Is the Presence of 6 or Fewer Crypt Apoptotic Bodies Sufficient for Diagnosis of Graft Versus Host Disease? A Decade of Experience at a Single Institution

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Abstract: Histopathology assessment is crucial for the diagnosis of graft versus host disease (GVHD), as the presence of crypt apoptosis is the cardinal criterion required. However, crypt apoptosis is not limited to GVHD; it also occurs in other conditions such as infection, drug reaction, or inflammatory reactions unrelated to GVHD. To better determine whether the presence of 6 or fewer apoptotic bodies is sufficient for the diagnosis of GVHD, we retrospectively reviewed 78 colon biopsies from 66 patients who received either hematopoietic stem cell (HSCT) or cord blood cell transplantation and whose colon biopsies exhibited apoptotic bodies. Among them, 41 cases contained 6 or fewer apoptotic bodies in the colon biopsy. These biopsies were compared with 141 colon biopsy controls that showed no significant pathologic changes as well as 16 colon biopsies with cytomegalovirus colitis from patients without a history of bone marrow transplantation. Among the 41 cases reviewed, 7 patients had coexisting GVHD in other organs (skin or liver). However, gastrointestinal symptoms of at least 4 HSCT patients whose colon biopsies contained 6 or fewer apoptotic bodies completely resolved in the absence of further intervention for GVHD. The discrepancy between pathologic findings and the clinical course may be due to confounding factors, such as infection or medication-induced injury. Our data suggest that identifying 6 or fewer crypt apoptotic bodies in colon biopsies from HSCT patients is worth reporting in order to alert the clinicians of the possibility of GVHD but not sufficient to render a diagnosis on the pathologic grounds alone. The colon biopsies containing 6 or fewer apoptotic bodies represent a heterogenous group. We suggest this group to be classified as indeterminate for GVHD, instead of diagnosing GVHD outright. Synthesis of all clinical, endoscopic, and pathologic information, including the status of infection, coexisting GVHD involvement in the other organs, and medication, is essential for confirmation of the diagnosis of GVHD.

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Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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Key Words: graft versus host disease, colon, cytomegalovirus colitis, apoptosis, transplantation

(*Am J Surg Pathol* 2013;37:539–547)

Transplantation, either hematopoietic stem cell (HSCT) or cord blood cell, is a potentially curative therapy in the management of immunohematopoietic disorders. However, the efficacy of this treatment is greatly impaired by graft versus host disease (GVHD), a donor-derived immune response against recipient antigens, resulting in significant morbidity and mortality.[1] The gastrointestinal (GI) tract is one of the main target organs involved by GVHD. Unfortunately, GI GVHD may present with a variety of nonspecific symptoms; diagnosis based on clinical presentation alone is not reliable or specific.[2] Gross endoscopic discordance with histologic findings exists[3,4]; therefore histopathology assessment is crucial to make a diagnosis of GVHD.

The presence of crypt apoptosis is the cardinal criterion for the diagnosis of GVHD according to the Consensus of National Institutes of Health.[5–9] However, crypt apoptosis is not limited to GVHD; it is also present in other conditions such as infection, drug reaction (nonsteroidal anti-inflammatory drugs),[10] or inflammatory reactions unrelated to GVHD,[11] and it is even seen in unremarkable GI biopsies. Most cases of GVHD are fairly straightforward to diagnose. They exhibit relatively diffuse crypt apoptosis in multiple fragments with or without crypt dropout. However, some cases show only rare crypt apoptosis, 1 or 2 total crypt apoptotic bodies in the entire specimen. In this setting it is unclear whether the crypt apoptosis is a manifestation of minimal GVHD or whether it is due to other factors (drug reaction, infection, etc). Rare crypt apoptosis also poses a difficult problem for the clinicians. Undertreatment of GVHD may lead to enteric failure and possibly death, whereas overimmunosuppressing the patient may lead to equally bad results.

To establish minimal criteria for a histologic diagnosis of GVHD, we retrospectively reviewed 78 biopsies from 66 patients who received either HSCT or cord blood cell transplantation and had GI symptoms and crypt apoptosis in the colon biopsy. In this study, we sought to

determine whether the presence of 6 or fewer apoptotic bodies is sufficient for a diagnosis of GVHD by correlating the colon biopsy findings with the clinical course. Furthermore, as a minimum number of 7 apoptotic bodies per 10 contiguous crypts is widely accepted for acute cellular rejection in small bowel transplant, we tried to determine whether such a minimum number could be applicable in the setting of HSCT.

MATERIALS AND METHODS

Patients

A pathology database search was performed to identify all colon biopsies with a diagnosis of GVHD from 2000 to 2011 at our institution. This study was approved by the Institutional Review Board. The major GI symptoms at the time of endoscopy were diarrhea, abdominal pain/cramping, and nausea/vomiting. The original hematologic diagnoses for which transplantation was performed included acute myelogenous leukemia (n = 17), acute lymphocytic leukemia (n = 15), myelodysplastic syndrome (n = 11), chronic lymphocytic leukemia (n = 5), non-Hodgkin lymphoma (n = 5), chronic myelogenous leukemia (n = 3), chronic myelomonocytic leukemia (n = 2), Hodgkin lymphoma (n = 2), multiple myeloma (n = 2), T-cell lymphoma (n = 2), mantle cell lymphoma (n = 1), and paroxysmal nocturnal hemoglobinuria (n = 1).

