Histopathology

Histopathology 2016, **69**, 802–811. DOI: 10.1111/his.13003

Diagnostic phrasing is independently correlated with the decision to treat for graft-versus-host disease: retrospective review of colon biopsies with rare apoptosis

Daniel J Rowan, Christopher P Hartley, Luis F Carrillo-Polanco, Kiyoko Oshima & Catherine E Hagen

Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, USA

Date of submission 18 March 2016
Accepted for publication 28 May 2016
Published online *Article Accepted* 30 May 2016

Rowan D J, Hartley C P, Carrillo-Polanco L F, Oshima K & Hagen C E (2016) *Histopathology* **69**, 802–811. DOI: 10.1111/his.13003

Diagnostic phrasing is independently correlated with the decision to treat for graft-versus-host disease: retrospective review of colon biopsies with rare apoptosis

Aims: The risks of immunosuppression and the non-specific nature of rare crypt apoptosis has led to debate over the lower threshold for histological diagnosis of colonic graft-versus-host disease (GVHD). A recent study proposed the diagnostic category of indeterminate for GVHD (iGVHD) for cases with six or fewer apoptotic bodies per 10 crypts. Our aim was to assess colon biopsies with iGVHD histology to determine whether the diagnosis was retrospectively predictive of the decision to treat, and to correlate these findings with endoscopic and clinical findings.

Methods and results: A retrospective search was performed for colonic biopsies taken to evaluate for GVHD from 2008 to 2014. Biopsies were blindly reviewed for the maximum number of apoptotic bodies per 10 contiguous crypts, evidence of crypt dropout, and ulceration. Clinical information was collected through chart review. One hundred and twenty-two biopsies from 84 transplant patients were included. Forty-seven cases met the histological criteria for iGVHD. Patients with an original diagnosis of iGVHD were more likely to be managed conservatively than those with a diagnosis of grade 1 GVHD (25% versus 0%). Eight symptomatic patients reclassified as iGVHD had resolution of symptoms without increased immunosuppression. A clinicopathologically similar group of 10 patients with iGVHD histology, normal or subtle endoscopic findings and no evidence of GVHD at other organ sites were treated with increased immunosuppression. On multivariate analysis, the original diagnostic category was the most significant predictor of the decision to treat.

Conclusion: The use of the diagnostic category iGVHD alerts clinicians to the presence of minimal crypt apoptosis, and allows treatment based on clinical judgement.

Keywords: apoptotic colopathy, gastrointestinal, stem cell transplantation

Introduction

Graft-versus-host disease (GVHD) is a common complication of stem cell transplantation, often involving the gastrointestinal (GI) tract, skin, and liver. GVHD has significant associated morbidity and mortality, and requires immunosuppressive therapy for resolution. Unfortunately, immunosuppression also has associated morbidity and mortality, often related to the risk of viral infection.[1]

The diagnosis of GVHD is based on a combination of clinical, endoscopic and histological features. Biopsy may not be necessary in straightforward cases, but, in instances when the clinical symptoms are atypical or other causes are considered in the

Address for correspondence: Catherine Hagen, Department of Pathology, Medical College of Wisconsin, 9200 W. Wisconsin Ave, Milwaukee, WI 53226, USA. e-mail: cahagen@mcw.edu
D.J.R. and C.P.H. contributed equally to this work.

differential diagnosis, biopsy is essential for confirmation of the correct diagnosis.[2] Histologically, GI GVHD is characterized by crypt apoptosis and, in more severe cases, crypt dropout and mucosal ulceration. Crypt apoptosis by itself is non-specific and can also be seen as a result of infection or drug-induced injury. Thus, cases with only rare crypt apoptosis raise a diagnostic dilemma for pathologists.[3–9]

Several histological grading systems have been proposed over the years. The 1974 Lerner system is commonly used, and requires the presence of a single apoptotic body to establish a diagnosis of GVHD.[10] Studies in recent years have challenged the lower threshold for a diagnosis of GVHD. Some have proposed using more than one or more than two apoptotic bodies per biopsy fragment, whereas others have proposed using more than six apoptotic bodies per 10 contiguous crypts, which is similar to the criterion used for the diagnosis of acute cellular rejection in small-bowel transplants.[11]

Lin *et al.* proposed the diagnostic category of indeterminate for GVHD (iGVHD) for colonic biopsies with six or fewer apoptotic bodies per 10 contiguous crypts; cases with more than six apoptotic bodies per 10 contiguous crypts were considered to be grade 1 GVHD. In this article, the group described four symptomatic patients with the histological appearance of iGVHD in whom clinical resolution was achieved without steroid therapy, challenging the idea that rare crypt apoptosis is sufficient for a diagnosis of GVHD. The study also found rare crypt apoptosis in ~20% of normal colon biopsies from non-transplant patients, supporting the non-specific nature of rare crypt apoptosis.[12]

