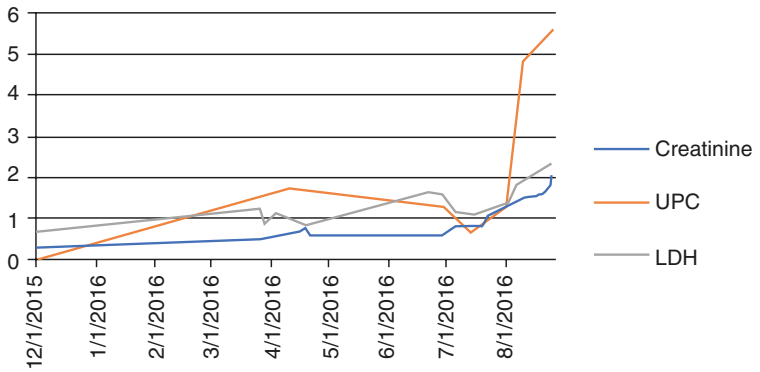

Fig. 19.1 Kidney Function tests. Soluble C5b9, CH50, and AH50 were within normal ranges

without complication and had successful engraftment 19 days after transplant. A few months after engraftment, he began to develop progressive edema and hypertension that was difficult to control despite the use of amlodipine, carvedilol, and lisinopril. He was eventually admitted roughly 6 months after transplantation due to headache and continued difficulty with blood pressure control. At that time, he was found to have significant proteinuria. He was discharged home after blood pressure was better controlled with close follow-up to monitor his blood pressure and proteinuria. In the outpatient setting, he continued to struggle with worsening proteinuria, rising creatinine, and an elevated LDH (Fig. 19.1). He developed significant abdominal pain and diarrhea of unclear etiology. Given the clinical picture and laboratory findings, a kidney biopsy was performed.

Pathology Images and Relevant Laboratory Values

Pathology: Step sections of the renal biopsy stained with H&E, PAS, Jones silver, and Trichrome stains included one core comprised predominantly of medulla and a second core that was entirely cortex. The sampled kidney contained up to 45 glomeruli which displayed a range of thrombotic microangiopathic changes including dilated blood filled capillaries with focal thrombus formation; mesangiolysis; bloodless glomeruli with fibrillary mesangial expansion; and narrowed peripheral capillary lumens due to endothelial swelling and thickened walls with basement membrane duplication, highlighted by PAS and silver stains. Occasional fragmented erythrocytes were seen and rare glomerular arterioles contained fibrin thrombi. Larger vessels were unremarkable (Figs. 19.2, 19.3, and 19.4). About half of the sampled cortex had interstitial fibrosis associated with tubular atrophy; protein casts expanded some tubules. The epithelium lining a subset of intact tubules was vacuolated and foamy, consistent with acute injury. Regenerative tubular epithelial changes were characterized by nuclear enlargement and pleomorphism but frank viral inclusions were not present.

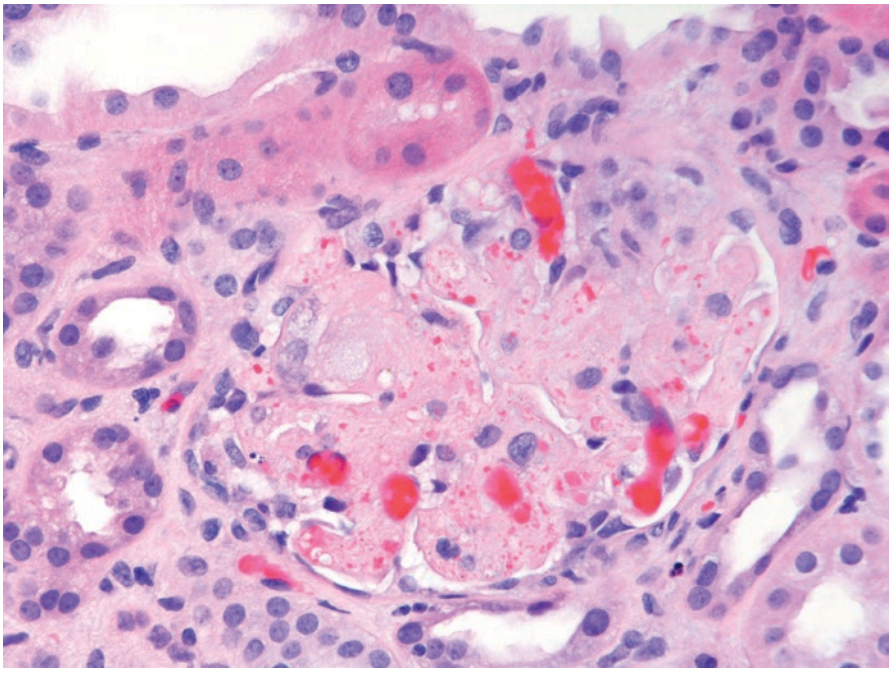

Fig. 19.2 Most lumens of this hypocellular glomerulus are occluded by markedly swollen endothelial cells, thickened capillary walls, and fibrillary mesangium with few foam cells that extend into the capillaries (H&E)