The cytomegalovirus (CMV) colitis group consisted of 16 cases identified from 2000 to 2011 in non-HSCT patients with age ranging from 22 to 65 years (mean, 47 y). The 16 cases included 6 liver transplants, 4 kidney transplants, 1 heart transplant, 1 small intestine transplant, 1 pancreas and kidney transplant, 1 lung transplant, 1 end-stage renal disease, and 1 breast carcinoma. All these cases demonstrated convincing viral inclusions on hematoxylin and eosin (H&E) sections with or without the immunohistochemical stain for CMV. These cases were also compared with the biopsies from a control group of 141 consecutive colon biopsies collected from January to October of 2009 from the patients who either had GI symptoms or underwent a screening colonoscopy for colon polyps without a history of HSCT. The ages in the control group ranged from 20 to 78 years (mean, 47 y). These control biopsies showed no evidence of viral inclusions or other significant pathologic changes.

Clinical Data at the Time of Colon Biopsies

The clinical chart of each GVHD patient was reviewed in detail. Data collected included the length of time between transplantation and the colon biopsy, the original hematologic diagnosis for which transplantation was performed, the patient's response before or after additional GVHD therapy, the positive biopsies concurrently taken from other organs (skin and/or liver), and the evidence of infections including cultures or serology of viral, fungal, or bacterial etiology.

The pretransplant cytoreductive regimens for the HSCT patients varied depending on the individual's primary disease. However, the patients were usually preconditioned with cytarabine, daunorubicin, fludarabine, cyclophosphamide, cytoxan, and melphalan. After transplantation, the patients were usually given baseline antiviral prophylaxis and anti-GVHD prophylaxis in various combinations of tacrolimus, sirolimus, cyclosporine, methotrexate, antithymocyte globulin, or prednisone. Most of the patients diagnosed as GVHD were treated by either increasing the current dose of the immunosuppressant medication (steroids, tacrolimus, and/or sirolimus) or by initiating additional medicines, including steroids, mycophenolate mofetil (Cellcept), tacrolimus, and/or sirolimus.

Histologic Assessment

All colon biopsies and immunohistochemical stains were reviewed by 1 GI pathologist (J.L.), who was blinded to the patients' clinical diagnoses. All biopsy tissues were formalin fixed and paraffin embedded. The H&E specimens were prepared on 2 slides with 4 to 8 series sections in our histology laboratory. The following histopathologic features were assessed: presence or absence of apoptosis, number of apoptotic bodies per 10 contiguous crypts, loss of crypt, ulceration, and the presence of viral inclusions. The GVHDs were classified as grades 1 to 4 according to the most common system developed by Lerner et al in 1974.[12] In this system, grade 1 lesion is defined as having a single cell apoptosis within the glandular epithelium in the crypt (Fig. 1A). GVHD grade 2 is composed of both apoptosis and loss of crypt. Grade 3 lesions are designated as continuous loss of crypt, and grade 4 lesions show diffuse ulceration with surface denudation.

Immunohistochemistry

Immunohistochemical staining analyses for CMV were performed in some of these cases to rule out the possibility of concurrent viral infection, although there was no evidence of viral inclusions identified on either routine H&E sections or with special stains in the GVHD group. Briefly, 4-μm-thick sections were cut from formalin-fixed paraffin-embedded blocks, deparaffinized, and immunohistochemically stained. The staining was carried out with mouse monoclonal anti-CMV (Dako, Carpinteria, CA; 1:1 dilution).

Statistical Analysis

Categorical data were compared using the χ^2 test. Continuous data were compared using the Student t test. A P value of <0.05 was considered statistically significant.

RESULTS

Demographics of the GVHD, CMV Colitis, and Control Groups

As shown in Table 1, a total of 78 colon biopsies with a diagnosis of GVHD were identified from 66 patients whose age ranged from 20 to 73 years (mean, 47 y).

