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Authors

Im, Jin S
Abraham, Susan C
Saliba, Rima M
et al.

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Histologic Grade 1 Is Associated With Increased Non-Relapsed Mortality In Lower Gastrointestinal Graft Versus Host Disease

Jin S. Im, M.D., Ph.D.[1], **Susan C. Abraham, M.D.**[2], **Rima M. Saliba, Ph.D.**[1], **Gabriela Rondon, M.D.**[1], **William A. Ross, M.D.**[3], **Asif Rashid, M.D., Ph.D.**[2], **Elizabeth J. Shpall, M.D.**[1], **Uday Popat, M.D.**[1], **Muzaffar H. Qazilbash, M.D.**[1], **Chitra Hosing, M.D.**[1], **Betul Oran, M.D.**[1], **Nina Shah, M.D.**[4], **Priti Tewari, M.D.**[5], **Yago Nieto, M.D.**[1], **Partow Kebriaei, M.D.**[1], **Richard E. Champlin, M.D.**[1], **Amin M. Alousi, M.D.**[1]

[1] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas M.D. Anderson Cancer Center

[2] Department of Pathology, The University of Texas M.D. Anderson Cancer Center

[3] Department of Gastroenterology, The University of Texas M.D. Anderson Cancer Center

[4]Department of Pediatric Oncology, Baylor College of Medicine

[5]Department of Medicine, Helen Diller Family Comprehensive Cancer Center, University of California

Abstract

Histologic confirmation is considered a standard practice to diagnose gastrointestinal graft versus host disease (GI GVHD) and is often used in making treatment decisions. A histologic grade is often determined in cases that are diagnosed with GI GVHD. Although extensive crypt loss (histologic grade 4) is associated with high non-relapse mortality (NRM), the prognostic value for the more common grade 1 is poorly understood. As clinical decisions are made on the degree of histologic evidence, it is important to establish its prognostic significance. Therefore, we evaluated 309 patients who underwent endoscopic biopsy for suspected GI GVHD within 6 months post-transplant between 2009–2012. The presence of histologic grade 1 was associated with increased NRM (HR 2.7, p=0.02) when compared to one of negative biopsy in patients with lower but not isolated upper GI GVHD. Multivariate competing-risk regression analysis confirmed the independent impact of histologic grade 1 in patients with early clinical stages of lower GI GVHD (Stage 0–2)(HR=2.7, p= 0.044). When compared to advanced histological grades, histologic grade 1 did not lessen the adverse outcome for patients with advanced lower GI GVHD (Stage 3–4) (% CI NRM 84 %). In conclusion, the presence of histologic grade 1 is associated with increased NRM in patients presenting with lower GI GVHD (Stages 0–2) and is sufficient evidence for decision to initiate therapy. At the same time, histologic grade 1 does not lessen the markedly adverse impact of advanced lower GI GVHD (stage 3–4) and is not synonymous with “mild” GVHD.

Correspondence to: Amin M. Alousi, M.D., Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas M.D Anderson Cancer Center, 1515 Holcombe Blvd Unit 423, aalousi@mdanderson.org.

Introduction

The gastrointestinal (GI) tract is involved in approximately 50% of patients with acute graft versus host disease (GVHD) making it the second most commonly affected organ behind skin in patients undergoing allogeneic stem cell transplantation (AHCT)(1). GI GVHD is commonly divided into upper and lower GI disease based on clinical symptoms: persistent nausea, vomiting, and anorexia for the former, and diarrhea greater than 500cc per day for the latter. The clinical severity of lower GI GVHD is assessed according to modified Glucksberg criteria with stage 0 (diarrhea <500 cc per day), stage 1 (diarrhea ≥ 500 cc but <1000 cc per day), stage 2 (diarrhea ≥ 1000 cc but <1500 cc per day), stage 3 (diarrhea ≥1500 cc per day but <2000), and stage 4 (diarrhea ≥ 2000 cc per day or persistent abdominal pain and/or ileus) (2, 3). Patient with upper GI GVHD without manifestation of lower GI symptoms have a high rate of response to systemic steroids with or without a “topical” (minimally absorbed) oral steroid, and have a lower risk for NRM(4–7). In contrast, lower GI GVHD is associated with a lower rate of response to systemic steroids when compared to other sites, and worse NRM especially in patients with steroid refractory disease(1, 4, 8–10). Clinical stage for patients with lower GI GVHD has been shown to be predictive for GVHD outcome as advanced clinical stage 3–4 hase a higher NRM than early clinical stage 1–2 (1, 11, 12). On the contrary, the prognostic value of histologic grading system has been poorly validated with clinical outcomes.