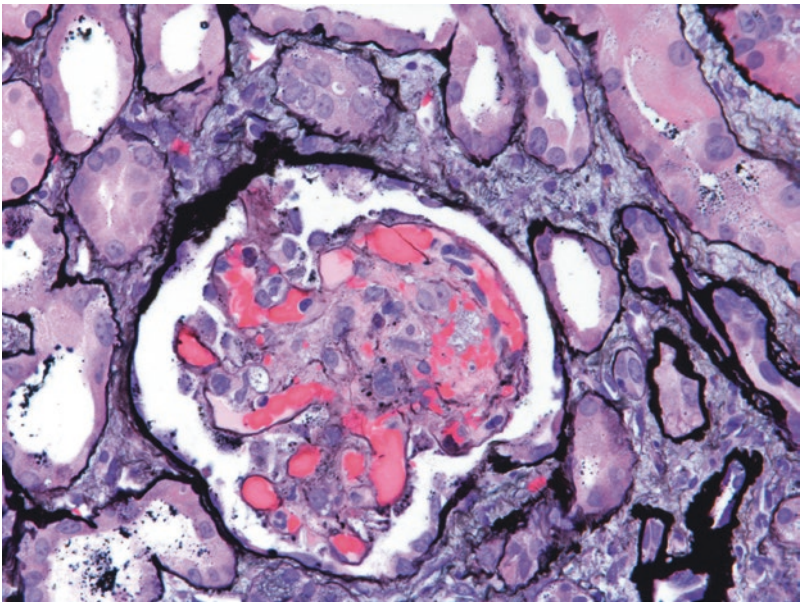

Fig. 19.3 Mesangiolysis is associated with aneurysmal dilation of the glomerular capillaries which are filled with blood and fibrin. Rare inflammatory cells are noted in the capillary lumens (Jones methenamine silver)

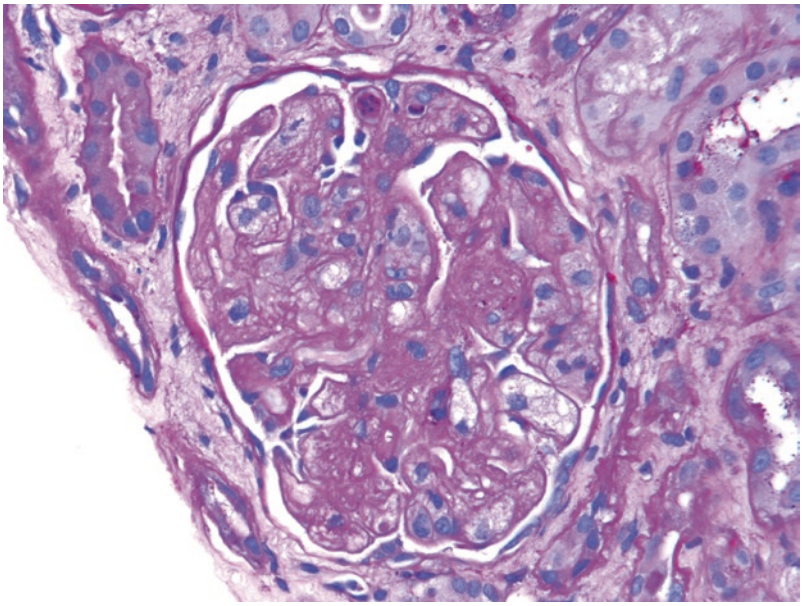

Fig. 19.4 The glomerulus has swollen endothelium, segmental membrane duplication, and expanded fibrillary mesangium with foam cells, all of which contribute to obstruction of the capillary lumens (PAS)

Diagnosis

Our patient was diagnosed with thrombotic microangiopathy (TMA) based on his pathology. He was quickly transitioned from tacrolimus to sirolimus in an attempt to halt the progression of his disease. A gut biopsy was obtained 10.5 months post-transplant which showed mild active GVHD and focal mucosal hemorrage suggestive of TMA. Unfortunately, he continued to have worsening proteinuria (UPC max of 4.64), hypertension, and declining kidney function. Over the subsequent 2 months, he had progressive loss of kidney function and eventually developed end-stage kidney disease despite increasing steroids and an 8-week course of eculizumab. He transitioned from continuous renal replacement therapy (CRRT) to hemodialysis and is now maintained on peritoneal dialysis.

Key Pathology Features and Relevant Laboratory Values

- Labs/clinical history features for TMA:
  - Anemia and thrombocytopenia
  - Hypertension requiring >2 antihypertensive medications
  - Proteinuria >/=300 mg/g creatinine

  - Schistocytes on smear
  - Elevated LDH
  - Decreased haptoglobin
  - Could have elevated C5b9 but is not required for diagnosis
- More common pathologic findings described in the kidney after HSCT
  - TMA
  - Membranous nephropathy
  - Minimal change
  - FSGS
  - BK nephropathy

Differential Discussion

The multiple causes of post-HSCT kidney injury include (but are not limited to) infections, nephrotoxic medications, GVHD, and thrombotic microangiopathy (TMA). Many of these disease processes present similarly and concurrently. Further investigation, e.g., labs and pathologic evaluation, is therefore needed to better delineate the cause of injury and to assist in guiding treatment.