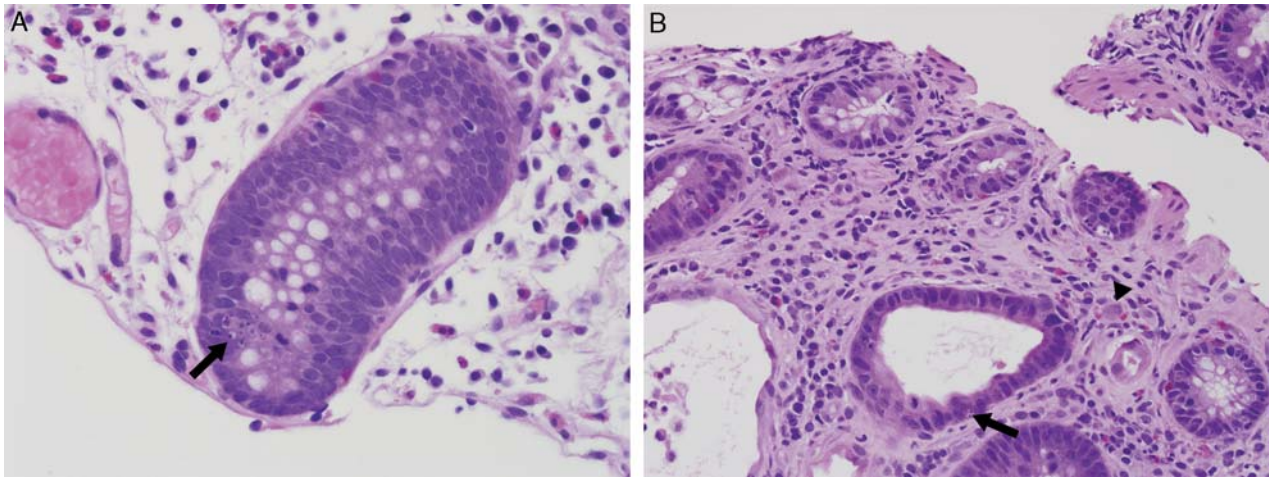

FIGURE 1. Histopathologic features of apoptosis. A, High-power magnification showing apoptosis in a nontransplanted patient with unremarkable colon biopsy. The apoptotic body (arrow) is indicated. B, High-power magnification showing apoptosis in patients with CMV colitis. Both apoptotic body (arrow) and viral inclusion (arrowhead) are appreciated.

Fifty-five patients had only a single biopsy in the study; 11 patients had >2 biopsies during different hospital visits. Among these 66 patients, 58 had undergone allogeneic HSCT, and 8 had undergone cord blood transplantation. Of the 78 biopsies, 53 had grade 1 lesions, 5 had grade 2 lesions, 12 had grade 3 lesions, and 8 had grade 4 lesions. The median posttransplant time of colon biopsy was 165 days (range, 13 to 1765 d). Of the 78 biopsies, 6 biopsies were taken <21 days after the transplant (cases 3, 12, 17, 18, 28, and 29).

Among 66 GVHD patients, 44 (66.7%) were male. Of the control group of 141 patients, 92 (65.3%) were male. Of the 16 CMV colitis patients, 9 (56.3%) were male. The sex composition was not significantly different among these groups ($P > 0.05$). The average age of the patients in the GVHD group was 47 years (range, 20 to 73 y), which showed no significant difference when compared with the mean age of 49 years for the controls (range, 20 to 82 y; $P > 0.05$) or the mean age of 47 years for the CMV colitis group (range, 22 to 65 y; $P > 0.05$).

Histopathologic Features of the Colon Biopsies in GVHD, CMV Colitis, and Control Groups

As shown in Table 2, the mean number of apoptotic bodies per 10 contiguous crypts was 11.3 for the GVHD group (range, 1 to 55 per 10 contiguous crypts). The mean number of apoptotic bodies per 10 contiguous crypts was positively correlated with the degree of GVHD, with an increasing number of apoptosis seen in the higher-grade lesions (mean of 5.2 in grade 1, 9.4 in grade 2, 22.5 in grade 3, and 37.3 in grade 4). The presence of rare apoptosis (1 to 6 apoptotic bodies per 10 contiguous crypts) was present in 73.6%, 60%, 16.7%, and 0% of the grades 1 to 4 GVHD lesions, respectively. More than 10 apoptotic bodies per 10 contiguous crypts were observed in 13.2%, 20%, 83.3%, and 100% of the grades 1 to 4 GVHD lesions, respectively.

Interestingly, all CMV colitis biopsies demonstrated the presence of crypt apoptosis with 75% of the cases in the range of 1 to 6 apoptotic bodies per 10 contiguous crypts and 18.8% with >10 apoptotic bodies per 10 contiguous crypts. There was no significant difference in the mean apoptotic bodies between the GVHD and CMV colitis groups ($P > 0.05$). CMV colitis also showed the full spectrum of pathologic changes seen in GVHD, including loss of crypts (31.3%), continuous loss of crypts (31.3%), and ulceration (12.5%).

Strikingly, the presence of a single or rare crypt apoptosis was seen in 21.3% (30 of 141) of the normal controls with a mean of 0.2 per 10 contiguous crypts and a maximum count of up to 5 per 10 contiguous crypts. Twenty-eight biopsies (93.3%) showed only a single crypt apoptotic body. No case exhibited >6 apoptotic bodies per 10 contiguous crypts. The mean apoptotic numbers of both GVHD and CMV colitis groups were significantly increased compared with the normal control group ($P < 0.05$ in both).

TABLE 1. Demographics of the GVHD, CMV Colitis, and the Control Groups

	Control	GVHD	CMV Colitis
No. patients (no. biopsies)	141 (141)	66 (78)	16 (16)
Mean age (range) (y)	49 (20-82)	47 (20-73)	47 (22-65)
Female to male ratio	49:92	22:44	7:9

There is no statistical significance between the study groups or between the study group and the controls ($P > 0.05$).

Correlation of Presence of 6 or Fewer Apoptotic Bodies in Colon Biopsies With the Patients' Clinical Course

Of the 78 GVHD colon biopsies, 44 showed the presence of 6 or fewer apoptotic bodies per 10 contiguous crypts, which met the criteria for the diagnosis of possible or definitive GVHD according to the National Institutes of Health Consensus.[5] Among them, 41 cases had appropriate clinical follow-up, whereas 3 cases were lost

TABLE 2. Histopathologic Features of the Colon Biopsies in the Study and the Control Groups