It is routine practice to perform histologic confirmation of acute GI GVHD through upper and/or lower endoscopy with random biopsies. These biopsy results are often used in making decisions on initiation or continuation of treatment with systemic steroids. The current NIH consensus guidelines recommend 3 histologic diagnostic criteria: negative, possible, and likely GVHD on the level of histologic evidence present on the biopsy specimen and any mitigating clinical factors which may confound the histologic findings(13). A histologic grade is often determined in cases that are deemed diagnostic of GI GVHD, with a modification of the system for colonic GVHD described by Lerner et al(14, 15): grade 1, the presence of increased apoptotic epithelial cells without crypt loss; grade 2, isolated crypt loss or micro-abscess; grade 3, 2 or more contiguous crypt loss; grade 4, extensive crypt loss with mucosal denudation. Although extensive crypt loss (histologic grade 4) is associated with advanced clinical stage of GI GVHD and early transplant related mortality(16–18), the prognostic value for the more common histologic grade 1 has been poorly understood. In addition, some centers do not report a histologic grade in endoscopic biopsy results in an effort to avoid confusion with the prognostically validated clinical stage(1, 11, 12)..

As the majority of biopsy specimens represent histologic grade 1 changes, clinical decisions are being rendered based on this degree of histologic evidence. Therefore, it is important to establish prognostic significance of histologic grade 1. The primary objective of this study is to determine the prognostic significance for histologic grade 1 in patients with suspected GI GVHD. Our hypothesis is that histologic grade 1 is associated with increased NRM post transplantation when stratified for presenting clinical stage, supporting its use in clinical decision with respect to treatment in patients with suspected GI GVHD. To test our hypothesis, we evaluated 309 consecutive patients who underwent an initial endoscopic

biopsy for suspected GI GVHD within 6 months from transplantation, and assessed the impact of histologic grade 1 on NRM and its correlation to presenting clinic stage of GI GVHD.

Materials and Methods

Patients

A total of 1415 patients underwent a first AHCT between January 2009 and December 2012, and 341 of 1415 patients underwent endoscopic evaluation and biopsy for suspected GI GVHD that occurred within 6 months from transplantation. Sixteen (of 341) patients with suspected GI GVHD were excluded due to preceding relapse or progression of disease prior to the endoscopic evaluation for suspected GI GVHD. An additional 16 patients were excluded because biopsy specimens were not available for pathologic review, therefore, a total of 309 patients were included for this study. The information on demographics, transplantation details and presenting GI symptoms that prompted endoscopic evaluation were extracted from the medical record. Our institutional review board approved this retrospective study, and waived the requirement for informed consent.

Clinical stage of GI GVHD

The clinical stage of GI GVHD at the time of endoscopic biopsy for all 309 patients was determined according to modified Glucksberg grading system (2, 3). Patients with isolated upper GI symptoms (persistent nausea and/or anorexia without diarrhea) were categorized into isolated upper GI GVHD, and remaining patients with lower GI GVHD symptoms were staged as clinical stage 0–4 as described above (2, 3).

Endoscopic evaluation

Routinely patients underwent endoscopic evaluation within 2–3 days from the initial presentation of GI symptoms after excluding infection such as C. Difficile colitis. The majority of patients with suspected GI GVHD underwent endoscopic biopsy from both the upper and lower GI tract to maximize diagnostic sensitivity and specificity for GI GVHD as previously published(19, 20). Flexible sigmoidoscopy was preferred over colonoscopy due to the ability to avoid a bowel preparative regimen and previously published reports suggesting that histologic diagnosis from random biopsies of the rectum correlate with the identification of GI GVHD in more proximal sites such as the terminal ileum(20–22). Patients who presented with isolated upper GI symptoms underwent upper GI endoscopy with or without lower GI endoscopy, and patients who presented with predominantly diarrhea underwent lower GI endoscopic biopsy with or without upper GI endoscopic biopsy. Therefore, a total of 151 patients underwent both upper and lower GI endoscopic biopsy, 75 patients underwent upper GI endoscopy biopsy only, and 83 patients received lower GI endoscopy biopsy only. Among 234 patients who underwent lower GI endoscopy, 33 patients (14.1%) received colonoscopy and 201 patients (85.9%) underwent sigmoidoscopy. Multiple random biopsies were obtained from the body and antrum of the stomach and from the duodenum on upper endoscopy, sigmoid-rectum from flexible sigmoidoscopy, and ascending and descending colon and terminal ileum in cases when complete colonoscopy was performed. Lower endoscopy was performed without standard

bowel preparation for all flexible sigmoidoscopy, and selected colonoscopy when patients presented with voluminous diarrhea.

