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Association between histopathological alterations and diarrhea severity in acute intestinal graft-versus-host disease

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Abstract

Gastrointestinal (GI) acute graft-versus-host disease (aGVHD) remains one of the most important complications of allogeneic hematopoietic cell transplantation (allo-HCT). The diagnosis of this complication is largely dependent on clinical symptoms, but GI biopsies are warranted in most cases, due to the multitude of potential causes that coexist in patients with a clinical suspicion of this complication. In addition, several lines of evidence support that the GI is not only a target organ in aGVHD, but also a key mediator of the pathogenesis of this condition. Controversy exists on whether histopathological findings are associated with clinical severity. Crypt loss is a relatively straightforward histological finding of GI aGVHD, whose presence has been associated with disease severity in a previous study.

In order to independently validate this association, we retrospectively evaluated all histological changes from 25 patients with confirmed GI aGVHD who underwent allo-HCT in our center from 2008 to 2014. Clinical, laboratory, and histological data were obtained from the medical records and pathological reports. All GI biopsies were reviewed by 2 investigators blinded to clinical data, who classified GI aGVHD according to the presence of severe crypt loss.

The proportion of patients with grades I–II and III–IV aGVHD patients in our population were 45.5% and 54.5%, respectively. The most common histological alterations were isolated apoptotic bodies, present in 80% of colon biopsies with aGVHD. Severe crypt loss, corresponding to grades III–IV aGVHD was associated with higher stool volumes ($P = .02$) and increased diarrhea duration ($P = .02$), but not with response to steroids or mortality.

In this study, we independently validated that the presence of severe crypt loss, a reliable and simple parameter to grade the extension of GI aGVHD, is associated with disease severity in GI aGVHD.

Abbreviations: aGVHD = acute graft-versus-host disease, allo-HCT = allogeneic hematopoietic cell transplantation, allo-HSCT = allogeneic hematopoietic stem cell transplantation, CLN = colonoscopy, CMV = cytomegalovirus, GI = gastrointestinal, GVHD = graft-versus-host disease, HD = high dose, HLA = human leukocyte antigens, LD = low dose, MMF = micophenolate mofetil, TRM = transplant-related mortality, UGE = upper gastrointestinal endoscopy.

Keywords: crypt loss, graft versus host disease, histopathological, intestinal

1. Introduction

Acute graft-versus-host disease (aGVHD) is one of the main complications of allogeneic hematopoietic cell transplantation (allo-HCT) which, despite improvements in conditioning and supportive care, remains lethal for a large proportion of patients.[1,2] The diagnosis and grading of aGVHD is based on classical criteria that considers signs and symptoms affecting the skin, liver, and gastrointestinal (GI) tract.[3–5] Severity of aGVHD according to these criteria is associated with important outcomes, and patients with grades III–IV aGVHD present a significantly higher mortality.[6] In the GI tract, aGVHD manifests predominantly as an intense diarrhea that can evolve to bleeding, severe abdominal pain, and ileus.[2]

In the last decade, several lines of evidence indicated that the GI tract presents a prominent role in aGVHD, not only as a major target organ, but also as a mediator of the pathogenesis of this condition. Severity of aGVHD has also been associated with the integrity of the epithelial barrier in a study with 38 allo-HCT recipients, suggesting that barrier breakdown in the early phases of aGVHD could facilitate T-cell mediated response that characterizes this condition.[7] In addition, intestinal cells were identified as key mediators of the pathogenesis of aGVHD,[8] and characteristics of the microbiota were associated with aGVHD severity and mortality.[9] Finally, some of the most promising and important biomarkers of aGVHD identified in the last years have their function directly or indirectly associated with the GI tract.[10–12]

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Although the diagnosis of GI aGVHD relies mostly on clinical symptoms, GI biopsies are important due to the multitude of alternative causes for diarrhea in those patients. However, controversy exists on the precise association between histopathological findings and clinical severity.[7] In particular, the presence of crypt loss has been previously associated with aGVHD disease,[13] but this observation has not been independently validated. Here, we retrospectively evaluated the histological findings in a consecutive cohort of patients who underwent allo-HCT, in whom a clinical suspicion of aGVHD resulted in the performance of GI biopsies.