		GVHD (%)					
	Controls (n = 141) (%)	Total (n = 78)	Grade 1 (n = 53)	Grade 2 (n = 5)	Grade 3 (n = 12)	Grade 4 (n = 8)	CMV Colitis (n = 16) (%)
Apoptosis involvement	21.30	100	100	100	100	100	100
Mean apoptosis/10 contiguous crypts, N (range)	0.2 (0-5)	11.3* (1-55)	5.2 (1-35)	9.4 (2-25)	22.5 (3-55)	37.3 (15-50)	7.9* (2-33)
1-6 apoptosis/10 contiguous crypts	21.30	57.00	73.60	60.00	16.70	0	75.00
7-10 apoptosis/10 contiguous crypts	0	10.10	13.20	20.00	0	0	6.20
> 10 apoptosis/10 contiguous crypts	0	32.90	13.20	20.00	83.30	100	18.80
Loss of crypt	0	32.90	0	100	100	100	31.30
Continuous loss of crypts	0	25.30	0	0	100	100	31.30
Ulcer	0	10.10	0	0	0	100	12.50

*$P < 0.05$ compared with the controls.

to follow-up and were not included in the study. Forty-one biopsies were taken from 39 patients, in which 2 patients had 2 colon biopsies during different hospital visits. As shown in Table 3, 37 cases had grade 1 GVHD, 2 had grade 2 GVHD, and 2 had grade 3 GVHD. The clinical chart and laboratory results of each patient were reviewed in detail. These 41 GVHD cases containing 6 or fewer apoptotic bodies were further classified depending on the grade of GVHD, the status of infection, and the response to the further GVHD treatment.

The first scenario involved 3 patients who had 6 or fewer apoptotic bodies, no evidence of infection at the time of biopsy, and resolved GI symptoms without any significant intervention for GVHD (cases 1 to 3). One patient (case 1) had been prescribed with anti-GVHD medication; however, her symptoms were completely resolved before she received the medicine. Patient 2 was thought to have residual disease from the previous GVHD and was on baseline anti-GVHD medication; her symptoms improved without additional treatment. The condition of the third patient (case 3), who was thought to be allergic to Bactrim, had improved without further treatment. However, as this biopsy was taken <21 days after transplant, the apoptosis was more likely related to chemoradiation. Strictly speaking, the GI symptoms of at least 1 patient in this group were completely resolved in the absence of further intervention for GVHD.

The second group included 9 patients who had 6 or fewer apoptotic bodies with no evidence of infection at the time of the biopsy (cases 4 to 13), and their GI symptoms resolved after receiving advanced GVHD treatment. Among them, 1 patient (case 5) had coexisting biopsy-proven skin GVHD; the remaining patients did not show GVHD involvement in other organs.

The third group consisted of 5 patients who had 6 or fewer apoptotic bodies and evidence of variable infections, including those caused by Candida, adenovirus, BK virus, vancomycin-resistant Enterococcus, and *Clostridium difficile* (cases 13 to 17). These patients received treatment for the infections; however, their GI symptoms were completely resolved without any further intervention for GVHD (not more than prophylaxis). None of them had concurrent GVHD involvement in either skin or liver. However, 2 biopsies were taken <21 days after the transplant (cases 16 and 17), in which the apoptosis was more likely related to chemoradiation. Therefore, strictly speaking, the GI symptoms of at least 3 patients in this group were fully resolved in the absence of advanced intervention for GVHD.

The fourth group included 11 patients who had 6 or fewer apoptotic bodies, evidence of infection, and resolved GI symptoms after additional GVHD and respective infection treatment (cases 18 to 28). The infectious agents included candida, adenovirus, BK virus, *C. difficile*, CMV, herpes, pseudomonas, and staphylococcus. Among them, 2 patients (cases 21 and 27) had concomitant GVHD involvement either in the liver or skin. The symptoms of patient 18 had already improved before the initiation of prednisone therapy (Table 3).

The fifth group comprised 9 patients who had 6 or fewer apoptotic bodies with evidence of infection; they did not respond to the aggressive treatment for infection and GVHD and eventually expired (cases 29 to 37). The infectious agents included Candida, Streptococcus, Enterococcus, Klebsiella, HHV6, adenovirus, *C. difficile*, CMV, Herpes, Pseudomonas, and Staphylococcus. Among them, 3 patients had biopsy-proven GVHD involvement in liver or skin (cases 31, 32, and 33). Cases 35 and 37 were suspicious of liver involvement; however, the biopsies were not performed to differentiate between GVHD and hepatitis C-induced liver injury. The skin GVHD lesion of patient 34 responded to sirolimus, and his diarrhea was thought to be related to adenovirus infection. The other 4 patients (cases 29, 30, 34, and 36) who presented with severe infection and multiple organ failure did not have coexisting GVHD in other organs.

Lastly, 6 or fewer apoptotic bodies were also seen in 2 cases of grade 2 GVHD and 2 of grade 3 GVHD. Two patients had grade 2 GVHD (cases 38 and 39); 1 of them did not have infection. Their GI symptoms were improved after receiving aggressive intervention for GVHD of tacrolimus and/or corticosteroids. However, the other 2 patients with grade 3 GVHD (cases 40 and 41), who had simultaneous GVHD in the skin and/or the liver but no

TABLE 3. Correlation of 6 or Fewer Apoptosis Per 10 Contiguous Crypts in Colon Biopsies With the Patients' Clinical Course