Histologic evaluation

Endoscopic biopsy specimens were retrieved from the archive for pathologic review to determine the histologic grade of GI GVHD. A single pathologist (SA) who was experienced in GI GVHD retrospectively reviewed all biopsy specimens in blind fashion for clinical information. The overall histologic grade for GI GVHD was defined as the maximum grade identified in any biopsy sites. Histologic grade was determined according to a criteria modified from previously reported grading system (14, 15, 23): the presence of 4 or more apoptotic bodies per a biopsy fragment for grade 1, crypt micro-abscess or single crypt drop-out for grade 2, loss of more than two consecutive crypts for grade 3, and extensive loss of crypts or total denudation for grade 4. A biopsy fragment with 3 or fewer apoptotic bodies (formerly *suggestive of* or *cannot exclude* GVHD) was classified as "negative" (histologic grade 0) for this analysis. In order to be considered as "negative" for GVHD, the absence of apoptotic bodies (histologic grade 0) was required for all biopsy fragments.

Statistical analysis

Overall survival was estimated using the Kaplan-Meier method. The cumulative incidence of NRM was estimated considering disease progression or death attributed to the underlying malignancy as a competing risk. Predictors for increased NRM were evaluated using competing risks regression analysis. Factors found significant on univariate analysis were considered in multivariate analysis using backward elimination. Overall survival and NRM were estimated in landmark analysis starting on the date of GI biopsy. The association between clinical stages and histologic grades was assessed using Pearson's chi-squared test. Statistical significance was defined with p value less than 0.05. All statistical analysis was performed using STATA 11 (StataCorp LP, College Station, TX).

Results

Patient characteristics

A total of 309 patients met the study inclusion eligibility criteria and their characteristics are described in Table 1. Briefly, the median age of patients at transplantation was 52 years (range, 2 to 75 years) and nine patients were younger than 18 years. Sixty -six percent (65%) of patients underwent allogeneic stem cell transplantation for acute leukemia or myelodysplastic syndrome, followed by 15% for lymphoma and 10% for chronic lymphocytic leukemia. Roughly half of the patients (51%) received a transplant from a matched unrelated donor, 28% from a matched related donor, and 20% from an alternative donor either umbilical cord or haploidentical donor. Peripheral blood was the most common graft source (57%), followed by bone marrow (29%) and umbilical cord (12%). The majority of patients (68%) received a myeloablative conditioning regimen and received GVHD prophylaxis consisting of tacrolimus (94%) in combination with methotrexate (69%) and/or mycophenolate mofetil (25%). The average time from transplant to the diagnosis of

GI GVHD (date of endoscopic biopsy) was 63 days (range, 12 to 178 days). Median follow up for survivors was 38 months.

Clinical and histologic characteristics

The distribution of clinical stage vs. histologic grade is summarized in supplementary Table 1 and Figure 1. Among 309 eligible patients with suspected GI GVHD, 112 (36%) patients presented with isolated upper GI symptoms without lower GI symptoms, and 197 (64%) patients presented with lower GI symptoms with or without upper GI symptoms. Among 197 patients with lower GI symptoms, 33 (17%) patients presented with diarrhea but in volumes of less than 500 cc per day and were referred as clinical stage 0 in this analysis. There were 77 (39%) patients with stage 1, 35 (18%) patients with stage 2, 17 (9%) patients with stage 3, and 35 (18%) patients with stage 4 lower GI symptoms at the time of initial endoscopic evaluation.

Maximum histologic grade 1 was the most common in our study subjects and occurred in 101 of 309 cases (33%), followed by negative biopsy in 82 of 309 cases (26%), grade 3 in 53 cases (17%), grade 4 in 45 cases (15%), and grade 2 in 28 cases (9%). In patients with isolated upper GI symptoms, negative biopsy was the most common histologic findings with 50 of 112 cases (45%), followed by histologic grade 1 with 43 of 112 cases (38%). There were a small number of patients with isolated upper GI GVHD who were found to have histologic grade 2 (11 of 112 cases:10%), grade 3 (6 of 112 cases: 5%) or grade 4 (2 of 112 cases: 2%). In contrast, in patients with lower GI GVHD symptoms, histologic grade 1 was the most common with 58 of 197 cases (29%), followed by grade 3 with 47 cases (23%), grade 4 with 43 cases (21%), negative biopsy with 32 cases (16%), and grade 2 with 17 cases (9%).