In practice at our institution, we have adopted the category of iGVHD for biopsies with six or fewer apoptotic bodies per 10 contiguous crypts. The aims of this study were to assess colon biopsies with one to six apoptotic bodies per 10 contiguous crypts, and to determine whether the diagnostic phrasing was retrospectively predictive of the decision to treat and patient outcome. We also applied the histological criteria for iGVHD to a cohort of patients with colonic biopsy prior to 2013, and correlated these findings with endoscopic findings and the presence of GVHD at other organ sites in order to identify a cohort of patients who could potentially be spared increased immunosuppression.

Materials and Methods

PATIENT POPULATION

This study was approved by the Institutional Review Board. A retrospective search of our pathology database was performed for colonic biopsies taken to evaluate for GVHD from 2008 to 2014. Cases diagnosed as iGVHD were retrieved from 2013 to 2014. A cohort of cases from 2008 to 2013 was also retrieved to retrospectively apply iGVHD criteria. Solid organ transplant patients, patients who had no available clinical information, asymptomatic patients and patients with evidence of cytomegalovirus (CMV) colitis on biopsy were excluded. Patients who had an original diagnosis of iGVHD or grade 1 GVHD on colonic biopsies, but who had an original diagnosis of grade 2–4 GVHD based on upper GI tract biopsies, were excluded to enrich for patients with overall mild disease, as the main focus of this study was to evaluate the lower diagnostic threshold for GVHD based on colonic biopsy.

CLINICAL INFORMATION

Clinical information, including underlying disease, symptoms at time of biopsy, time from transplantation, treatment regimens, evidence of GVHD at other organ sites, endoscopic findings, clinical evidence of infection, and patient outcome, was collected from chart review. Increased immunosuppression following endoscopy was defined as either initiation of steroid therapy or an increase in the dose of immunosuppressant agents that the patient was receiving prior to endoscopy. Symptom resolution was defined as resolution of symptoms within 1 month of treatment. Endoscopic findings were categorized into three groups: (i) normal; (ii) subtle findings, including loss of vascular pattern, oedema, melanosis coli, and/or patchy erythema; and (iii) ulcer or exudate, including diffuse erythema. A skin or liver biopsy diagnostic of GVHD or characteristic clinical features such as skin rash or elevated aminotransferases and/or alkaline phosphatase were considered to be evidence of GVHD at extracolonic organ sites.

HISTOLOGICAL ASSESSMENT

All biopsies were retrospectively reviewed blindly by one pathologist with experience in GI pathology (C.H.). Colonic biopsies were assessed for the maximum number of apoptotic bodies per 10 contiguous crypts, evidence of crypt dropout, and evidence of ulceration. Biopsies with one to six apoptotic bodies per 10 contiguous crypts and an absence of crypt dropout or ulceration were considered to be diagnostic of iGVHD. Biopsies with more than six apoptotic bodies per 10 contiguous crypts or evidence of crypt dropout or ulceration were considered to be

diagnostic of GVHD, and regraded according to the Lerner system: grade 1 = crypt apoptosis (more than six apoptotic bodies per 10 contiguous crypts) without evidence of crypt loss; grade 2 = individual crypt loss; grade 3 = contiguous crypt loss; and grade 4 = extensive crypt loss with ulceration (Figure 1). Biopsies with no evidence of crypt apoptosis were considered to be negative for GVHD. If a patient had more than one colonic biopsy specimen per endoscopy, the biopsy with the highest overall grade was used for analysis. The number of colonic biopsy fragments per endoscopy and the number of tissue levels examined were recorded. The original diagnosis was later collected from the corresponding pathology report. Corresponding upper GI tract biopsies for colonic biopsies reclassified as iGVHD or normal were reviewed and regraded similarly to the colonic biopsies. Previously performed CMV immunohistochemical stains were reviewed.

STATISTICAL ANALYSIS

ANOVA was used to compare continuous variables between groups, and the chi-square test or Fisher's exact test was used to compare categorical variables by use of the PRISM statistical program (GraphPad, San Diego, CA, USA). For comparison of categorical and continuous independent variables with the categorical binary outcome variable (decision to treat), univariate and multivariate logistic regression were used (Microsoft Excel 2010, Redmond, WA, USA).