Case	GVHD, Grade	Apoptosis/ 10 Contiguous Crypts	21 d After Transplant	Infection Before or After Colon Biopsy	Infection During Colon Biopsy	Clinical Note to Evaluate the Treatment of GVHD
Category 1. No infection, no treatment for GVHD, better						
1	1	2	Yes	No	No evidence of infection	"Diarrhea has resolved and abdominal pain has improved; no clinical evidence of GVHD"
2	1	4	Yes	No	No evidence of infection	"GVHD in liver and GI before; could be left over from previous GVHD and stools gotten better"
3	1	5	No	No	No evidence of infection	"Allergy to Bactrim, f/u no N/V without treatment"
Category 2. No infection, treated for GVHD, better						
4	1	2	Yes	No	No evidence of infection	Getting better
5	1	2	Yes	No	No evidence of infection	"GVHD in skin and treated; stool becoming more solid"
6	1	3	Yes	No	No evidence of infection	Getting better
7	1	3	Yes	No	No evidence of infection	Getting better
8	1	2	Yes	No	No evidence of infection	Getting better
9	1	2	Yes	No	No evidence of infection	Getting better
10	1	1	Yes	No	No evidence of infection	Getting better
11	1	3	Yes	No	No evidence of infection	Getting better
12	1	6	No	No	No evidence of infection	Getting better
Category 3. Infected, no treatment for GVHD, better						
13	1	2	Yes	Yes	Candida	"Resolved on its own, the patient did well afterwards"
14	1	2	Yes	Yes	Adenovirus	"No treatment is necessary at this time as all his symptoms resolved"
15	1	3	Yes	Yes	BK virus	"Was doing very well after his scope, no bowel movement"
16	1	3	No	Yes	VRE	"GI symptoms resolved without glucocorticosteroids which militates against the diagnosis of GVHD"
17	1	6	No	Yes	C. diff	"Skin rash related to medicine, not GVHD; diarrhea after finishing course of treatment for C. diff colitis. This had improved on its own without any significant intervention, so GVHD was thought to be very unlikely"
Category 4. Infected, treated for GVHD, better						
18	1	2	Yes	Yes	Pneumonia; thrush	"He received three doses of it but had already had improvement his symptoms before the initiation of prednisone therapy"
19	1	2	Yes	Yes	CMV	Getting better
20	1	2	Yes	Yes	CMV	Getting better
21	1	2	Yes	Yes	Herpetic esophagitis, C. diff	"Had GVHD in liver; felt to a touch of liver GVHD, resolved"
22	1	3	Yes	Yes	Pseudomonas, HSV	Getting better
23	1	4	Yes	Yes	BK virus and gram$^+$ bacteremia	Getting better
24	1	2	Yes	Yes	gram$^+$ cocci and adenovirus	Getting better
25	1	3	Yes	Yes	C. diff	Getting better
26	1	1	Yes	Yes	C. diff	Getting better
27	1	1	No	Yes	Coagulase$^-$ Staphy and BK virus	"Grade 2 skin GVHD as well; diarrhea has resolved"
28	1	1	No	Yes	Adenovirus and BK virus	Getting better
Category 5. Infected, treated for GVHD, died						
29	1	2	Yes	Yes	CMV and *Strept agalactiae*	Renal insufficiency; died
30	1	2	Yes	Yes	Candida, Enterococcus, CMV, Klebsiella	Died due to sepsis and multiorgan failure
31	1	6	Yes	Yes	CMV	GVHD in liver; multiorgan failure; died
32	1	1	Yes	Yes	HSV1, adenovirus	GVHD in liver; died
33	1	3	Yes	Yes	Adenovirus and fungi	"Grade 2 GVHD of skin responding to siro, diarrhea related to viral infection rather than GVHD"; died
34	1	3	Yes	Yes	HHV6 and C. diff	Multiorgan failure; died
35	1	2	Yes	Yes	Yeast in blood culture, hepatitis C	Elevated LFT, ?GVHD in liver versus Hepatitis C; multiorgan failure; died
36	1	2	Yes	Yes	Coagulase$^-$Staph and CMV	Died due to CMV
37	1	1	Yes	Yes	C. diff; gram$^+$ cocci; hepatitis C	Elevated LFT, ?GVHD in liver versus hepatitis C; died of multiorgan failure
Category6. Grade 2, treated for GVHD, better						
38	2	2	Yes	Yes	Klebsiella, *E. faecium*	Getting better
39	2	2	Yes	No	No evidence of infection	Getting better

TABLE 3. (*continued*)

Case	GVHD, Grade	Apoptosis/ 10 Contiguous Crypts	21 d After Transplant	Infection Before or After Colon Biopsy	Infection During Colon Biopsy	Clinical Note to Evaluate the Treatment of GVHD
Category 7. Grade 3; treated for GVHD, died						
40	3	6	Yes	No	No evidence of infection	GVHD in skin and liver; diarrhea slightly improve but eventually died
41	3	3	Yes	No	No evidence of infection	Grade 2 GVHD in skin and liver; no improvement, died

Clinicians' original wordings in notes were incited in quotation marks.
C. diff indicates *C. difficile*; coagulase$^-$, coagulase negative; *E. faecium*, *Enterococcus faecium*; gram$^+$, gram positive; HHV6, human herpesvirus 6; HSV, herpes simplex virus; LFT, liver functional test; Staphy, Staphylococcus; Strept, Streptococcus; VRE, vancomycin-resistant Enterococcus.

evidence of infection, were refractory to the GVHD treatment and subsequently passed away.

Correlation of presence of >6 Apoptotic Bodies in Grade 1 GVHD Colon Biopsies With the Patients' Clinical Course

Of the 53 grade 1 GVHD colon biopsies, 13 cases showed presence of 6 or more apoptotic bodies per 10 contiguous crypts. As shown in Table 4, these cases were further classified depending on the status of infection and the response to the further GVHD treatment.