Adenovirus and BK virus are common infectious causes of kidney injury after transplantation and can present with progressive elevations in serum creatinine similar to that seen in our patient [1, 2]; however, the urinalyses are often bland. Both of these infections can be monitored via serum viral load levels and further confirmed on biopsy. Antibody staining for adenovirus and BK virus shows reactivity in the nuclei of tubular epithelial cells. These infections can cause tubule epithelial cell injury, nuclear enlargement with inclusions, and subsequent interstitial inflammation. As these diseases progress, further debris and tubular damage can be appreciated. BK viremia has been associated with TMA, though the nature of this relationship is unknown. Fungal infections can also occur in patients after HCT but are distinguished by the presence of fungal elements (i.e., hyphae, spores) on biopsy, usually associated with localized necrosis.

Transplant-associated thrombotic microangiopathy (TA-TMA), as in our patient, is well described as a cause of significant kidney injury in patients after hematopoietic cell transplantation (Fig. 19.5). A strong clinical suspicion for TA-TMA is warranted in a patient with proteinuria, elevated LDH, and difficult-to-control hypertension [3–5]. Serum creatinine may or may not be elevated. The inciting mechanism is endothelial injury leading to activation of the coagulation system, formation of thrombin, and deposition of fibrin. Pathologic kidney findings include mesangiolysis, activation and injury of endothelial cells, expansion of the subendothelial space, and occlusion of the capillary lumens with debris and thrombi [6, 7]. Arteriolar C4d staining can be positive in some patients, suggesting a possible role of complement activation from endothelial injury [8].

There are many proposed etiologies of TA-TMA which include calcineurin inhibition (CNI), total body irradiation, GVHD, and complement activation [9]. CNI

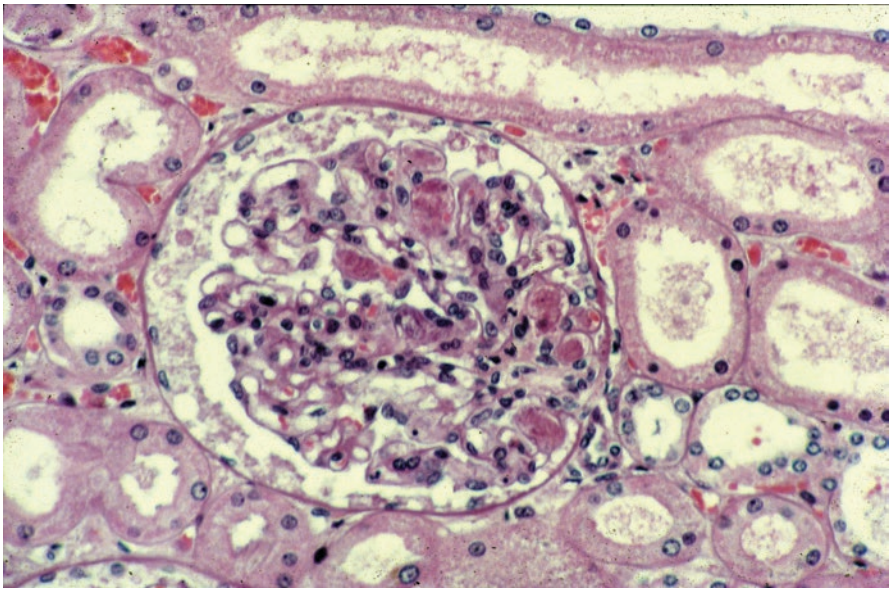

Fig. 19.5 Transplant-associated thrombotic microangiopathy: the glomerular capillary loops are distended by microthrombi

has long been considered a cause of TMA due to the vasoconstrictive effects of the pharmaceutical class leading to decreased renal blood flow and presumed thrombosis [10] (Fig. 19.6). However, TA-TMA is not consistently associated with calcineurin inhibition across all studies [9, 11]. Moreover, some patients appear to have resolution of their TA-TMA with increasing doses of calcineurin inhibitors (CNIs) used to treat their GVHD [12]. Multiple studies have shown increased incidence of TA-TMA in patients with active GVHD and in those who did not receive high-dose conditioning [3, 11, 13–15]. Beyond supportive care including avoiding further nephrotoxicity and controlling blood pressure, the best treatment for TA-TMA is unclear. There is growing interest in eculizumab as this is an effective therapy for TMA in other disease processes associated with abnormal complement activation such as atypical HUS. Initial trials with eculizumab in patients with TA-TMA have had promising results in patients with markers of complement activation such as elevated serum levels of soluble C5b9 but have not shown consistent efficacy in patients who lack these findings [16, 17]. These studies have been small; thus, far and further evaluation is ongoing. Currently, we do not recommend the use of eculizumab in patients without elevated serum levels of soluble C5b9 and clinical and/or pathologic findings consistent with TA-TMA.