2. Methods

This was a retrospective single-center observational study that analyzed clinical and histopathology data from patients who received an allo-HCT between April 2007 and February 2014 at University of Campinas, Brazil. All consecutive patients with a clinical suspicion of GI aGVHD submitted to upper gastrointestinal endoscopy (UGE) or colonoscopy (CLN) in the post-HCT period, for whom GI biopsies were available, were eligible for the study. Patients were identified from the electronic database of GI biopsies from our institution, through a search using the expression. "GVHD" in the test request. Demographic and clinical data were obtained from the records of the HCT Unit and from the electronic laboratory database.

The study was approved by the local Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

2.1. Transplantation procedure and definitions

Conditioning regimens were classified as high (HD) or low dose (LD) based on previously published criteria.[14] Pharmacologic GVHD prophylaxis consisted of at least 2 agents, including a calcineurin inhibitor. No donor grafts were depleted of T cells, and all procedures were performed from human leukocyte antigens (HLA) matched related donors. aGVHD was diagnosed and graded according to classical criteria.[3–5] Data from the frequency of bowel movements and stool volume were obtained from the medical and nurse records of the HCT unit.

2.2. Evaluation of histological findings

Initially, histological findings of biopsies were obtained from the original pathology reports. Our institutional protocol recommends that in the evaluation of patients with suspected GI aGVHD, at least 1 biopsy sample is obtained from each colon portion ascending, transverse, and sigmoid, as well as from other areas with macroscopic alterations, unless limited by technical issues. All specimens were stained with hematoxylin and eosin. In these reports, histological findings described as compatible with GI aGVHD included: presence of apoptotic bodies, crypt abscesses, crypt loss of individual or contiguous crypts, or total mucosa denudation or ulceration. Although these alterations are used in the classical grading system used to classify the histological findings of aGVHD,[15–17] grading was not included in all original reports. Therefore, all cases were reviewed by 2 investigators (LNGC and RBC) that were blinded to the clinical outcome. In this review, the histological findings were graded using a modification of the grading system described by Lerner et al[18]: grade I—isolated apoptotic bodies with not crypt loss; grade II—isolated crypt loss; grade III—loss of ≥2 contiguous crypts; grade IV—extensive crypt loss with mucosal denudation. According to the definition used in the study that associated crypt loss with poorer outcomes, severe crypt loss was defined as grades III or IV GVHD.[13]

2.3. Statistical analyses

Results are expressed as means, medians, standard deviation, and ranges. Categorical and continuous variables are compared using the Fisher exact or Mann–Whitney *U* test, respectively. Graphpad Prism v6.0 (GraphPad Software, Inc., California) was used to analyze and present the data.

3. Results

During the study period, a total of 203 patients were submitted to allo-HCT in our Center. GI biopsies due to a clinical suspicion of aGVHD were performed in 25 patients, which were included in this retrospective analysis. The clinical and demographic characteristics of these patients are presented in Table 1. All but one of these patients included in this analysis presented aGVHD clinical grades III or IV according to the Glucksberg clinical classification. Median time to the confirmation of GI aGVHD diagnosis was 75.5 days (28–162). Treatment regimens are shown in Table 2. Six patients (24%) presented steroid-refractory aGVHD. One-year transplant-related mortality (TRM) was 40% among all grades III–IV aGVHD patients. All 6 steroid-refractory patients died within the 1st year after HCT.

Biopsies were compatible with the clinical suspicion of aGVHD in 22/25 (88%) patients. In total, specimens were available from duodenum (n=15 patients), ileum (n=10 patients), and colon (n=21 patients). The distribution of biopsies and the presence of findings associated with GI aGVHD per site are shown in Table 3. Histological findings associated with GI aGVHD in our study population are shown in Table 4. The proportion of histological grades I–II and III–IV GI aGVHD were

Table 1

Demographic and clinical parameters of the study population.