The first group included 3 patients who had >6 apoptotic bodies with no evidence of infection at the time of the biopsy (cases 1 to 3), and their GI symptoms resolved after receiving advanced GVHD treatment. Among them, 1 patient (case 1) had coexisting GVHD in the skin and the eye; the remaining patients did not show GVHD involvement in other organs.

The second group consisted of 1 patient who had up to 18 apoptotic bodies and evidence of CMV infection (cases 4). Even though this patient's GVHD treatment was reduced, the GI symptoms were completely resolved after treatment for CMV colitis. Although this patient had GVHD involvement in the skin shortly before, the etiology of the diarrhea is CMV colitis not GVHD.

The third group included 6 patients who had >6 apoptotic bodies, evidence of infection, and resolved GI symptoms after additional GVHD and respective infection treatment (cases 5 to 10). The infectious agents included respiratory syncytial virus, BK virus, CMV, and Epstein-Barr virus. Among them, 2 patients (cases 6 and 9) had concomitant GVHD involvement in the skin. Although patient 10 received further GVHD treatment and responded well, clinically it was considered a soft finding.

The fourth group comprised 3 patients who had >6 apoptotic bodies with evidence of infection but did not response to the aggressive treatment for infection and GVHD and eventually expired (cases 11 to 13). The infections included fungal esophagitis, CMV colitis, and aspergillosis. Among them, 2 patients had biopsy-proven GVHD involvement in the skin (cases 11 and 13).

DISCUSSION

Histopathologic assessment is a crucial component in diagnosing GVHD, although it can be highly suspected on clinical grounds alone. The biopsies showing abundant crypt apoptosis or higher-grade lesions are generally less problematic from a diagnostic perspective. However, microscopic diagnosis of GVHD can be difficult in the setting of a single or rare apoptosis; given the fact that apoptosis is a nonspecific histologic finding. In our series, 21.3% of the unremarkable colon biopsies in nontransplanted populations showed a single or rare crypt apoptosis. CMV colitis, a well-known confounding condition in the HSCT setting, demonstrates the full spectrum of histologic features mimicking GVHD, from crypt apoptosis, to loss of crypt/gland, to mucosal ulceration. Besides CMV, other infectious etiologies, including *C. difficile*, are also known to be related to apoptosis.[13,14] Unequivocal apoptosis is present in inflammatory processes unrelated to GVHD, including Crohn disease.[10] In addition, colitis with apoptosis has been associated with extracorporeal photopheresis,[15] sodium phosphate bowel preparation,[16] proton pump inhibitors,[3] nonsteroidal anti-inflammatory drugs,[9] and mycophenolate mofetil, a medication to treat GVHD.[17,18] Therefore, a challenging question for practicing pathologists is whether a single or rare crypt apoptosis is sufficient to diagnose GVHD.

In our study combining the pathologic findings and the clinical course, presence of 6 or fewer crypt apoptotic bodies in colon biopsy was not sufficient to render a diagnosis of GVHD on histologic grounds alone. Our data provide the clinical evidence, for the first time to our knowledge, that at least 1 patient without infection and 3 patients with infection, who received treatment for the infection, had completely resolved GI symptoms without advanced intervention for GVHD (4 of 41 cases, 9.8%). Given the fact that GVHD has not been reported to resolve on its own without further treatment, the presence of a single or rare apoptosis in these biopsies is likely due to other confounding factors, such as infection or medication, rather than GVHD itself. Given that apoptosis is also seen in approximately 20% of the unremarkable colon biopsies in nontransplanted patients, it is not

TABLE 4. Correlation of >6 Apoptotic Bodies Per 10 Contiguous Crypts of Grade 1 GVHD in Colon Biopsies With the Patients' Clinical Course

Case	GVHD, Grade	Apoptosis/10 Contiguous Crypts	21 d After Transplant	Infection Before or After Colon Biopsy	Infection During Colon Biopsy	Clinical Note to Evaluate the Treatment of GVHD
Category 1. No infection, treated for GVHD, better						
1	1	35	Yes	No	No evidence of infection	Getting better; GVHD in skin and eye
2	1	8	Yes	No	No evidence of infection	Getting better
3	1	8	Yes	No	No evidence of infection	Getting better
Category 2. Infected, treated for infection, but no treatment for GVHD, better						
4	1	18	Yes	Yes	CMV	"His steroid was reduced, on ganciclovir IV"; grade 2 GVHD of skin shortly before
Category 3. Infected, treated for infection and GVHD, better						
5	1	8	Yes	Yes	Respiratory syncytial virus pneumonia	Getting better
6	1	11	Yes	Yes	BK virus	Getting better; had GVHD of skin
7	1	8	No	Yes	CMV	Getting better
8	1	15	Yes	Yes	BK virus	Getting better
9	1	10	Yes	Yes	Epstein-Barr virus	Getting better; had grade 2 GVHD of skin
10	1	11	Yes	Yes	Epstein-Barr virus	"The colonoscopy did show rare crypt apoptosis consistent with grade 1 GVHD, however, it was considered to be a soft finding and we will continue to monitor, especially in light of her large amount of diarrhea"
Category 4. Infected, treated for infection and GVHD, died						
11	1	12	Yes	Yes	Fungal esophagitis	"Her symptoms have not improved whatsoever on relatively high doses of steroids. Cause of diarrhea is not clear."; GVHD of skin; died
12	1	8	Yes	Yes	CMV, aspergillosis	Died due to progressive invasive aspergillosis
13	1	19	Yes	Yes	CMV	GVHD of skin; immunosuppression was increased; died

Clinicians' original wordings in notes were incited in quotation marks.

reliable to predict GVHD on the basis of the pathologic finding of a single or rare crypt apoptosis alone.