Higher histologic grades (3–4) was associated with advanced clinical stages (3–4) (Pearson correlation coefficient χ^2=47.6, p<0.0001), while negative biopsy occurred more frequently in isolated upper GI GVHD and early clinical stage lower GI GVHD (0–1) (Pearson correlation coefficient χ^2=26.8, p<0.0001). Interestingly, maximum histologic grade 1 was fairly evenly distributed in all clinical stages: 43 of 112 isolated upper GI GVDH (38%), 10 of 33 stage 0 (30%), 27 of 77 stage 1 (35%), 9 of 35 stage 2 (26%), 3 of 17 stage 3 (18%), and 9 of 35 (26%) patients with stage 4 lower GI GVHD manifestations.

Impact of histologic grade 1 on GI GVHD outcome

As the isolated upper GI GVHD has a favorable outcome with lower NRM compared to lower GI GVHD(1, 4–7), we evaluated the impact of histologic grade 1 on NRM for patients with isolated upper GI GVHD separately (Table 2 and Figure 2). In patients with isolated upper GI GVHD, the presence of histologic grade 1 did not predict for higher NRM when compared with negative biopsy with a cumulative incidence (CI) NRM of 26% [95% Confidence Interval (95% CI) of 20–46%] and 30% (95% CI: 20–46) for grade 1 and 0 respectively [Hazard ration (HR) 0.8, p=0.6]. Advanced histologic grade 3–4 in patients with isolated upper GI GVHD symptoms was associated with increased NRM [CI NRM of 75%; (95% CI: 50–100) vs. 30%; (95% CI: 20–46), HR=3.7, p=0.005], while histologic grade 2 did not increase NRM (HR=0.6, p=0.5) compared to negative biopsy. However, it

should be noted that the number of patients with isolated upper GI GVHD symptoms who were found to have histologic grade 2 and 3/4 changes was relatively small for statistical analysis. In patients with isolated upper GI GVHD symptoms, the only risk factor for higher NRM identified in univariate analysis was the receipt of cord blood transplant (CBT) which was found to be associated with significantly increased NRM when compared to recipients of a matched related donor (MRD) (HR 3.1, p=0.025). In multivariate analysis, histologic grade 1 in upper GI GVHD was not associated with increased NRM (HR 1.04, p=0.9) while receipt of a CBT remained a predictor for higher NRM in these patients with histologic 0 or 1 grades (HR 12.3, p<0.001).

Among patients with lower GI GVHD symptoms, NRM in patients with histologic grade 1 (CI NRM of 52%; 95% CI: 40–69, HR=2.6, p=0.02), grade 3 (CI NRM of 49%; 95% CI: 37–66, HR=2.7, p=0.02), or grade 4 (CI NRM of 61%; 95% CI: 48–77, HR=4.1, p=0.001) was significantly increased compared to NRM in patients with negative biopsy (CI NRM of 22%; 95% CI: 11–43). Histologic grade 2 was associated with lower NRM (HR 0.5, p=0.5) but this was not statistically significant (Table 2 and Figure 2). To assess the independent impact of histologic grade 1 versus 0 on NRM, the primary objective of this study, subsequent analyses were focused on the subset (N=90) of patients with histologic grade 1 or 0 (Table 3 and 4, and Figure 3). Univariate analysis in this subset of patients revealed that, in addition to histologic grade 1, clinical stage 3–4 compared to clinical stage 0 (HR 12, p=0.001), multi-organ GVHD (HR 2.8, p=0.005), and alternate donor compared to matched related donor transplantation (HR 2.1, p=0.05) were associated with significantly increased NRM. Histologic grade 1 was associated with increased NRM compared to histologic grade 0 in patients with clinical stage 0–2 lower GI GVHD (CI NRM: 43% (95% CI:30–62) vs 18% (95% CI:8–39), HR 2.55, p=0.06), and in patients with advanced clinical stage 3–4 lower GI GVHD (CI NRM: 83% (95% CI:65–100) vs 67% (95% CI:30–100), HR=1.7, p=0.04) (Figure 3). However, only three of 15 patients with clinical stage 3–4 had a negative biopsy limiting the assessment of the independent effect of histologic grade 1 versus 0 in this subset of patients. Multivariate analysis (Table 4) confirmed that histologic grade 1 in early clinical stage 0–2 lower GI GVHD (HR 2.7, p=0.044), advanced clinical stage lower GI GVHD (HR 9.6, p<0.001), and multi-organ GVHD (HR 2.5, p=0.013) were independent significant predictors of NRM among patients with histologic grade 0 or 1 lower GI GVHD.