Results

DEMOGRAPHIC INFORMATION

One hundred and twenty-two biopsies from 84 transplant patients and 97 endoscopy procedures were included in the study; 11 patients had two sets of biopsies included in the study, and one patient had three sets of biopsies. Thirty-seven patients were male, and 47 were female; the mean patient age at time of biopsy was 53.6 years (range: 22–73 years). Sixty-four patients underwent peripheral blood stem cell transplantation, 17 underwent bone marrow transplantation, and three underwent cord blood transplantation. Seventeen of the stem cell transplant patients underwent autologous stem cell transplantation, with indications for transplantation including multiple myeloma (n = 14) and non-Hodgkin's lymphoma (n = 3). The original indications for allogeneic transplantation were as follows: acute myeloid leukaemia (n = 30), multiple myeloma (n = 8), non-Hodgkin's lymphoma (n = 16), acute lymphoblastic leukaemia (n = 6), myelofibrosis (n = 2), myelodysplastic syndrome (n = 2), classical Hodgkin lymphoma (n = 1), acute promyelocytic leukaemia (n = 1), and aplastic anaemia (n = 1).

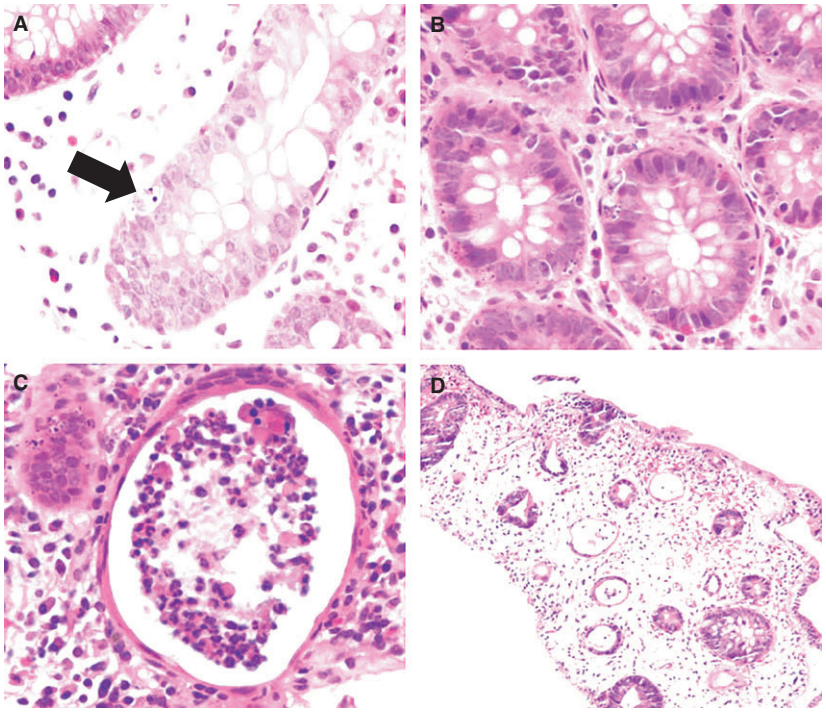

Figure 1. (A) A case with rare crypt apoptosis (arrow) histologically classified as indeterminate for graft-versus-host disease. **(B–D)** Cases with definitive evidence of graft-versus-host disease showed abundant apoptosis (more than six apoptotic bodies per 10 crypts) (B), single crypt dropout **(C)**, and contiguous crypt dropout **(D)**.

The average time from transplantation to endoscopy was 287 days (range: 9–3650 days). Twelve biopsies were performed <21 days post-transplantation, and two biopsies were performed on day 21 post-transplantation. The majority of biopsies performed <21 days post-transplantation ($n = 8$) were from autologous stem cell transplant patients.

HISTOLOGICAL FEATURES OF COLON BIOPSIES AND DECISION TO TREAT

The original pathological diagnoses were as follows: 18 negative for GVHD, eight iGVHD, 43 grade 1 GVHD, and 28 grade 2–4 GVHD. The mean number of apoptotic bodies per 10 crypts increased with increasing grade. In patients with an original diagnosis of negative for GVHD, the mean apoptotic body count per 10 crypts was 1.2, whereas the count was 2.5 for iGVHD, 5.4 for grade 1 GVHD, and 10.1 for grade 2–4 GVHD ($P < 0.0001$) (Table 1).

After blind review and reclassification, 47 cases met the histological criteria for iGVHD: 12 of 18 negative cases, eight of eight iGVHD cases, 26 of 43 grade 1 GVHD cases, and one of 28 grade 2–4 GVHD cases. One case originally diagnosed as grade 2 GVHD was reclassified as iGVHD on retrospective review, owing to lack of crypt dropout and the presence of three apoptotic bodies per 10 contiguous crypts. The decision to treat patients for GVHD was inversely correlated with the original GVHD grade; 50% of patients with biopsies negative for GVHD, 25% of patients with iGVHD biopsies and 0% of patients with grade 1–4 GVHD biopsies were untreated ($P < 0.0001$). Symptom resolution was similar among all diagnostic categories ($P = 0.81$) (Table 1).

