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ORIGINAL ARTICLE

Prospective Evaluation of Acute Graft-Versus-Host Disease

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Abstract
Background Graft-versus-host disease (GVHD) is a common complication of allogeneic bone marrow transplantation. Severe GVHD carries significant morbidity and mortality and remains one of the leading causes of treatment failure. Unfortunately, intestinal GVHD may present with a variety of non-specific symptoms and diagnosis based on clinical presentation is often inaccurate; biopsy is therefore needed for definitive diagnosis. At present, the optimal endoscopic approach to the diagnosis of gastrointestinal GVHD remains uncertain.
Aims The primary aims of our study were: (1) to evaluate the yield of upper versus lower endoscopy, and (2) to determine which anatomic regions were most likely to provide a histologic diagnosis.
Methods We conducted a prospective study of 27 consecutive patients who had undergone stem cell transplantation within the past 100 days and were referred to the Yale Gastrointestinal Procedure center between August 2002 and February 2006 for the evaluation of suspected acute GVHD. All patients underwent standardized endoscopic evaluation of the upper and lower gastrointestinal tract with biopsies. The diagnostic yield of upper versus lower endoscopy was compared in all patients.
Results GVHD was identified in 18 of the 27 patients (67%). Of those with GVHD, 15 patients (83%) had diffuse intestinal involvement. Six of 10 patients (60%) with an endoscopically normal EGD had GVHD on biopsies of the upper gastrointestinal tract. Six of 13 (46%) patients with an endoscopically normal appearing colonoscopy had GVHD on colonic biopsies. Two of 18 (11%) patients had isolated GVHD of the upper intestinal tract and 1 (6%) had isolated colonic GVHD. Rectal biopsy alone identified 89% (16 of 18) of GVHD cases and all 16 cases of GVHD with colonic involvement. A diagnosis of GVHD was not altered by the additional performance of biopsy of the proximal colon or terminal ileum.
Conclusions In the present study, the majority of cases of acute GVHD demonstrate diffuse upper and lower gastrointestinal involvement with rectal, sigmoid, gastric and duodenal biopsies having similarly diagnostic yield. Based on our findings, we recommend starting with flexible sigmoidoscopy with rectal biopsy alone in patients who are poor candidates to undergo full colonoscopy with sedation or in those in whom GVHD is strongly suspected based on clinical findings. However, more extensive evaluations may be necessary to rule out infection and should be considered in those with no contraindications to sedation and in whom other differential diagnoses are also being considered.

Keywords Graft-versus-host disease (GVHD) · Bone marrow transplantation · Endoscopy

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Introduction

Acute graft-versus-host disease (GVHD) occurs in 17–78% of patients who undergo allogeneic stem cell transplantation [1]. Severe GVHD results in significant morbidity and mortality and is the leading cause of treatment failure in patients undergoing transplantation for low-risk malignancy [2]. Acute GVHD typically involves the intestinal tract, skin and/or liver. Intestinal GVHD may present with a variety of non-specific symptoms including nausea, vomiting, abdominal cramping, anorexia and diarrhea. Diagnosis based on clinical presentation alone is often inaccurate [3].

Endoscopy in the setting of acute GVHD often reveals normal appearing mucosa or non-specific findings [4], thus endoscopic biopsy is needed for definitive diagnosis. Intestinal GVHD may be limited to the upper or lower intestinal tract in up to one-third of patients [5, 6], suggesting that both colonoscopy and upper endoscopy with biopsy may be required [7]. More recent studies have subsequently suggested that distal colonic biopsy alone may be sufficient in patients presenting with diarrhea [8].

The optimal endoscopic approach to the diagnosis of gastrointestinal GVHD remains uncertain. We conducted a prospective, standardized endoscopic evaluation of the upper and lower gastrointestinal tract with biopsies in patients with suspected acute GVHD. The primary aim of our study was to evaluate the yield of upper versus lower endoscopy. We also sought to determine which anatomic regions were most likely to provide a histologic diagnosis.

Patients and Methods

This prospective study consisted of 27 consecutive patients who had undergone bone marrow transplantation in the previous 7–100 days and were referred to the Yale Gastrointestinal Procedure Center between August 2002 and February 2006 for endoscopic evaluation of suspected acute GVHD. All patients were referred by the oncology service and had symptoms suggestive of gastriointestinal GVHD including abdominal pain, nausea, vomiting, diarrhea, dysphagia or anorexia. Patients were excluded if they had a prior history of gastrointestinal GVHD, were less than 18 years of age, had a platelet count <50,000, or an INR $\geq$1.5. Prior to endoscopy, the predominant gastrointestinal symptoms were recorded. Upper endoscopy (EGD) and colonoscopy (or sigmoidoscopy) were performed in all patients using a standardized biopsy protocol. Table 1 shows the baseline characteristics of our patient population.