However, we cannot exclude the possibility that a single or rare crypt apoptosis might represent the early event or mild form of GVHD. In our series, the biopsy-proven GVHD involving the other organs (liver or skin involvement in 7 of 41 cases, 16.6%) suggests that a single or rare crypt apoptosis in the GI tract, although not sufficient to render the final diagnosis, is worth reporting in order to alert the clinicians of the possibility of GVHD. A recent study found that up to 11% of biopsies diagnosed as GVHD showed only a single isolated crypt apoptotic body.[19] Among these 9 cases, 4 lacked any confounding factors of GVHD with coexisting GVHD in the skin and 1 responded to the GVHD treatment; therefore, the authors suggested that in the appropriate clinical setting, a single apoptotic cell could possibly represent GVHD. In our study, a single apoptotic body was found in 7.7% of colon biopsies (6 of 78 cases). Among them, 2 had biopsy-proven GVHD involvement in the liver or skin. Given the apparent correlation of improved outcome with early diagnosis, it is worth reporting the subtle changes of a single or rare crypt apoptosis in a colon biopsy to remind the clinicians of the possibility of GVHD.

Although apoptosis is not specific, colon biopsies showing features of GVHD lesions of grade >1 are more likely to represent true GVHD. One of the grade 2 GVDH patients in our study had no evidence of infection, and the GI symptoms were completely resolved after further GVHD treatment. The other 2 grade 3 GVHD patients had no evidence of infection and had concomitant GVHD involvement in the skin or liver. Most of these GVHD cases of >grade 1 lesion lack the confounding factors, which suggest that the rare apoptosis seen in the GI tract, in the presence of GVHD involvement in other organs, probably represents the true GVHD.

Cases with infectious conditions superimposed on GVHD do coexist. In our study, at least 7 patients (17.1%) showed evidence of both infection and GI symptoms with additional biopsy-proven GVHD involvement in the liver or skin. Unfortunately, a majority of our study cases had evidence of infection (71.4%); among them, most had no extra GI GVHD involvement (76.7%). Therefore, a single or rare crypt apoptosis in these GI biopsies poses the highest degree of difficulty as far as the diagnosis of GVHD is concerned. In this setting, the presence of a single or rare crypt apoptosis can represent true GVHD, infection, or both. So far, reliable markers to distinguish true GVHD and the confounding

TABLE 5. Suggested Modified Histopathologic Criteria for Grading GVHD in Colon Biopsies

Indeterminate for GVHD
Rare crypt apoptosis (≤6 apoptotic bodies per 10 contiguous crypts)
Grade 1 GVHD
Increased crypt apoptosis without crypt/glands loss (≥7 apoptotic bodies per 10 contiguous crypts)
Grade 2 GVHD
Loss of individual crypt/gland with crypt apoptosis
Grade 3 GVHD
Loss of 2 or more contiguous crypts/glands with crypt apoptosis
Grade 4 GVHD
Extensive crypt loss with mucosal denudation or ulceration with crypt apoptosis

mimickers are lacking. This scenario challenges both the clinicians and pathologists.

The diagnosis of grade 1 GVHD is very difficult on pure morphologic grounds given that a single or rare crypt apoptosis is not specific. The current diagnostic criteria for GVHD are based on the presence of even a single apoptotic body in the colon biopsy. On the basis of our knowledge of the clinicopathologic data from this study, we think that the current pathologic diagnostic approach—the minimum number of apoptotic bodies required in a GI biopsy—needs further clarification. Setting this minimum number must consider 2 basic requirements: specificity and sensitivity. From our study, we know that 1 apoptotic body is not specific for GVHD, which cannot separate the GVHD group from the normal controls. As the apoptotic number in our control group ranges from 1 to 5 per 10 contiguous crypts, as shown in Table 5, we suggest to set the minimal number as 7. To render a diagnosis of grade 1 GVHD, the minimum number of apoptotic bodies is suggested to be >6 per 10 contiguous crypts, which is similar to the criteria used for acute cellular rejection in small bowel transplant. However, we do not consider this is a magic number to draw a line to completely separate GVHD from the confounding factors. As shown in Table 4, 1 patient who had CMV infection had up to 18 apoptotic bodies per 10 contiguous crypts in the colon biopsy, and the symptoms were resolved without further GVHD treatment. Similar to other dilemmas in reality, we have to evaluate the pros and cons to balance between sensitivity and specificity. As only 1 case was falsely diagnosed as GVHD, we consider it reasonable to recommend 7 as the minimal criteria.

The colon biopsies containing 6 or fewer apoptosis represent a heterogenous group. Given the fact that the presence of even a single apoptotic body can be characteristic of GVHD, this group is better to be classified as indeterminate. This strategy allows pathologists to confidently render a diagnosis of indeterminate for GVHD in biopsies showing a single or rare crypt apoptosis, and meanwhile allows clinicians to consider the other causes of apoptosis by consolidating all clinical, endoscopic, and pathologic data before reaching a final diagnosis of GVHD. Therefore, some patients with such minimal changes in colon biopsy might not need further intervention for GVHD. Of course, the proposed criteria, the minimum required apoptotic number, needs to be validated in large series studies of colon biopsies in HSCT patients from multiple transplantation centers by combining both the pathologic findings and the clinical course.