Characteristics	n=25
Patient age, median (range), y	49 (17–68)
Patient gender, no. male/female	19/6
Diagnosis at HCT, n	
Acute leukemia	7
Chronic myeloid leukemia/myelofibrosis	2
Lymphomas	5
Chronic lymphocytic leukemia	3
Multiple myeloma	4
Myelodisplastic syndrome	1
Severe aplastic anemia/PNH	2
Sickle cell disease	1
Donor-female/male-recipient, n (%)	13 (52%)
Conditioning regimen, n	
High dose	10
Low dose	15
Graft source, n	
Bone marrow	4
Mobilized blood	21
Clinical of diagnosis acute GVHD, n	
Grades I–II	1
Grades III–IV	24

GVHD=graft-versus-host-disease, HCT=hematopoietic cell transplantation, PNH=paroxysmal noctumal hemoglobinuria.

Table 2

Clinical characteristics of patients with biopsies compatible with aGVHD.

Clinical characteristics of GI aGVHD	n = 22
GI aGVHD stage*	
0–1	3
2–3	11
4	6
GI aGVHD diagnosis day, median (range)	74 (28–162)
Peak daily stool volume, mL, median (range)	2800 (0–8650)
Duration of diarrhea, days, median (range)	26 (0–124)
Treatment regimen, n (%)	
Steroids	16 (73%)
Other**	6 (23%)

GI aGVHD = gastrointestinal acute graft-versus-host-disease.
* According to Glucksberg et al.[4] Data not available for 2 patients.
** Anti-thymocyte globulin (n = 2), micophenolate mofetil (n = 1), Alentuzumab (n = 2); Basiliximab (n = 1).

10/22 (45.5%) and 12/22 (54.5%), respectively. Clinical and demographic differences between these groups are shown in Table 5. Patients with histological grade III–IV GI aGVHD presented higher stool volume and diarrhea length (Fig. 1). No differences were observed in the frequency of steroid-refractory aGVHD and TRM.

4. Discussion

Acute GVHD still represents a leading cause of non-relapse mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT), and a major restriction for allo-HSCT success.[19] Therefore, identification and validation of aGVHD biomarkers is very important for the management of this condition. The main finding of our study was that a specific characteristic of GI biopsies, namely the presence of severe crypt loss, can provide information on clinical relevance in aGVHD.

The characterization of aGVHD-specific alterations in GI biopsies of patients submitted to allo-HCT is challenging due to the coexistence of several conditions that can result in GI pathology. The spectrum of these alterations includes treatment-related mucositis, which is more frequent in the first 2 to 3 weeks after HCT, infections, and immunosuppressive-associated colitis. Since aGVHD usually manifests after the 3rd week of HCT, the differential diagnosis with chemotherapy-induced mucositis is less complex. On the other hand, cytomegalovirus (CMV) infection is a very prevalent complication of allo-HCT, whose time of presentation overlaps with that aGVHD. In one series, CMV infection always presented within 100 days of transplantation,[19] which is the very period when aGVHD is more prevalent. The limitations of the histological assessment of these cases are illustrated by study that demonstrate that CMV infection alone is regarded as sufficient to cause apoptosis of intestinal epithelial cells,[16,20] as well as by the recent demonstration that CMV infection can be identified by molecular biology techniques in samples lacking typical viral inclusions, or even CMV immunopositivity.[21,23] In our cohort, concomitant CMV infection was diagnosed in 11/22 (50%) of patients based on antigenemia and polymerase chain reaction (PCR). Among these patients, histological finding suggesting CMV infection were reported in 7/11 patients. Since additional techniques capable to refine the differential diagnosis of these conditions such as immunohistochemistry or PCR were not performed, we are not able to discuss the impact of CMV infection in our results. Micophenolate mofetil (MMF) was not frequently used in our patients, and we do not expect MMF-associated colitis to be a relevant confounder in our study.