Endoscopic Protocol

During colonoscopy, four biopsies for light microscopy were performed in each of the following segments: rectum, sigmoid, transverse, right colon and terminal ileum (when intubated) using standard-sized biopsy forceps. During sigmoidoscopy, four biopsies for light microscopy were performed in each of the following segments: rectum and sigmoid. During EGD, four biopsies for light microscopy were performed in each of the following segments: gastric body, gastric antrum, and the second portion of the duodenum and two biopsies were performed from the distal esophagus. A single biopsy was performed for electron microscopy from the second portion of the duodenum and sigmoid colon.

Endoscopic mucosal abnormalities were recorded at the time of endoscopy which included erythema, edema, erosions, ulcerations and/or mucosal sloughing. Additional biopsies were performed if any significant abnormalities were seen based on the judgment of the attending gastroenterologist. All biopsies were evaluated by one of two expert gastrointestinal pathologists at Yale-New Haven Hospital (D.J., M.R.). The histologic diagnosis of GVHD was established using NIH consensus criteria [9]. Briefly, the minimal criteria required to make a diagnosis of GVHD required increased apoptosis of the epithelial cells in the mucosa (squamous epithelium in esophagus, crypt/glandular epithelium in colon, small bowel and stomach). The other findings which are variably present, but not essential to make a diagnosis of GVHD include lymphoplasmacytic lamina propria infilrate, ulceration, crypt loss and fibrosis. Since there is no accepted "gold-standard" for the diagnosis of GVHD, a final diagnosis was made after clinicopathologic correlation in each patient.

This study was approved by the Yale School of Medicine Human Investigation Committee and all patients gave their consent to participate in the study.

Results

A total of 27 patients were enrolled in the study. Patient demographic characteristics are shown in Table 1. Of the 27 patients, 22 underwent EGD and colonoscopy and 5 had EGD and sigmoidoscopy. The mean time of endoscopy after bone marrow transplantation was 37 days (range 21–93 days). There were no complications due to the performance of endoscopic biopsies. A histologic diagnosis via light microscopy of GVHD was identified in 18 patients (67%). Of those with GVHD, 15 patients (83%) had diffuse involvement (present on all biopsies taken in the upper and lower gastrointestinal tract). Esophageal sparing was present in 5 patients with diffuse involvement and only 3

Table 1 Patient baseline characteristics

Patient characteristics	$n = 27$
Mean age, years (range)	47 (21–70)
Gender: female, *n* (%)	13 (48)
Days since stem cell transplant mean (range)	37 (20–93)
Disease requiring transplantation	
Acute myeloid leukemia	7
Chronic myelogenous leukemia	2
Acute lymphoblastic leukemia	1
Aplastic Anemia	2
Myeloma	1
Non-Hodgkins Lymphoma	5
Hodgkins	3
Other Lymphoma	6
Type of transplant	
Peripheral blood stem cell transplant *n* (%)	21 (78)
Bone marrow transplant *n* (%)	6 (22)
Extra-intestinal GVHD	
Total (*n*)	7
Skin	5
Liver	1
Both	1
Main presenting symptom	
Diarrhea	21
Large volume	14
Small volume	7
No Diarrhea	6
Nausea/vomiting or anorexia	3
Abdominal pain	2
Dysphagia	1

patients (17%) had isolated (limited to *either* the upper *or* lower gastrointestinal tract) GVHD. All 3 patients with isolated GVHD presented with diarrhea, 2 had upper intestinal involvement only and 1 with colonic involvement only.

Histologic findings of GVHD in the esophagus included increased apoptosis of sqaumous epithelial cells. The inflammatory infiltrate in all cases was minimal to none. In the colonic and small bowel biopsies, increase in apoptosis in the crypt epithelium was noted in all cases. The inflammatory infiltrate was minimal to mild in all cases, and no differences were noted with respect to different sites in the intestines. Significant crypt distortion or loss was not seen in any of the cases. In the stomach, the increased apoptosis was noted in the glands. Occasionally, a few of the glands showed mild cystic dilatation containing cellular debris in the lumen. The inflammatory infiltrate in the stomach, similar to esophageal biopsies, was none to minimal, and no differences were noted in the antral and corpus biopsies with regards to GVHD. Other histologic diagnoses identified included CMV esophagitis which was identified in one patient with diffuse (both upper and lower involvement) GVHD and gastritis in one patient without GVHD. All 7 patients with extra-intestinal GVHD were found to have gastrointestinal GVHD (5 of the 7 had upper/lower gastrointestinal involvement).