Table 1. Correlation of original pathological diagnosis with apoptotic count, histological reclassification, treatment, and symptom resolution

Original diagnosis	Mean apoptotic bodies per 10 crypts (range)	Reclassified as iGVHD, *n* (%)	Not treated, *n* (%)	Symptom resolution, *n* (%)
Negative (18/97)	1.2 (0–4)	12/18 (67)	9/18 (50)	16/18 (89)
iGVHD (8/97)	2.5 (1–5)	8/8 (100)	2/8 (25)	7/8 (88)
Grade 1 GVHD (43/97)	5.4 (0–22)	26/43 (60)	0/43 (0)	36/43 (84)
Grade 2–4 GVHD (28/97)	10.1 (0–27)*	1/28 (4)	0/28 (0)	22/28 (79)

GVHD, graft-versus-host disease; iGVHD, indeterminate for graft-versus-host disease.

*One case with grade 4 GVHD had diffuse mucosal ulceration with nearly complete loss of crypts, and therefore had zero crypt apoptosis present.

Nine patients with colon biopsies negative for GVHD were treated with increased immunosuppression. Six patients had evidence of GVHD at other organ sites (four skin and two liver). Two patients were treated on the basis of endoscopic findings suggestive of GVHD, including erythema and oedema, despite negative biopsies. One patient was treated empirically despite the negative colonic biopsy because of severe GI symptoms, including nausea, vomiting, and diarrhoea.

The average number of biopsy levels examined was 9.1 (range: 3–16), and the average number of biopsy fragments per endoscopy was 10.9 (range: 3–26). There was no significant difference in the number of biopsy fragments examined across diagnostic categories ($P = 0.33$). Immunohistochemical staining for CMV had been performed on 82 of 97 sets of biopsies, and gave negative results in all cases. No histological evidence of viral inclusions was seen in the remaining 15 sets of biopsies.

CORRELATION OF HISTOLOGICAL AND ENDOSCOPIC FINDINGS

Reports were available for 72 endoscopies, including 30 of the endoscopies for patients reclassified into the iGVHD category. Endoscopic findings in iGVHD patients were almost exclusively normal or subtle (93%). Eighty-nine per cent of patients with more than six apoptotic bodies per 10 contiguous crypts without crypt dropout (grade 1 GVHD) and 50% of patients with histological evidence of crypt loss and/or ulceration (grade 2–4 GVHD) had normal or subtle endoscopic findings.

CORRELATION OF HISTOLOGICAL FINDINGS WITH EVIDENCE OF GVHD AT OTHER ORGAN SITES

The majority of patients (63.9%) had evidence of GVHD at other organ sites. The most common site of GVHD outside of the colon was the skin, followed by the liver. Other rare organ sites of involvement included the mouth, kidney, eye, and lung. The prevalence of extracolonic GVHD was similar among groups regardless of the histological findings on colonic biopsies ($P = 0.62$) (Table 2).

Table 2. Correlation of histological reclassification and evidence of graft-versus-host disease (GVHD) at other organ sites

Colonic GVHD grade	Evidence of GVHD outside of the colon, *n* (%)	Organ site, *n* (%)			
		Skin	Upper GI tract	Liver	Other sites*(%)
Negative (*n* = 8)	6 (75)	4 (50)	0 (0)	3 (37.5)	1 (12.5)
iGVHD (*n* = 47)	27 (57.4)	20 (42.6)	4 (8.5)	5 (10.6)	2 (4.3)
Grade 1 (*n* = 9)	6 (66.7)	5 (55.6)	3 (33.3)	2 (22.2)	1 (11.1)
Grade 2–4 (*n* = 33)	23 (69.7)	15 (45.5)	6 (18.2)	12 (36.4)	1 (3)
Total (*n* = 97)	62 (63.9)	44 (45.4)	13 (13.4)	22 (22.7)	5 (5.2)

GI, gastrointestinal; iGVHD, indeterminate for GVHD.
*Other sites include the mouth, kidney, eye, and lung; some patients had evidence of GVHD at multiple organ sites.

Fifty-six patients had corresponding upper GI tract biopsies, including 27 patients reclassified as iGVHD on colonic biopsy, and six patients with normal colonic biopsies. Overall, 13 patients had diagnostic features of GVHD on upper GI tract biopsies. Of the patients with iGVHD findings on colonic biopsies, three had normal upper GI tract biopsies, 20 had iGVHD histology, three had findings consistent with grade 1 GVHD, and one had evidence of grade 3 GVHD. The one patient with grade 3 GVHD in the upper GI tract was originally diagnosed as having grade 1 GVHD, but, on retrospective review, an area of contiguous crypt dropout was noted. Of the patients with normal colonic biopsies, two had normal upper GI tract biopsies and four had findings of iGVHD.