In addition to TA-TMA, nephrotic syndrome is another potential manifestation of kidney injury after HSCT. Nephrotic syndrome can occur as soon as 2 months after transplantation and as late as years after transplantation. The pathologic findings in this population are most often membranous nephropathy

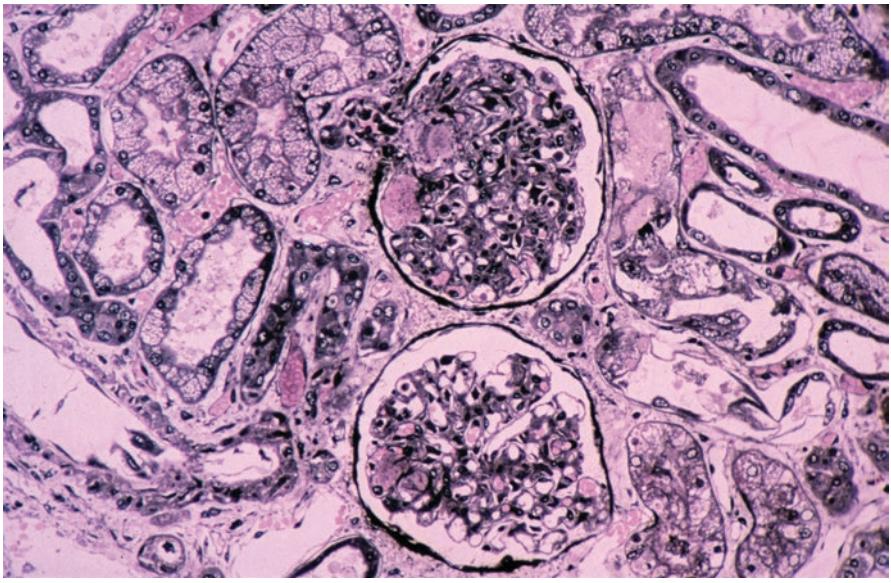

Fig. 19.6 A Jones methenamine silver stain of autopsy kidney demonstrates severe cyclosporine nephrotoxicity/transplant-associated microangiopathy (TMA). Nephrotoxicity occurred prior to the development of assays used to monitor the drug levels. The glomerular hilus and an afferent arteriole contain microthrombi. The capillary loops are small and have focal splitting of the basal layer. There is some mesangial widening or sclerosis. The markedly edematous interstitium contains dilated proximal tubules with marked cytoplasmic vacuolization. Other tubules have necrotic sloughed epithelium

(63%) and minimal change disease (MCD, 19%) [18]. In patients with membranous nephropathy, the subepithelial deposits are thought to be antibody-antigen complex deposition. MCD is thought to be T-cell mediated, though the pathophysiology in post-HSCT patients is unclear. Both disorders typically appear in conjunction with GVHD in other organ systems or when weaning immunosuppression. Less commonly, patients have also developed FSGS, proliferative glomerulonephritis (GN), cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA) GN, and IgA nephropathy. These causes of nephrotic syndrome have been associated with chronic GVHD and tapering of immunosuppression, but again, the pathophysiology remains unclear. Treatment of nephrotic syndrome after HSCT includes reinitiation of high-dose steroids, calcineurin inhibitors, or rituximab often with improvement and resolution of nephrotic syndrome [19–23].

Research to understand and to define GVHD in the kidney is ongoing utilizing mouse and rat models [24, 25]. Kidney infiltration by CD3+ cells, including CD8+ and CD4+ cells, plus CD68+ macrophages has been seen in rat models with associated peritubulitis, interstitial inflammation, capillaritis, glomerulonephritis, and renal dysfunction in the absence of immune deposition. The kidney inflammation in these rats showed temporal correlation with the appearance of GVHD in the skin,

liver, and gastrointestinal tract [24]. Mouse models have also shown increased gene expression in the kidney of proteins associated with antigen presentation and innate immune response [25].

Teaching Points

- Kidney changes associated with GVHD have a diverse array of presentations including endothelial injury in the form of TMA, interstitial nephritis, tubulitis, and nephrotic syndrome with membranous nephropathy, minimal change disease, and FSGS.
- Kidney biopsy is important to make a diagnosis and to guide therapy.
- It is important to rule out other causes of kidney injury including infectious etiologies and BK and adenovirus; viral copy numbers in the blood should be checked.
- A strong clinical suspicion for TA-TMA is needed when a patient has hypertension and proteinuria, associated with anemia, thrombocytopenia, and elevated LDH, regardless of elevations in serum creatinine. This may be a manifestation of GVHD in the kidney.
- Proteinuria, complement activation, and elevated levels of soluble C5b9 are often present in the setting of TA-TMA.
- The mechanisms by which GVHD contributes to kidney injury are not completely understood. Further investigation to elucidate the contributions of T cells, macrophages, cytokines, and gene expression of kidney proteins associated with antigen presentation and immune response is needed.
- Kidney biopsy tissue is needed to define and establish potential pathologic criteria for GVHD-associated kidney injury.

Questions

1. What are pathologic findings typical for BK nephropathy, and how would you differentiate this from TMA?
2. What are the possible presentations of GVHD in the kidney?
3. What are the potential causes of TA-TMA in the kidney?