For the group as a whole (n=309), the presence of histologic grade 4 was associated with significantly higher NRM when compared to those with a negative biopsy [CI NRM of 63% (95% CI: 50–78) versus NRM 27% (95% CI: 19–39), HR=3.4, p<0.001]. Next we sought to determine if the presence of a lower histologic grade (histologic grade 1) lessened the impact on NRM when compared to those with a higher histologic grade (histologic 3 or 4). For patients with early lower GI GVHD (clinical stage ≤ 2), those with maximum histologic grade 1 had a NRM of 43% (95% CI:8–39) compared to grades 3 or 4 NRM of 51% (95% CI: 40–66), HR-0.6, p-value 0.1. For patients with advanced lower GI GVHD (clinical stage 3 or 4), 12 of 52 patients had histologic grade 1. NRM for those with histologic grade 1 was not statistically different compared to histologic grade 3 or 4 [83% (95% CI: 65–100) vs 61% (95% CI: 46–81) HR=1.9, p-value 0.1]. Interestingly, patients with histologic grade 2 GI GVHD appeared to have better outcome with respect to histologic grade 1, 3, and

4 (Table 2 and Figure 2), however, it should be noted that maximum histologic grade 2 occurred in relatively infrequent number (28 out of 309 patients (9%)) representing small subgroup of GI GVHD. Further validation of prognostic significance for histologic grade 2 in GI GVHD is required.

Finally, we sought to determine the minimum number of positive biopsy sites for histologic grade 1 that associated with an increased NRM in the subset of patients with lower GI GVHD symptoms with a maximum histologic grade of 1 (Table 5). The ratio of positive sites for histologic grade 1 over total sites sampled from patients with histologic grade 1 lower GI GVHD (N=58) was represented in histogram (Figure 4). Patients with one third or less of biopsy sites positive for histologic grade 1 behaved more similarly to those with negative results at all sites sampled (HR=1.1, p-value 0.9). In contrast, those with greater than one third positive for histologic grade 1 had significantly increased NRM compared to those with negative biopsy(HR= 3, p-value 0.009).

Discussion

To date, the clinical application of endoscopic biopsy results for patients with suspected acute GI GVHD has been of limited use beyond the confirmation of clinical diagnosis of GI GVHD. This is because there has not been a clear demonstration on how histologic grades correlate to corresponding clinical severity and transplant outcomes. Higher histologic grades (3–4) of GI GVHD have been shown to correlate to clinical severity and poor outcome of GI GVHD(16) and can be useful in the treatment decision for patients with GI GVHD. However, clinical implication of the most common histologic findings of grade 1 or 0 (negative biopsy) has not been clearly understood making it difficult to adopt in decision-making process for patients with suspected GI GVHD. Therefore, we sought to investigate whether histologic grade, particularly grade 1, can provide additional prognostic value with clinical stage of GI GVHD.

The most common method to determine the histologic grade is based on a modification of the classification proposed by Lerner et al (14) where four grades were given depending on the degree of epithelial cell damages. In contrast to histologic grades 2–4 where distinct histologic features are present, the diagnosis of histologic grade 1 GI GVHD has been challenging due to the variability in interobserver interpretation of “increased apoptosis”. Thus, the most recent update from the NIH consensus conference in 2014 stated that a minimum of more than one epithelial apoptosis per biopsy piece is sufficient for the diagnosis of GI GVHD in an appropriate clinical setting(13). As mild epithelial apoptosis (1 or 2 apoptotic cells) can be present in other conditions such as infection, inflammation, and certain drugs such as mycophenolate mofetil and is not specific to GVHD (24–26), some investigators have advocated more stringent thresholds, for example, more than 6 apoptotic bodies per 10 consecutive intestinal crypts for diagnosis of histologic grade 1 (27). However, this overly stringent strategy raises the concern of not being sensitive enough to detect GI GVHD when present. In the current study, we attempted a balance between sensitivity and specificity in the diagnosis of histologic grade 1 GI GVHD by setting the presence of 4 or more epithelial apoptosis per biopsy fragment as a diagnostic threshold. Therefore, the presence of 3 or less epithelial apoptosis per biopsy fragment was considered as histologic

grade 0 (negative), and an additional 40 of 309 study subjects were classified as having histologic grade 0 accordingly. Among these 40 patients, the majority of these patients presented with isolated upper GI GVHD (N=22) or early clinical stage 0 or 1 lower GI GVHD (N=15), and did not have the increased NRM compared to patients with “negative biopsy” (HR=0.8, p= 0.7 for upper GI GVHD, HR=1.05, p=0.9 for lower GI GVHD). Importantly, the inclusion of these patients into the negative category did not diminish the prognostic implications for grade 1 histology with respect to increase NRM. Furthermore, mycophenolate mofetil did not impact the diagnosis of histologic grade 1 (the presence of 4 or more apoptosis) as there were similar incidences of histologic grade 1 among patients who received mycophenolate mofetil (30%, 28 of 78) and who did not (33%, 77 of 231). Despite this rather stringent diagnostic criterion for histologic grade 1, the sensitivity from lower GI endoscopic biopsy was well preserved at 80.7 %, which was further increased to 84.1% with addition of upper GI endoscopic sampling. This is comparable, if not better, to the previous report by Wild et al, where a study to evaluate the diagnostic yield of sites and symptoms based biopsies from a cohort of 169 patients with acute GI GVHD demonstrated that the sensitivity improved to 82% when both upper and lower endoscopic biopsies were taken (19).