SYMPTOM RESOLUTION IN IGVHD PATIENTS

Of the 47 patients reclassified into the iGVHD category, 39 (83%) were treated with increased immunosuppression. Thirty-four (87.2%) of these patients had resolution of their symptoms after treatment. Four patients (10.3%) did not respond to therapy, and one patient was lost to follow-up. Five autologous stem cell transplant patients were reclassified into the iGVHD category; all were treated for GVHD, with resolution of symptoms. Four patients within the iGVHD category had biopsies performed <21 days following transplantation, and two of these had concurrent evidence of skin GVHD; all were treated for GVHD, with resolution of symptoms.

Eight patients who met the diagnostic criteria for iGVHD were not treated with increased immunosuppression. One of the eight untreated iGVHD patients was receiving mycophenolate at the time of biopsy (case 5, Table 3). Full clinical and histological characteristics of this patient population are summarized in Table 3.

CORRELATION OF TREATMENT, PRESENCE OF GVHD AT OTHER SITES AND ENDOSCOPIC FINDINGS IN PATIENTS WITH IGVHD HISTOLOGY

To further subclassify the iGVHD group, endoscopic findings were correlated with the decision to increase immunosuppression and the presence of GVHD at other sites. Four patients with iGVHD histology, normal or subtle endoscopic findings and no evidence of GVHD at other sites were not treated with increased immunosuppression, and all four had resolution of their symptoms (cases 4–7, Table 3). Ten patients with iGVHD histology were treated despite lack of evidence of GVHD at other sites and normal or subtle endoscopic findings (Table 4). Symptoms resolved in nine of the patients after treatment. The patient without symptom resolution was treated for *Clostridium difficile* infection. One additional patient in this cohort was treated for *C. difficile* infection, but the remaining eight patients had no evidence of GI infection. Only one of the 10 patients was receiving mycophenolate at the time of biopsy.

Three patients with normal or subtle endoscopic findings and evidence of possible extracolonic GVHD were not treated with increased immunosuppression. One patient had eight apoptotic bodies per 10 contiguous crypts in the ileum. The other two patients had evidence of a skin rash. All had resolution of symptoms without further treatment for GVHD. Clinical information for these patients is summarized in Table 3 (cases 1, 2, and 8).

On univariate analysis, endoscopic findings ($P = 0.046$) and original histological grade ($P < 0.001$) were the only clinicopathological

Table 3. Summary of histological and clinical findings in eight untreated patients with indeterminate for graft-versus-host disease (iGVHD) histology

Case	Original histological Dx	Upper GI Bx findings	Days from TPX	Type of TPX	Extraintestinal GVHD	Evidence of GI infection	Endoscopic findings	Symptoms	Clinical Dx
1	iGVHD	Grade 1 GVHD in ileum (8 ap./10)	43	Allo-PBSCT	No	CMV NAAT-positive (CMV IHC-negative); started on ganciclovir	Normal	Three loose stools/day, nausea and emesis; diarrhoea resolved. Given odansetron and prochlorperazine; nausea improved	Aetiology unknown; possible CMV
2	iGVHD	iGVHD in ileum (2 ap./10)	150	Allo-BMT	Yes, skin rash with prior Bx confirming skin GVHD; resolved 1 month prior to colonoscopy	No	Mild erythema and punctate haemorrhage in TI, patchy oedema in caecum	Four watery stools/day; diarrhoea spontaneously resolved following colonoscopy. No further treatment given	Aetiology unknown
3	Negative	NA	26	Allo-PBSCT	Yes, skin rash on shins; thought to be a drug reaction	CMV NAAT-positive; started on ganciclovir	NA	Diarrhoea (severity unspecified); resolved following CMV treatment	CMV infection
4*	Negative	iGVHD in ileum (2 ap./10)	37	Allo-PBSCT	No	No	Normal	Fever, nausea, diarrhoea (maximum volume of 475 ml), abdominal pain	Fever of unknown origin
5*	Negative	NA	46	Allo-BMT	No	No	Normal	Occasional loose stools with incontinence, nausea, and anorexia; PEG tube placed for enteral feeding	Aetiology unknown
6*	Negative	iGVHD in ileum (2 ap./10)	66	Allo-PBSCT	No	*Clostridium difficile*, treated with vancomycin	Areas of punctate erythema	Ten to 12 loose stools/day; resolved following treatment for *C. difficile*	*C. difficile* infection
7*	Negative	NA	65	Allo-PBSCT	No	No	Normal	Three loose stools/day; diarrhoea resolved without further treatment	Aetiology unknown
8	Negative	NA	170	Allo-BMT	Yes, skin rash; resolved with triamcinolone cream	CMV NAAT-positive 4 months previously; negative at time of colonoscopy	Normal	Initially, the patient had abdominal pain and was treated for ileus; started having loose stools. Diarrhoea resolved without further treatment	Aetiology unknown