Answers

1. Answer: Injury with BK typically manifests as tubular epithelial injury, whereas TMA causes endothelial epithelial injury. BK also has characteristic nuclear enlargement with inclusions.
2. Answer: Potential pathologic findings of GVHD in the kidney biopsy include TA-TMA, membranous nephropathy, minimal change disease, and FSGS. Clinical findings of TA-TMA include anemia, thrombocytopenia, increased LDH, proteinuria, and hypertension. In addition, edema, proteinuria, and hypoalbuminemia are the presenting symptoms of nephrotic syndrome.

3. Answer: TA-TMA etiologies include GVHD, total body irradiation, abnormal complement system activation, abnormalities in the complement pathway, and calcineurin inhibitors, primarily in combination with sirolimus.

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Manifestations of Chronic GVHD in Other Organ Systems

20

Cecilia C. S. Yeung and Howard M. Shulman

Thymus

The thymus is at the center of the pathophysiology of GVHD. Immunologic attack on the thymus by acute GVHD (aGVHD) injures the thymic proliferative zone in the outer cortex, impairing normal immunologic recovery and contributing to immunodeficiency [1] (Figs. 20.1 and 20.2). Consequently, there is failure of the thymic dependent selection in the medulla zone to eliminate autoreactive T-cell clones. Auto- and alloreactive CD4+ T-cell populations produce IL-17A, which maintains the inflammatory cell population, simultaneously inducing a loss of regulatory cell populations with increased B-cell activating factors and fibrogenic stimuli driven by activated macrophages leading to sclerosis [2].

Myasthenia Gravis

Perhaps it is not surprising that myasthenia gravis (MG), an autoimmune disorder linked to thymic disorder in the non-HSCT setting, should occasionally occur in chronic GVHD (cGVHD). MG is considered an autoimmune disorder with the

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

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

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

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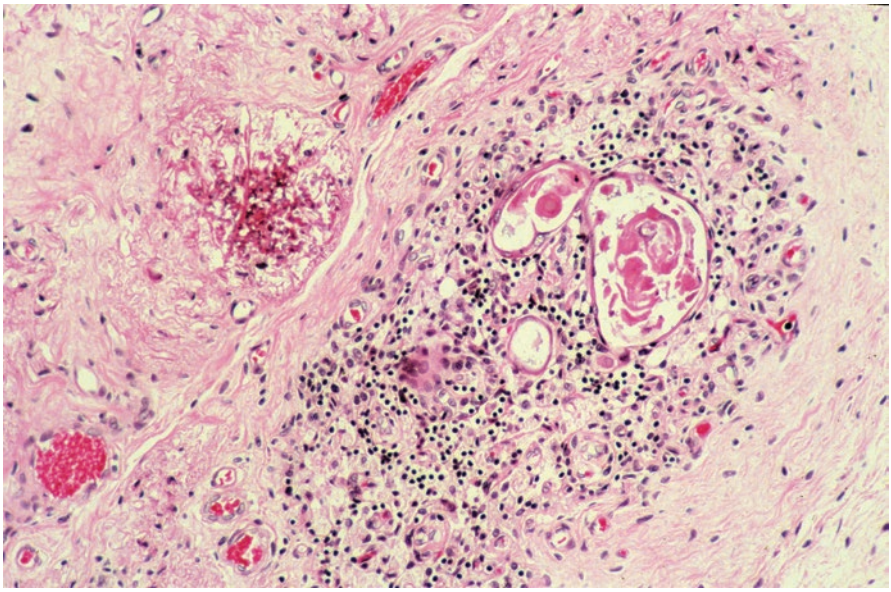

Fig. 20.1 This is an image from a thymus at autopsy from a 13-year-old patient with extensive cGVHD untreated with IS who was 350 days s/p HLA-matched sibling allo-transplant. There is extensive lymphodepletion, loss of cortico-medullary distinction, and cystic degeneration of Hassall's corpuscles. Loss of thymic influence is a major event in the pathophysiology of cGVHD

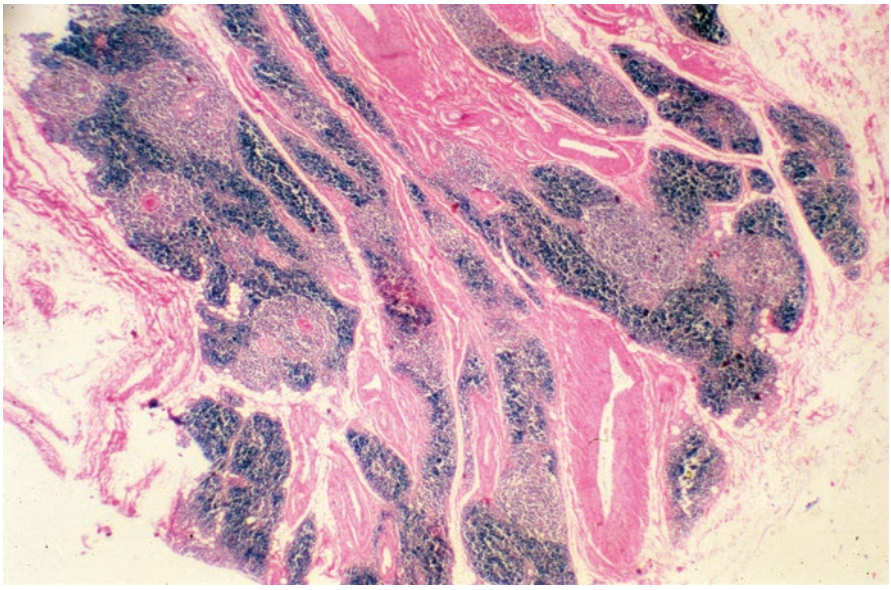

Fig. 20.2 This is a normal adolescent thymus from a 16-year-old patient who was 3 years s/p HLA-matched sibling allo-transplant without evidence of GVHD. The patient died from an auto accident unrelated to the transplant. Note the thymus has normal architecture with development of medulla and cortex regions typical of pediatric patients