With our new diagnostic criteria for histologic grade 1 of GI GVHD, we assessed histologic grades of endoscopic biopsies from 309 patients with acute or late acute GI GVHD, and evaluated histologic grade 1 for its clinical correlation and impact on NRM. While there is a strong correlation between higher histologic grade 3–4 and advanced clinical stage 3–4 and with negative biopsies and early clinical stage 0–1 of GI GVHD, there was no correlation for histologic grade 1 to any clinical stage of GI GVHD as the prevalence of histologic grade 1 was fairly similar in all clinical stages of GI GVHD. For this reason, it is important to emphasize that histologic grade 1 is not synonymous with “mild” GVHD and its presence can be seen even in patients with advanced clinical grade. Importantly, the presence of histologic grade 1 did not lessen already adverse outcome for patients with advanced clinical stage 3–4 lower GI GVHD. This message is an important one as reporting the presence of “mild” GVHD changes in patients with advanced lower GI GVHD symptoms may incorrectly be construed by clinicians as representing a “milder” form of GVHD, which is not the case. At the same time, it is imperative that for patients presenting with lower GI symptoms, identification of grade 1 histology should not be dismissed (and warrants treatment) as its presence is associated with increased NRM. Our data also suggest that strong clinical correlation is needed when making treatment decisions on patients with histologic grade 1 identified only a few of the biopsy fragments (<1/3) as these patients behave similar to those with negative biopsies at all sites with respect to NRM.

The implication of grade 1 histology in patients with isolated upper GI GVHD is less clear. These patients have a low baseline risk for NRM making it difficult to validate grade 1 histology for this population. A future analysis into whether GVHD therapy was subsequently needed in patients with isolated upper GI symptoms and who had a negative biopsy may help to clarify the prognostic significance (including the negative predictive value) for this group of patients. It may be that endoscopic evaluation for patients with isolated upper GI GVHD is not sensitive enough to warrant routine practice and a trial of empiric therapy is sufficient.

Prior to this report, few studies have been conducted to investigate the prognostic value and/or its clinical correlation for histologic grading in GI GVHD. Melson et al evaluated 23 patients with lower GI GVHD who underwent diagnostic colonoscopy and investigated the impact of extensive crypt loss on GVHD outcome(16). In their study, the presence of epithelial apoptosis was confirmed from seventeen patients (73.9%), 6 patients (26.0%) had minimal crypt loss (histologic grade 1–2), and 11 patients (47.8%) had severe crypt loss (histologic grade 3–4). Severe crypt loss (histologic grade 3–4) was associated with clinical stage 2–4 and steroid refractory disease when compared to those with histologic grade 0–2 changes. However, clinical correlation of histologic grade 0–2 was not assessed in their study likely due to small number of study subjects. In a more recent study by Abraham et al, 201 patients with either (or both) upper and lower GI GVHD underwent histologic confirmation through endoscopic biopsies from solely the upper GI tract based on the assumption that maximum histologic grade in GI GVHD would be obtained from duodenum and small bowel regardless of symptom sites (18, 28). In contrast to our study, there was no correlation observed between histologic grades and clinical severity, but histologic grade 4 was re-confirmed to have worse overall survival. However, their findings are difficult to compare to our study because the majority of our patients underwent endoscopic biopsies of the lower GI tract with or without upper endoscopy and we used a rather stringent diagnostic criteria for histologic grade 1 vs negative biopsy to improve specificity. It has been previously shown that advance histologic grades are more commonly seen in biopsies from the lower GI tract (a finding which we saw as well, *data not shown*) (17). As such, there might have been false negative cases (or down grading of maximum histologic grade) affecting final outcome analysis in the study by Abraham et al. due to the sampling limited to upper GI track alone. Further, their analysis did not separate patients with isolated upper GI GVHD from those with lower symptoms likely resulting in an underestimation of the impact of histologic grade 1 in patients with lower GI GVHD.