Allo, allogeneic; ap./10, apoptotic bodies per 10 crypts; BMT, bone marrow transplantation; Bx, biopsy; CMV, cytomegalovirus; Dx, diagnosis; GI, gastrointestinal; GVHD, graft-versus-host disease; IHC, immunohistochemistry; NA, not applicable; NAAT, nucleic acid amplification test; PBSCT, peripheral blood stem cell transplantation; PEG, percutaneous endoscopic gastrostomy; TI, terminal ileum; TPX, transplant.

*Cases comprise a subset of four untreated patients with iGVHD histology, no evidence of extracolonic GVHD, and available endoscopy reports with normal/subtle findings.

Table 4. Management, presence of graft-versus-host disease (GVHD) at other sites and endoscopic findings in patients reclassified as indeterminate for GVHD

	Not treated		Treated		
Endoscopic findings	No GVHD at other sites	Evidence of GVHD at other sites	No GVHD at other sites	Evidence of GVHD at other sites	Total
Normal	3	2	3	8	16
Subtle	1	1	7	3	12
Ulcer/ exudate	0	0	0	2	2
Total	4	3	10	13	30

variables that were significantly associated with the decision to treat. On multivariate analysis, the original diagnostic category remained significantly correlated with the decision to treat [$P = 0.01$, odds ratio (OR): 27.9, 95% confidence interval (CI): 2.4–324.5]; younger age was also significantly correlated with the decision to treat on multivariate analysis ($P = 0.04$, OR: 0.8, 95% CI: 0.71–0.99) (Table 5).

Discussion

The diagnosis of GVHD depends on a combination of clinical, endoscopic and histological features. Historically, the presence of one apoptotic body has been considered to be sufficient for the diagnosis of colonic GVHD.[3,10] Because of the low specificity of rare apoptosis and the risk posed by increased immunosuppression, the minimum criterion for the diagnosis of GVHD has recently been questioned.[11] Lin *et al.* proposed an iGVHD category, defined as having one to six apoptotic bodies per 10 contiguous crypts without evidence of crypt dropout, and described four symptomatic patients with iGVHD histology whose symptoms resolved without increased immunosuppression.[12]

We blindly re-examined colon biopsies taken to evaluate for GVHD from 97 endoscopy procedures for the maximum number of apoptotic bodies per 10 contiguous crypts. Nearly half of the cases (48.5%) within our cohort were reclassified within the iGVHD histological range, including cases originally diagnosed as negative for GVHD and grade 1 GVHD. Despite similar histological findings in patients originally diagnosed as negative for GVHD, iGVHD, and grade 1 GVHD, treatment decisions differed significantly between these diagnostic categories. Patients with an original diagnosis of negative for GVHD and iGVHD were more likely to be managed conservatively without increased immunosuppression (50% and 25%, respectively) than patients diagnosed with grade 1 GVHD (0%). Despite widely differing rates of treatment, the response to treatment was similar among the three groups. Furthermore, on multivariate analysis, the original diagnostic category was the most significant predictor of the decision to treat when other confounding variables were controlled for. This shows the impact of the pathologist's diagnostic language on the clinician's decision to treat. Whereas a clinician may feel obliged to increase immunosuppression in a patient who has a diagnosis of grade 1 GVHD, the clinician may be more likely to take a conservative approach to treatment in patients with a diagnosis of iGVHD. The diagnostic category of iGVHD potentially spares increased immunosuppression for some patients who would have previously been labelled as having grade 1 GVHD. Likewise, use of the diagnostic phrasing of iGVHD may increase the risk of treatment for some patients with rare apoptosis who would have been labelled as being negative for GVHD. However, these cases are best labelled as iGVHD, given that rare apoptosis may still represent GVHD. The diagnostic category iGVHD allows clinicians to treat patients with rare apoptosis on the basis of clinical judgement.