production of anti-acetylcholine receptor antibodies. In the HSCT setting, up to 20% of patients may develop anti-acetylcholine receptor antibodies [3], but only a small percentage will manifest symptoms of MG such as muscle weakness and easy fatigue [4]. Studies attribute the development of autoantibodies in MG to dysregulation of regulatory T cells [2, 5]. Transplant characteristics which may lead to higher incidence of MG include transplantation for aplastic anemia [6], recipients who have specific HLA antigens (Cw1, Cw7, and DR2) [7], and if there is increased peripheral blood OX40 CD4 T cells [5, 8]. Typical onset of symptoms occurs after tapering or discontinuation of immunosuppression. The symptoms of MG can be controlled by restarting therapy with steroids [7, 9].

Musculoskeletal System

Polymyositis has developed as a manifestation of cGVHD with presenting symptoms of muscle weakness, fever, and myalgia. This is relatively uncommon as a complication post-HSCT: the 5-year cumulative incidence previously was quoted at 0.55% [10]. Studies confirming the diagnosis include elevated CK, CKMB, aldolase, and troponin I, or an electromyographic examination demonstrating myopathic abnormalities [11]. Biopsy may reveal additional involvement of the adjacent fascia [12] or skin [13] (Fig. 20.3). Skeletal muscle biopsies will show varying degrees of interstitial fibrosis with infiltrating lymphocytes marching between muscle fibers (Fig. 20.4). There may be atrophy of the muscle cells, with reactive nuclear enlargement of muscle nuclei. Despite the marked muscle damage, most patients respond well to corticosteroid therapy.

Cardiac and Vascular System

When cGVHD affects the cardiovascular system, symptoms previously reported include arrhythmias, coronary artery disease, and myocarditis [14–18]. Endothelial cell injury and compromise of the vascular wall integrity have been associated with GVHD [19] and have been proposed as a useful indicator of GVHD development as early as day 7 posttransplant [20] (Fig. 20.5). A case series of 11 pediatric patients with cardiac GVHD described common symptoms as bradycardia, with bradyarrhythmias coinciding with periods of acute GVHD flares in other sites [14].

Central Nervous System

GVHD involving the central nervous system (CNS) is a very controversial and rare occurrence. It requires a diligent search for other causes, especially infectious agents including viruses. A case report by Polchlopek et al. described a 60-year-old man who presented post-second allo-transplant with impaired consciousness and psychomotor agitation who responded to treatment with intrathecal methylprednisolone [21]. At our own institution, we have performed an extensive workup on a patient who presented with headaches and memory disturbances, and subsequent MRI demonstrated diffuse

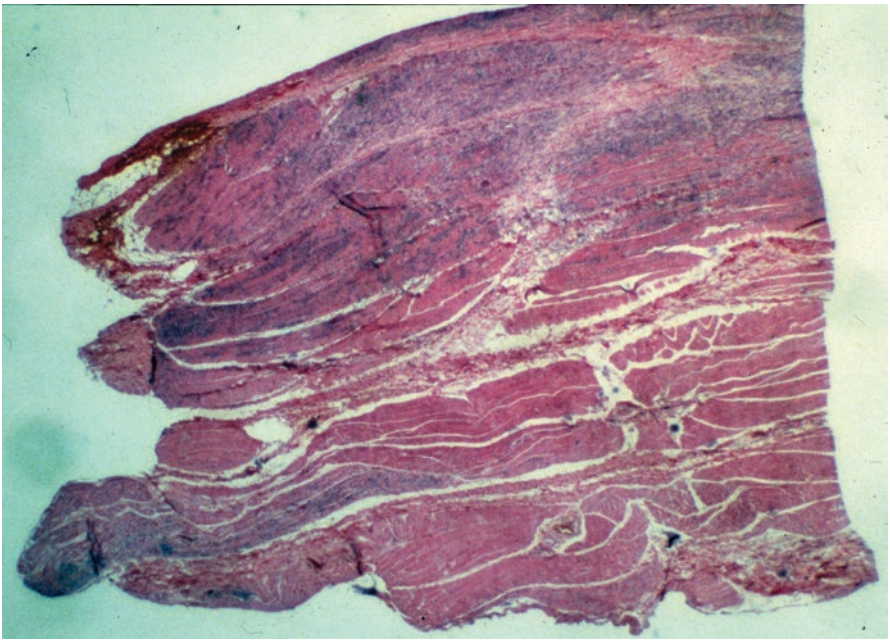

Fig. 20.3 This is a low-power image of a muscle biopsy showing polymyositis with a patchy distribution of dense endomycial chronic inflammation separated by uninvolved fascicles