Epithelial cell apoptosis is the hallmark histological feature of GI aGVHD, especially when observed in the deeper (proliferative) areas of intestinal crypts.[16] The system used to grade histological changes in GI GVHD is based on the presence of epithelial cell apoptosis and crypt loss.[16] Controversy exists on the number of apoptotic cells that should be identified to support the diagnosis of GI GVHD, and in other minimal criteria to support this diagnosis. Nguyen et al[22] retrospectively evaluated 85 biopsies of patients with GI aGVHD and concluded that a single apoptotic body is sufficient to support the diagnosis of this condition, in patients with associated skin GVHD. In contrast, Lin et al[23] demonstrated that the presence of 6 or fewer apoptotic bodies per 10 contiguous crypts were not sufficient to support the diagnosis of GI aGVHD, although an alert to clinicians about this possibility was recommended by the authors. Recent studies that tried to estimate the relative accuracy of

Table 3

Distribution of biopsies per site.

Biopsy site	Patients (n)	Presence of GI aGVHD, n (%)
Duodenum	15	15 (100%)
Ileum	10	10 (100%)
Colon*	21	19 (90%)

GI aGVHD = gastrointestinal acute graft-versus-host-disease.
* Of the 21 patients for whom colon biopsies were available, 19 had samples from both ascending, transverse and rectosigmoid portions. The remaining 2 patients had samples only from recto sigmoid.

Table 4

Histopathological alterations observed in GI biopsies compatible with GI aGVHD. (n = 22 patients).

Alterations	Duodenum (n = 15)	Ileum (n = 10)	Colon (n = 19)
Isolated apoptotic bodies	12/15 (80%)	4/10 (40%)	16/19 (84%)
Isolated crypt loss	0	0	2/19 (11%)
Contiguous crypt loss	4/15 (27%)	2/10 (20%)	4/19 (21%)
Mucosal denudation	3/15 (20%)	2/10 (20%)	6/19 (32%)
CMV-associated changes	3/15 (20%)	2/10 (20%)	3/19 (16%)
Histological grade III/IV	7/15 (47%)	4/10 (40%)	10/19 (53%)

CMV = cytomegalovirus, GI aGVHD = gastrointestinal acute graft-versus-host-disease.

Table 5

Clinical outcomes according to the presence of crypt loss.

	No severe crypt loss* (n = 10)	Severe crypt loss** (n = 12)	P****
Stool volume per day, peak, mL	1800 (0–4710)	3224 (1792–8650)	**.03**
Duration of diarrhea, d	16 (0–124)	24.5 (10–80)	**.02**
Day of aGVHD diagnosis, d	94.5 (47–162)	49 (38.5–108.5)	0.18
Steroid response, Yes/No	1/9	5/7	0.17
TRM (1 y), Yes/No***	3/6	7/4	0.37

GI aGVHD = gastrointestinal acute graft-versus-host-disease, TRM = transplant-related mortality.
* Corresponding to histological grades I and II GI GVHD.
** Corresponding to grades III and IV GI GHVD.
*** Deaths due to progressive disease (n = 2) excluded from the analysis.
**** *P* value from Mann–Whitney *U* of Fisher exact tests. Continuous data presented as median and range.
Bold values signify to highlight the statistically significant *P* value.