Eight symptomatic patients within our cohort fell into the diagnostic category of iGVHD, and did not receive increased immunosuppression. All eight patients had symptom resolution despite lack of treatment for GVHD and the presence of rare crypt apoptosis. These findings add support to Lin's previous report of four patients with iGVHD findings and symptom resolution without treatment,[12] further challenging the need to treat all transplant patients with rare crypt apoptosis. To further define a potential cohort of patients who could be spared therapy for GVHD, we correlated histological and endoscopic findings with the decision to treat and the presence of GVHD at other organ sites in the 30 iGVHD patients with available endoscopic reports. Within this subgroup, four symptomatic patients with no evidence of extracolonic GVHD and normal or subtle endoscopic findings had resolution of their symptoms without treatment. A similar group of 10 patients with histological findings of iGVHD, no evidence of GVHD at other organ sites and normal or subtle endoscopic findings were treated with increased immunosuppression. On the basis of the improvement of symptoms in the untreated group, we suggest that this

Table 5. Correlation of clinicopathological variables with decision to treat

Clinicopathological feature	Treated	Not treated	Univariate analysis *P*-value	Multivariate analysis OR	95% CI	*P*-value
Endoscopic findings, *n* (%)			**0.046**	9.3	0.8–97.7	0.06
Normal	24/31 (77.4)	7/31 (22.6)				
Subtle	21/24 (87.5)	3/24 (12.5)				
Ulcer/exudate	17/17 (100)	0/17 (0)				
Maximum original grade, *n* (%)			**<0.001**	27.9	2.4–324.5	**0.01**
Normal	9/18 (50)	9/18 (50)				
iGVHD	6/8 (75)	2/8 (25)				
Grade 1	43/43 (100)	0/43 (0)				
Grade 2	10/10 (100)	0/10 (0)				
Grade 3	10/10 (100)	0/10 (0)				
Grade 4	8/8 (100)	0/8 (0)				
Evidence of extracolonic GVHD, *n* (%)			0.18	0.8	0.04–14.6	0.89
Yes	57/62 (91.9)	5/62 (8.1)				
No	29/35 (82.9)	6/35 (17.1)				
On mycophenolate, *n* (%)			0.14	12.9	0.13–1239.6	0.27
Yes	27/28 (96.4)	1/28 (3.6)				
No	56/66 (84.8)	10/66 (15.2)				
Evidence of GI infection, *n* (%)			0.83	1.4	0.08–25.3	0.81
Yes	18/20 (90)	2/20 (10)				
No	68/77 (88.3)	9/77 (11.7)				
Age (years), mean (range)	52.7 (22–73)	60.2 (41–70)	0.05	0.8	0.71–0.99	**0.04**
Type of transplantation, *n* (%)			0.99			
Autologous	17/17 (100)	0/17 (0)				
Allogeneic	69/80 (86.2)	11/80 (13.8)				
Sex, *n* (%)			0.47			
Female	49/54 (90.7)	5/54 (9.3)				
Male	37/43 (86)	6/43 (14)				

CI, confidence interval; GI, gastrointestinal; GVHD, graft-versus-host disease; iGVHD, indeterminate for graft-versus-host disease; OR, odds ratio. Bold values are statistically significant, $p < 0.05$.

additional group of 10 patients could have potentially been spared increased immunosuppressive therapy. A group of patients with similar clinical, endoscopic and histological findings could be used as a starting point for future prospective studies investigating the need for treatment of patients with iGVHD histology.

In the 2014 National Institutes of Health (NIH) GVHD consensus guidelines, final diagnostic categories include not GVHD, possible GVHD, and likely GVHD. Possible GVHD is defined as 'evidence of GVHD but with other possible explanations'. Furthermore, cases with 'single or rare apoptotic epithelial

changes without other features of active GVHD and no alternative explanations' are placed in the likely GVHD category.[2] Unfortunately, full clinical information is not always available to pathologists at the time of biopsy review, or the clinical diagnosis may be uncertain. Four iGVHD patients in our cohort had no clinical explanation for crypt apoptosis, including lack of infection or mycophenolate use, and symptomatically resolved without treatment, arguing that these patients are best categorized as iGVHD or possible GVHD. In the future, iGVHD criteria could be incorporated into the NIH guidelines to provide histological criteria in addition to clinical criteria for distinguishing cases of possible (i.e. indeterminate) GVHD and likely GVHD.

Biopsies from both allogeneic and autologous stem cell transplant patients were included in this study. Two prior series of GI GVHD occurring in autologous stem cell transplant patients have been published from our institution, and the clinicians therefore have a specific awareness of GVHD occurring in this patient population.[13,14] Cases from autologous patients make up a significant proportion of colon biopsies taken to 'rule out GVHD' at our institution, and similarly to the situation for biopsies from allogeneic patients, the lower diagnostic threshold for GVHD in this patient population has not been firmly established.