In conclusion, we evaluated a large cohort of 309 patients with GI GVHD for clinical correlation and prognostic value of histologic grade. While histologic grade 3–4 correlated to advanced lower GI GVHD clinical stage (3–4), histologic grade 1 consisted of heterogeneous clinical stages of GI GVHD. Histologic grade 1 does not lessen the markedly adverse outcome of advanced lower GI GVHD clinical stages 3–4, and is associated with higher NRM in patients with lower GI GVHD clinical stages ≤ 2. Histologic grade 1 GVHD is associated with higher NRM in patients with lower GI GVHD with clinical stages ≤2 and provides important prognostic information independent of the clinical stage. Histologic grade should be considered along with clinical stage in evaluating new biomarkers for assessment of GVHD activity and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. MacMillan ML, Robin M, Harris AC, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. Biol Blood Marrow Transplant 2015;21:761–767. [PubMed: 25585275]
2. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304. [PubMed: 4153799]
3. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825–828. [PubMed: 7581076]
4. Pidala J, Chai X, Kurland BF, et al. Analysis of gastrointestinal and hepatic chronic graft-versus-host [corrected] disease manifestations on major outcomes: a chronic graft-versus-host [corrected] disease consortium study. Biol Blood Marrow Transplant 2013;19:784–791. [PubMed: 23395601]
5. Wakui M, Okamoto S, Ishida A, et al. Prospective evaluation for upper gastrointestinal tract acute graft-versus-host disease after hematopoietic stem cell transplantation. Bone Marrow Transplant 1999;23:573–578. [PubMed: 10217188]
6. Hockenbery DM, Cruickshank S, Rodell TC, et al. A randomized, placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease. Blood. 2007;109:4557–4563. [PubMed: 17244684]
7. Castilla C, Perez-Simon JA, Sanchez-Guijo FM, et al. Oral beclomethasone dipropionate for the treatment of gastrointestinal acute graft-versus-host disease (GVHD). Biol Blood Marrow Transplant 2006;12:936–941. [PubMed: 16920559]
8. Garnett C, Apperley JF, Pavlu J. Treatment and management of graft--host disease: improving response and survival. Ther Adv Hematol 2013;4:366–378. [PubMed: 24319572]
9. Ferrara JL, Levine JE, Reddy P, et al. Graft-versus-host disease. Lancet. 2009;373:1550–1561. [PubMed: 19282026]
10. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med 2010;363:2091–2101. [PubMed: 21105791]
11. Castilla-Llorente C, Martin PJ, McDonald GB, et al. Prognostic factors and outcomes of severe gastrointestinal GVHD after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2014;49:966–971. [PubMed: 24777184]
12. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. Blood. 1990;76:1464–1472. [PubMed: 2207321]
13. 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 2014 Pathology Working Group Report. Biol Blood Marrow Transplant 2015;21:589–603. [PubMed: 25639770]
14. 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. [PubMed: 4155153]
15. Washington K, Jagasia M. Pathology of graft-versus-host disease in the gastrointestinal tract. Hum Pathol 2009;40:909–917. [PubMed: 19524102]
16. Melson J, Jakate S, Fung H, et al. Crypt loss is a marker of clinical severity of acute gastrointestinal graft-versus-host disease. Am J Hematol 2007;82:881–886. [PubMed: 17570511]
17. Ma C, Maluf HM, Liu TC. Acute graft-versus-host disease is more prevalent and severe in the lower than the upper gastrointestinal tract. Hum Pathol 2015;46:1480–1487. [PubMed: 26243672]
18. Abraham J, Janin A, Gornet JM, et al. Clinical severity scores in gastrointestinal graft-versus-host disease. Transplantation. 2014;97:965–971. [PubMed: 24827766]
19. Wild D, Sung AD, Cardona D, et al. The Diagnostic Yield of Site and Symptom-Based Biopsies for Acute Gastrointestinal Graft-Versus-Host Disease: A 5-Year Retrospective Review. Dig Dis Sci 2015.
20. Sultan M, Ramprasad J, Jensen MK, et al. Endoscopic diagnosis of pediatric acute gastrointestinal graft-versus-host disease. J Pediatr Gastroenterol Nutr 2012;55:417–420. [PubMed: 22367336]

21. Ross WA, Ghosh S, Dekovich AA, et al. Endoscopic biopsy diagnosis of acute gastrointestinal graft-versus-host disease: rectosigmoid biopsies are more sensitive than upper gastrointestinal biopsies. Am J Gastroenterol 2008;103:982–989. [PubMed: 18028511]