Integrating all clinical, endoscopic, and pathologic information, including the presence or absence of infection, the presence of GVHD involvement in other organs, and medication, is essential to make a final diagnosis of GVHD. If a patient is highly suspected of GVHD, who also has GVHD involvement in the skin and/or liver in the absence of infection, then even a single or rare crypt apoptosis seen on the colon biopsy is highly suspicious for GVHD. Management of possible GVHD in transplantation patients requires a close interaction between or among clinicians and pathologists. Attempts should be made to identify other causes of apoptosis, such as infection or medication, before considering the diagnosis of GVHD. If no other causes are identified and GVHD is highly suspected, adding new immunosuppressant medication or increasing the current dose may be attempted under careful observation.

In summary, our study suggests that the presence of 6 or fewer crypt apoptotic bodies is not sufficient to render a diagnosis of GVHD in colon biopsies, given that apoptosis is a nonspecific finding seen in other confounding conditions of infection and medication. However, our data also demonstrate that, in the appropriate clinical setting, a single or rare crypt apoptosis should be mentioned in the pathologic report to alert the clinicians of the possibility of GVHD when all clinical, endoscopic, and pathologic data are taken into consideration. A consensus on the minimal number of apoptotic bodies required for GVHD diagnosis needs to be further clarified to better serve the needs of both pathologists and clinicians.

ACKNOWLEDGMENTS

The authors acknowledge Kerra Wagener and Tracey Bender for their assistance in the preparation of this manuscript.

REFERENCES

1. Iqbal NSD, Lazenby AJ, Wilcox CM. Diagnosis of gastrointestinal graft-versus-host disease. *Am J Gastroenterol*. 2000;95:3034–3038.
2. Appleton ALSL, Pearson AD, Green MA, et al. The need for endoscopic biopsy in the diagnosis of upper gastrointestinal graft-versus-host disease. *J Pediatr Gastroenterol Nutr*. 1993;16:183–185.
3. Yeo MKD, Kim YB, Oh TY, et al. Selective induction of apoptosis with proton pump inhibitor in gastric cancer cells. *Clin Cancer Res*. 2004;10:8687–8696.
4. Terdiman JPLC, Ries CA, Damon LE, et al. The role of endoscopic evaluation in patients with suspected intestinal graft-versus-host disease after allogeneic bone-marrow transplantation. *Endoscopy*. 1996;28:680–685.
5. Shulman HMKD, Lee SJ, Morton T, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. *Biol Blood Marrow Transplant*. 2006;12:31–47.
6. Bombí JANA, Carreras E, Ramírez J, et al. Assessment of histopathologic changes in the colonic biopsy in acute graft-versus-host disease. *Am J Clin Pathol*. 1995;103:690–695.
7. Snover DCWS, Vercellotti GM, Rank B, et al. A histopathologic study of gastric and small intestinal graft-versus-host disease following allogeneic bone marrow transplantation. *Hum Pathol*. 1985;16:387–392.

8. Sviland LPA, Eastham EJ, Hamilton PJ, et al. Histological features of skin and rectal biopsy specimens after autologous and allogeneic bone marrow transplantation. *J Clin Pathol*. 1988;41:148–154.
9. Sale GESH, McDonald GB, Thomas ED. Gastrointestinal graft-versus-host disease in man. A clinicopathologic study of the rectal biopsy. *Am J Surg Pathol*. 1979;3:291–299.
10. Samaha HSKG, Steele V, Rao CV, et al. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res*. 1997;57:1301–1305.
11. Cree IANS, Milne G, Beck JS. Cell death in granulomata: the role of apoptosis. *J Clin Pathol*. 1987;40:1314–1319.
12. Lerner KGKG, Storb R, Buckner CD, et al. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. *Transplant Proc*. 1974;6:367–371.
13. Brito GAFJ, Carneiro-Filho BA, Lima AA, et al. Mechanism of *Clostridium difficile* toxin A-induced apoptosis in T84 cells. *J Infect Dis*. 2002;186:1438–1447.
14. McCole DFEL, Laurent F, Kagnoff MF. Intestinal epithelial cell apoptosis following Cryptosporidium parvum infection. *Infect Immun*. 2000;68:1710–1713.
15. Bladon J. TP. Extracorporeal photopheresis induces apoptosis in the lymphocytes of cutaneous T-cell lymphoma and graft-versus-host disease patients. *Br J Haematol*. 1999;107:707–711.
16. Driman DK, Preiksaitis PH. Colorectal inflammation and increased cell proliferation associated with oral sodium phosphate bowel preparation solution. *Hum Pathol*. 1998;29:972–978.
17. Izeradjene K, Revillard J. Apoptosis of superantigen-activated T cells induced by mycophenolate mofetil treatment. *Transplantation*. 2001;71:118–125.
18. Parfitt JRJS, Driman DK. Mycophenolate mofetil-related gastrointestinal mucosal injury: variable injury patterns, including graft-versus-host disease-like changes. *Am J Surg Pathol*. 2008;32:1367–1372.
19. Nguyen CVKD, Choudhary C, Katz LC, et al. Is single-cell apoptosis sufficient for the diagnosis of graft-versus-host disease in the colon? *Dig Dis Sci*. 2008;53:747–756.