Overall, the iGVHD group is heterogeneous, with some patients having convincing clinical evidence of GVHD, some having an uncertain clinical picture, and some having alternative explanations for apoptosis, such as infection or drug use. Four patients within our iGVHD cohort had biopsies performed <21 days post-transplantation, and all were treated. GVHD is difficult to histologically diagnose in this time period, because the cytotoxic effects of conditioning chemotherapy and total body irradiation cause histological change that is indistinguishable from that caused by GVHD.[8] However, a subset of patients, especially autologous stem cell transplant patients, develop symptoms of GVHD soon after transplantation.[14] Depending on the extent of histological change and clinical context, biopsies with scattered crypt apoptosis in this time period may best be interpreted as iGVHD, given the confounding cytotoxic effects. Further studies to examine the evaluation of biopsies performed <21 days post-transplantation, especially in autologous stem cell transplant patients, are necessary.

Our study is limited by its retrospective nature, meaning that biopsy sampling was not performed in a standardized fashion in terms of sites sampled and number of biopsies taken. However, the numbers of biopsy fragments examined across diagnostic categories were statistically similar, and it is therefore unlikely that biopsy sampling was a source of diagnostic bias within our study.

The findings of this study support the use of iGVHD as a distinct diagnostic category with prognostic and treatment implications. The use of this diagnostic category alerts clinicians to the presence of minimal crypt apoptosis, and allows them to treat on the basis of clinical judgement.

Acknowledgements

IRB approval, including waiver of informed consent, was obtained for this work; initial approval 17 November 2014, study ID PRO00023473. No funding was received for this project.

Conflict of interest

The authors state that they have no conflicts of interest.

Author contributions

Daniel J. Rowan contributed to data collection, data analysis, and writing and review of the manuscript. Christopher P. Hartley contributed to design of the research study, data collection, data analysis, and writing and review of the manuscript. Luis F. Carrillo-Polanco contributed to data collection and review of the manuscript. K. Oshima contributed to design of the research study and review of the manuscript. Catherine E. Hagen contributed to design of the research study, data collection, data analysis, and writing and review of the manuscript.

References

1. Arnaout K, Patel N, Jain M *et al.* Complications of allogeneic hematopoietic stem cell transplantation. *Cancer Invest.* 2014; **32**; 349–362.
2. Jagasia MH, Greinix HT, Arora M *et al.* National Institutes of Health consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol. Blood Marrow Transplant.* 2015; **21**; 389–401e1.
3. Nguyen CV, Kastenberg DM, Choudhary C *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.
4. Washington K, Bentley RC, Green A *et al.* Gastric graft-versus-host disease: a blinded histologic study. *Am. J. Surg. Pathol.* 1997; **21**; 1037–1046.

5. Selbst MK, Ahrens WA, Robert ME *et al.* Spectrum of histologic changes in colonic biopsies in patients treated with mycophenolate mofetil. *Mod. Pathol.* 2009; **22**; 737–743.
6. Brito GA, Fujji J, Carneiro-Filho BA *et al.* Mechanism of *Clostridium difficile* toxin A-induced apoptosis in T84 cells. *J. Infect. Dis.* 2002; **186**; 1438–1447.
7. McCole DF, Eckmann L, Laurent F *et al.* Intestinal epithelial cell apoptosis following *Cryptosporidium parvum* infection. *Infect. Immun.* 2000; **68**; 1710–1713.
8. Epstein RJ, McDonald GB, Sale GE *et al.* The diagnostic accuracy of the rectal biopsy in acute graft-versus-host disease: a prospective study of thirteen patients. *Gastroenterology* 1980; **78**; 764–771.
9. Snover DC. Mucosal damage simulating acute graft-versus-host reaction in cytomegalovirus colitis. *Transplantation* 1985; **39**; 669–670.
10. Lerner KG, Kao GF, Storb R *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.
11. Shulman HM, Cardona DM, Greenson JK *et al.* NIH consensus development project on criteria for clinical trials in chronic graft-versus-host disease: II. The, Pathology Working Group Report. *Biol. Blood Marrow Transplant.* 2014; **2015**; 589–603.
12. Lin J, Fan R, Zhao Z *et al.* 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. *Am. J. Surg. Pathol.* 2013; **37**; 539–547.
13. Drobyski WR, Hari P, Keever-Taylor C *et al.* Severe autologous GVHD after hematopoietic progenitor cell transplantation for multiple myeloma. *Bone Marrow Transplant.* 2009; **43**; 169–177.
14. Cogbill CH, Drobyski WR, Komorowski RA. Gastrointestinal pathology of autologous graft-versus-host disease following hematopoietic stem cell transplantation: a clinicopathological study of 17 cases. *Mod. Pathol.* 2011; **24**; 117–125.