Endoscopic findings seen only in patients with GVHD included duodenal and colonic erosions in ($n = 1$) and ulceration of the terminal ileum (TI) ($n = 3$). Six of 10 patients (60%) with an endoscopically normal appearing EGD had GVHD on biopsies of the upper gastrointestinal tract. Six of 13 (46%) patients with an endoscopically normal appearing colonoscopy had GVHD on colonic biopsies. Non-specific findings of erythema and edema were seen in all the remaining endoscopic examinations.

Rectal biopsy alone identified 89% (16 of 18) of GVHD cases and all 16 cases of GVHD with colonic involvement. A diagnosis of GVHD was not altered by the additional performance of biopsy of the proximal colon or terminal ileum. A biopsy of the terminal ileum was performed in 9 patients, which identified GVHD in all 7 cases with GVHD at other intestinal sites. EGD with biopsies of the gastric body and duodenum identified all but one case (94%) of GVHD due to isolated colonic involvement in that patient. Biopsy of the antrum identified 89% of all GVHD cases. Electron microscopy was performed in 19 patients [duodenum and sigmoid (10), sigmoid (6), duodenum (3)], and did not identify any opportunistic infections or alter the histologic GVHD diagnosis in any patient. The one case of CMV esophagitis was identified on light microscopy.

Two of 6 (33%) patients presenting with isolated upper gastrointestinal symptoms (nausea, vomiting, dysphagia, dyspepsia) had GVHD (with diffuse involvement) as compared to 16 of 21 (76%) patients who presented with diarrhea as their predominant symptom. Both patients with isolated upper gastrointestinal GVHD presented with diarrhea as their primary symptom and had involvement of the gastric antrum, body and duodenum. Esophageal biopsy was positive in one and negative in the other. Neither patient had biopsy of the TI performed. The one patient with isolated colonic GVHD had sigmoidoscopy with positive biopsies of the rectum and sigmoid and also presented with diarrhea. Table 2 shows you the accuracy of GVHD by biopsy location.

Discussion

The optimal approach to the endoscopic diagnosis of patients with suspected acute GVHD, including the location of biopsies and the utility of clinical symptoms in directing biopsy location has been controversial [10].

Table 2 Accuracy of GVHD diagnosis by biopsy location

	n	+GVHD	−GVHD	Specificity	Sensitivity	NPV	PPV
Upper GI lesions							
Esophagus	27	11	7	100	61	56	100
Body	27	17	1	100	94.4	90	100
Antrum	27	16	2	100	88.8	81.8	100
Duodenum	27	17	1	100	94.4	90	100
Lower GI lesions							
Rectum	27	16	2	100	88.8	81.8	100
Sigmoid	27	16	2	100	88.8	81.8	100
Transverse	22	13	1	100	92.8	88.8	100
Proximal colon	22	13	1	100	88.8	88.8	100
TI	9	7	0	100	100	100	100

Gastrointestinal symptoms are frequently non-specific, thus a high level of suspicion for GVHD is necessary in patients who have undergone stem cell transplantation. The differential diagnosis in this population commonly includes infection, drug-induced mucosal injury, along with procedural artifacts. Endoscopic findings are also frequently normal or nonspecific [11], as was the case in our study. Many institutions perform both upper and lower endoscopy with biopsies in all patients presenting for the evaluation of possible GVHD. Early studies identified rectal biopsy as accurate for diagnosis [12]; however, subsequent studies found that GVHD may be isolated to the upper gastrointestinal tract [13] in up to one-third of cases. Gastric biopsies have been shown to be sensitive for the diagnosis [14]; however, upper intestinal biopsy alone may also be insufficient, even in patients with predominantly upper gastrointestinal symptoms [15].

Two recent studies have recommended initiating the evaluation of suspected GVHD with the performance of rectosigmoid biopsies. A prospective study of upper and lower intestinal biopsies in patients with suspected GVHD and diarrhea found biopsy of the rectosigmoid to have the highest yield of 82%. In this same study, EGD with sigmoidoscopy and colonoscopy with terminal ileum biopsy both had equivalent diagnostic yields of 94%. Given the ease of performance, low-risk profile and cost, sigmoidoscopy with biopsy of the rectum and sigmoid was suggested as the initial diagnostic test in patients with diarrhea [16].