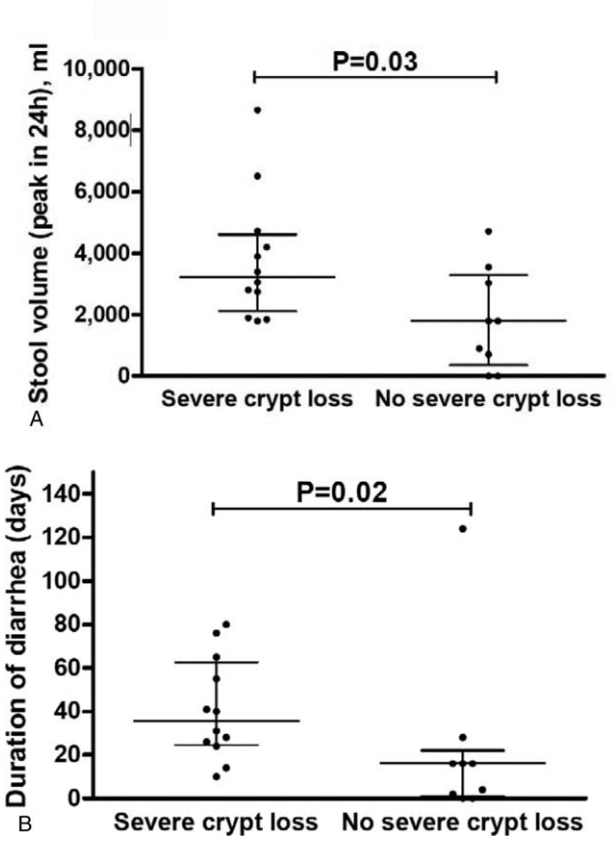

Figure 1. Clinical characteristics of GI aGVHD. Dot plots for the peak stool volume (mL) in 1 day (A) and for the duration of diarrhea in days (B) for patients with and without severe crypt loss. Mann–Whitney *U* test. aGVHD = acute graft-versus-host disease, GI = gastrointestinal.

several alterations observed in these patients, identified intra-cryptal apoptosis as the most reliable indicator of GI aGVHD, in particular when associated with other alterations that were more often present in patient with GI GVHD than in patients with non-GVHD related GI symptoms.[24] These additional alterations that were more often associated with GI aGVHD were pericryptal apoptosis, dilated crypts, irregular distribution of crypts, decreased lymphocytes, increased micro vessel network, focal fibrosis, presence of muciphages, mucosal ulceration, and/or reduced mucosal thickness. The updated NIH Consensus document on the pathological diagnosis of GI GVHD acknowledges this lack of definition, and questions the benefits of grading GI GVHD.[25] In our sample, apoptotic bodies were present in 80%, 40%, and 20% of patients in duodenum, ileum, and colon, respectively, and were key to the diagnosis of GI GVHD.

Controversy also exists on the association between histological findings with clinical severity, with limited demonstrations of the presence of such associations. In 2004, in a retrospective study with 95 patients who underwent gastroduodenal biopsy, Socie[26] demonstrated that the presence of ≥5 apoptotic bodies per field, or ≥20 neutrophils within the cellular infiltrate was associated with TRM. More recently, Paneth cell loss, counted in H&E stained sections, in at least 3 (40×) high power fields in the area with most Paneth cells, was also identified as a poor prognosis marker in GI GVHD.[11]

One frequent and relatively straightforward histological alteration in GI aGVHD is the presence of severe crypt loss. In 2007, Melson et al[13] also demonstrated that the presence of this alteration, which corresponds to grades III–IV histological changes, was associated with steroid-refractory aGVHD and mortality in a cohort of 23 patients who underwent colonoscopy. To our knowledge, this study was not validated in an independent population, and this was one of the main aims of our study. In our patients, histological alterations were not different that those commonly reported in other studies of GI aGVHD. Using a well-characterized cohort of patients we were able to show that severe crypt loss is associated with higher stool volume and increased diarrhea duration, which are clinical characteristics suggestive of increased severity. Although we did not find an association with response to steroids and mortality, our sample size was not powered to exclude such association.

Our study presents several limitations that need to be acknowledged by the reader. First, it included a low number of patients and used a retrospective design. GI aGVHD is a relatively infrequent condition, and most single center studies that addressed the association between clinical and histological parameters also used a retrospective design and enrolled <100 patients. On the other hand, the fact that our population consisted of a cohort of consecutive patients submitted to allo-HCT under standardized conditions (i.e., same staff, within a relatively short-time span), could be regarded as a strength of our study. A second limitation is one inherent to retrospective studies and refers to the fact that a standardized sample collection protocol was not used, and that endoscopies and pathology analyses were performed by multiple physicians. We tried to minimize the effect of this limitation by performing a histopathological review of all cases. Third, another important limitation was the fact that CMV immunohistochemistry was not performed when these cases were originally evaluated, which limits the evaluation of the effect of CMV infection on our results. Finally, additional clinical characteristics of GI aGVHD such as anorexia, nausea, and vomiting were not systematically described in all medical records. Since severe diarrhea is normally in the end of the severity spectrum of GI aGVHD, we believe that this limitation does not preclude the characterization of aGVHD severity in our population.

In conclusion, we described the most frequent histological alterations in the GI tract in a cohort of consecutive patients with the diagnosis of GI aGVHD, and validated the association between severe crypt loss with GI aGVHD severity in an independent population.

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