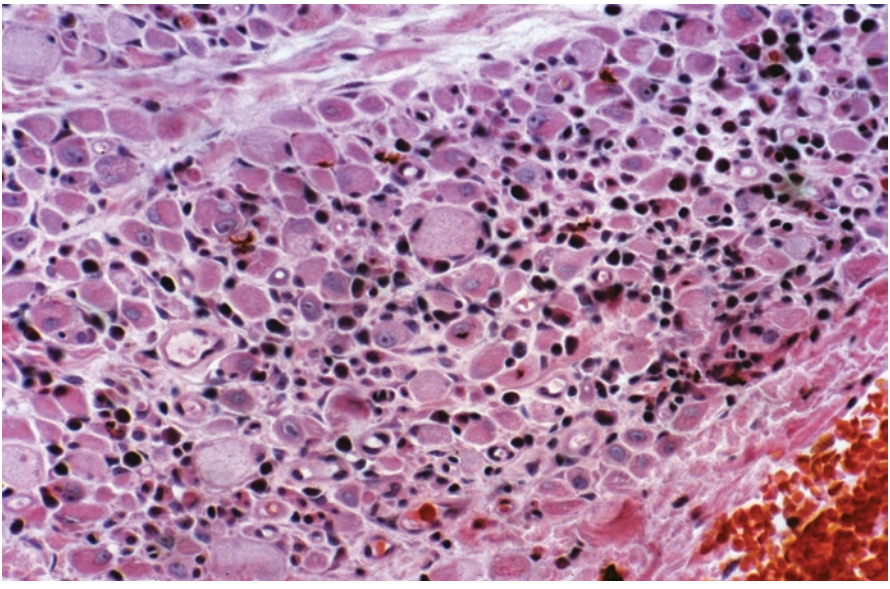

Fig. 20.4 This is a higher-power image demonstrating a polymyositis with marked myocyte destruction and predominantly lymphocytic inflammatory infiltrate that is diffusely percolating through this muscle biopsy

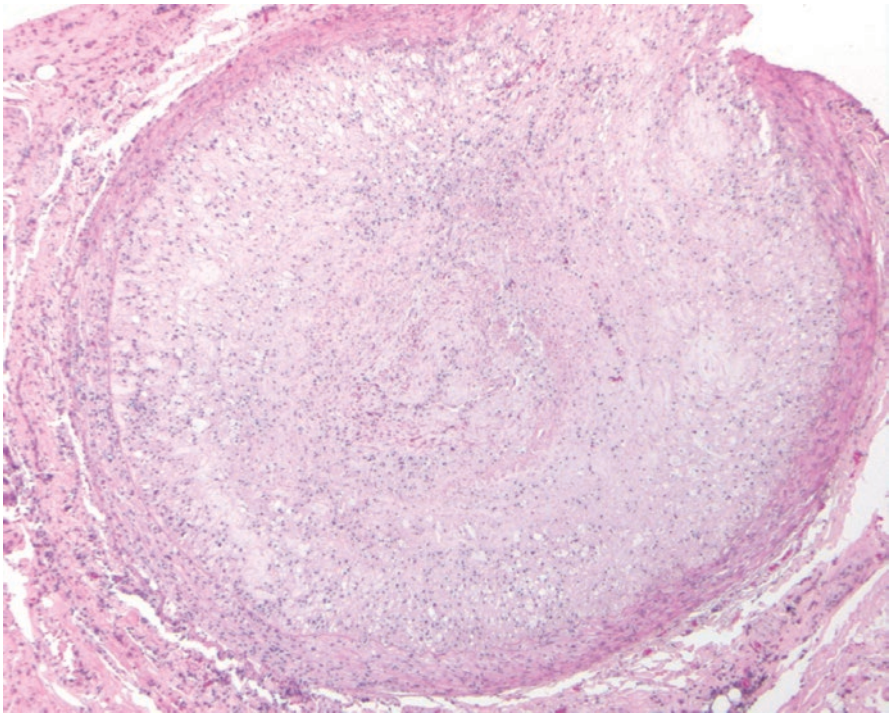

Fig. 20.5 This is a cross section of coronary artery from a 15-year-old patient who died of severe coronary insufficiency. The obliterated lumen and muscular wall are infiltrated by lymphoid cells, monocytes, and extracellular debris. This arteritis is similar to that observed in a rejected cardiac allograft

white matter signal abnormalities with restricted diffusion in the left insular cortex and hippocampus. Microbiology studies including PCR workup for viral agents and bacterial and fungal cultures were all negative. A magnetic resonance angiogram revealed no other large vascular deformities. Only when an exhaustive search for other etiologies of the CNS disturbance was ruled out did we make a diagnosis of compatible with GVHD involving the CNS (Fig. 20.6). Primate studies performed by Kaliyaperumal et al. showed that integrin-expressing CD8+ T-cell infiltration of the CNS can be resolved by immunosuppressive therapy [22] and further suggest that one of the proposed mechanisms of CNS dysfunction in GVHD is attributed to infiltrating T cells.