A retrospective review of 112 patients with suspected acute GVHD analyzed three biopsy sites: stomach, duodenum and recto-sigmoid. GHVD was identified in 81% of patients, two-thirds of whom had positive biopsies at all locations. We had similar findings and identified diffuse involvement of the upper and lower gastrointestinal tract in 83% of patients with acute GVHD, with biopsies positive at all locations except the distal esophagus (as expected with acute GVHD). Ross et al. found that recto-sigmoid biopsies yielded the greatest sensitivity (95.6%) and had the highest negative predictive value (84%) whether the patient presented with diarrhea, nausea or vomiting [17]. These authors also concluded that biopsy of the recto-sigmoid is the single best test for diagnosing gastrointestinal GVHD.

Our results provide further support for utilizing sigmoidoscopy with recto-sigmoid biopsy as the initial test in patients with suspected acute gastrointestinal GVHD. Our study is the first to analyze rectal and sigmoid biopsies separately, and we found that both sites had an equally high sensitivity of 88.8% and NPV of 81.8%. Although terminal ileum (TI) biopsies identified GVHD in all 9 of the 27 cases in which it was performed, additional cases of GVHD were not identified with the performance of proximal colon or TI biopsies.

The lack of additional diagnostic yield with proximal colon biopsies supports sigmoidoscopy rather than colonoscopy as the initial test, although in two of our patients the rectal biopsies were insufficient to make the diagnosis. Sigmoidoscopy can be performed with minimal or no sedation and can be performed with a lesser bowel preparation, especially if only rectal biopsies are required. While the utilization of sigmoidoscopy over colonoscopy increases patient convenience and safety as well as reduces cost, there is a possibility of sampling error and of missing some cases of GVHD as well other etiologies, especially infections which preferentially involve the right side of the colon. GVHD is a diagnosis of exclusion and, at times, the histologic changes are subtle or mild. While the changes of GVHD may be evident on distal colonic biopsies alone, more extensive evaluations may be important in excluding other diagnoses.

Contrary to the findings of Ross et al. [18], we found biopsies of the gastric body and duodenum to have a slightly higher sensitivity than recto-sigmoid biopsies (94.4 vs. 88.8%), due to the identification of two cases of isolated

upper GVHD (versus one isolated colonic case). Interestingly, both of these patients presented with diarrhea. Isolated upper GI symptoms of nausea and vomiting which were present in six patients were not predictive of isolated upper GI GVHD in our series. Factors to support including an EGD with sigmoidoscopy as the initial test include: (1) concern for missing opportunistic infections of the upper intestinal tract (we identified one patient with esophageal CMV); (2) identifying other common upper gastrointestinal pathology (esophagitis, peptic ulcer disease, etc.); (3) the presence of isolated upper GVHD (2 patients in our study); and (4) convenience for patients to have both procedures done at the same sitting. However, the finding that a rectal biopsy alone has a sensitivity of 88.8% would allow the performance of an unsedated bedside rectal biopsy with limited preparation to evaluate ill hospitalized patients, versus colonoscopy with bowel preparation and sedation.

In our study, the performance of electron microscopy (EM) of the sigmoid or duodenum did not alter the diagnosis of GVHD in any case and did not identify undetected opportunistic infection. However, the number of cases in this study may be too small to make any specific recommendations regarding EM. In our past experience, we have identified unsuspected viral infections, mostly adenovirus by electron microscopy in duodenal or colonic biopsies where the viral inclusions were not obvious on routine evaluation of histologic sections. Since specific antibodies for immunohistochemical staining are now available for many viruses, including antibodies for adenovirus, the cost-effectiveness of routine use of electron microscopy needs to be evaluated prospectively before making any strong recommendations.

There are several limitations to this study including the small study population, which limits the ability to evaluate the influence of electron microscopy. Additionally, we recognize the possibility that intestinal infections (particularly right-sided) and upper gastrointestinal infections can be missed with recto-sigmoid evaluation alone. Finally, sampling error may have occurred, especially given that in the majority of cases, the mucosa appeared endoscopically normal and, therefore, biopsies were not directed.

Conclusions

In this study, the majority of cases of acute GVHD demonstrated diffuse upper and lower gastrointestinal involvement with rectal, sigmoid, gastric and duodenal biopsies having similarly high diagnostic yield. These results support recent recommendations to initiate the evaluation of suspected intestinal GVHD with recto-sigmoid biopsies. Based on our findings, we recommend starting with flexible sigmoidoscopy with rectal biopsy alone in those patients who are either poor candidates to undergo full colonoscopy with sedation or in those patients in whom GVHD is strongly suspected based on clinical findings.

However, more extensive evaluations may be necessary to rule out infection and should be considered in those with no contraindications to sedation and in whom other differential diagnoses are also being considered.

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