Serositis

Serositis was first noted in an early series of HSCT patients who demonstrated a lymphoplasmacytic infiltrate and fibrosis in various serosal surfaces [23]. Since then, several other studies have described effusions or serositis as complications in their patients with incidences reported at approximately 0.5% [24–26].

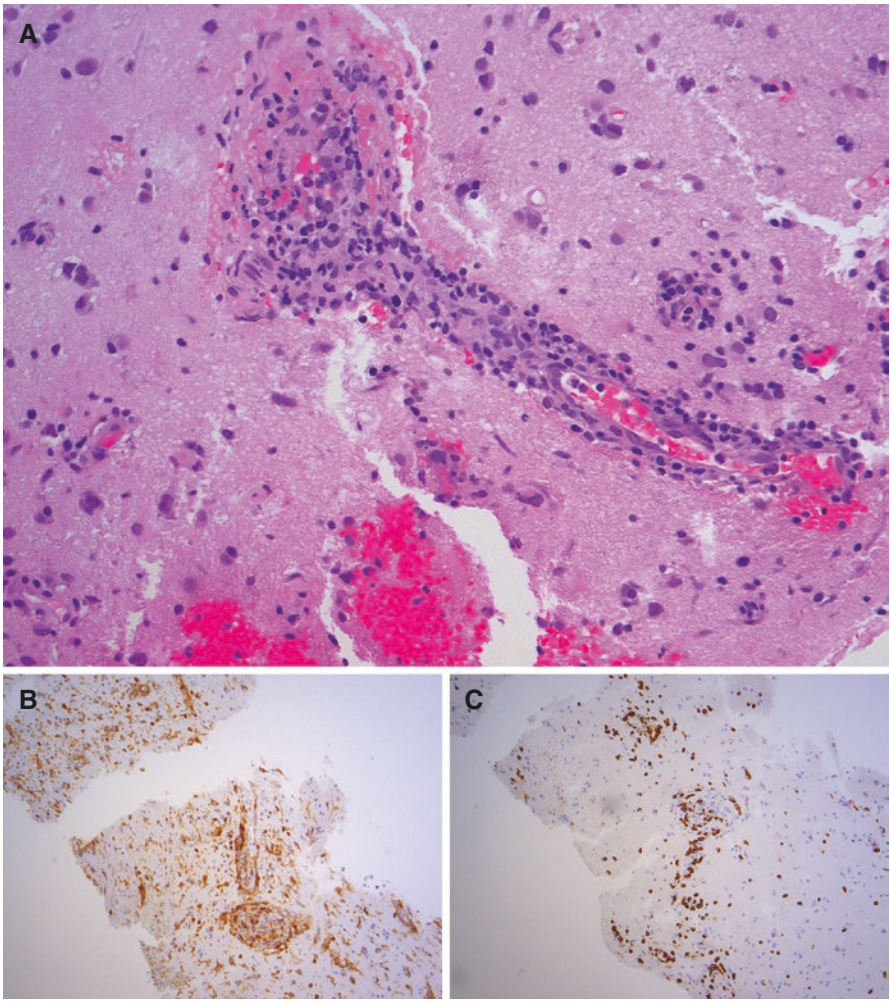

Fig. 20.6 Panel A shows a high-power image of a CNS biopsy from a posttransplant patient with high clinical suspicion of GVHD involving the CNS. Morphology demonstrates increased inflammatory cells mostly concentrated around the vessels with some edema. An extensive workup of infectious agents was negative; ultimately the pathology workup, findings, and clinical picture were interpreted to be most consistent with GVHD involving the CNS. Panel B shows a lower-power image of a CD4 immunohistochemistry, and Panel C shows a lower-power image of a CD8 immunohistochemistry demonstrating a mixed T-cell infiltrate that is concentrated in an around the CNS vessel (personal communication: C. Yeung)

Retroperitoneal Fibrosis

A very rare manifestation of cGVHD includes retroperitoneal fibrosis, a progressive dense fibrous growth entrapping the iliac vessels, ureters, aorta, and other adjacent structures. Clinically these patients may initially present as dull ache in the

abdomen, then edema, and obstructive uropathy. Retroperitoneal fibrosis can result in very severe to fatal outcomes with end-stage renal failure and ischemic bowel [27, 28]. This disorder has now been linked to elevated levels of IgG4 [29].

Bone Marrow

Patients in the early posttransplant period who have persistent cytopenias have a wide differential that includes graft rejection, relapse, infection, and poor engraftment, but cGVHD may also explain the persistent cytopenia. This phenomenon is more common with solid tumor allograft as a passenger leukocyte allograft. The most common cytopenia associated with cGVHD involving the marrow is thrombocytopenia and manifests similar to the clinical syndrome of idiopathic thrombocytopenic purpura [30]. Having higher numbers of B-cell progenitors early posttransplant (before day 30) has been associated with a lower chance of developing chronic GVHD and has been proposed as a possible predictor [31]. Eosinophilia is a common manifestation of aGVHD and cGHVD, reported in 15–44% of cGVHD cases [32].

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Index

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