22. Johansson JE, Nilsson O, Stotzer PO. Colonoscopy and Sigmoidoscopy are Equally Effective for the Diagnosis of Colonic Acute Graft-versus-Host Disease in Patients with Diarrhea after Allogeneic Stem Cell Transplantation: A Prospective Controlled Trial. Biol Blood Marrow Transplant 2015;21:2086–2090. [PubMed: 26183080]

23. CM f-P. Non-neoplastic lesions of the small intestine in Gastrointestinal Pathology: An Atlas and Text. 1999:439.

24. Liapis G, Boletis J, Skalioti C, et al. Histological spectrum of mycophenolate mofetil-related colitis: association with apoptosis. Histopathology. 2013;63:649–658. [PubMed: 24025088]

25. Tzankov A, Stifter G, Tschorner I, et al. Detection of apoptoses in gastro-intestinal graft-versus-host disease and cytomegalovirus colitis by a commercially available antibody against caspase-3. Pathol Res Pract 2003;199:337–340. [PubMed: 12908524]

26. Seidelin JB, Nielsen OH. Epithelial apoptosis: cause or consequence of ulcerative colitis? Scand J Gastroenterol 2009;44:1429–1434. [PubMed: 19958058]

27. 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. [PubMed: 23211294]

28. Socie G, Mary JY, Lemann M, et al. Prognostic value of apoptotic cells and infiltrating neutrophils in graft-versus-host disease of the gastrointestinal tract in humans: TNF and Fas expression. Blood. 2004;103:50–57. [PubMed: 12881313]

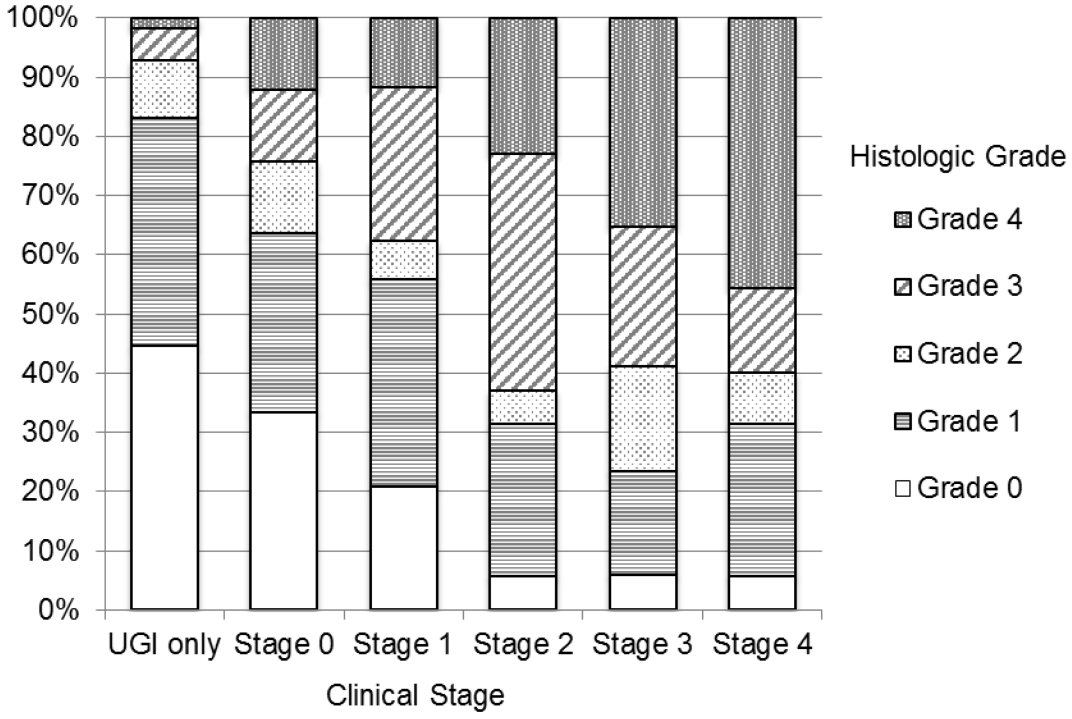

Figure 1.

Distribution of GI GVHD clinical stages vs histologic grade. A total of 309 eligible patients with suspected GI GVHD were grouped into isolated upper GI GVHD (N=112), and lower GI GVHD clinical stage 0 (N=33), 1 (N=77), 2 (N=35), 3 (N=17), and 4 (N=35). The distribution of histologic grades within each clinical stages were represented as percentage in y-axis. Histologic grade 0 is associated with UGI or clinical stage 0–1 GVHD (χ^2=26.8, p<0.001), and histologic grade 3 or 4 correlates to clinical stage 2–4 GVHD (χ^2=47.6, p<0.001).

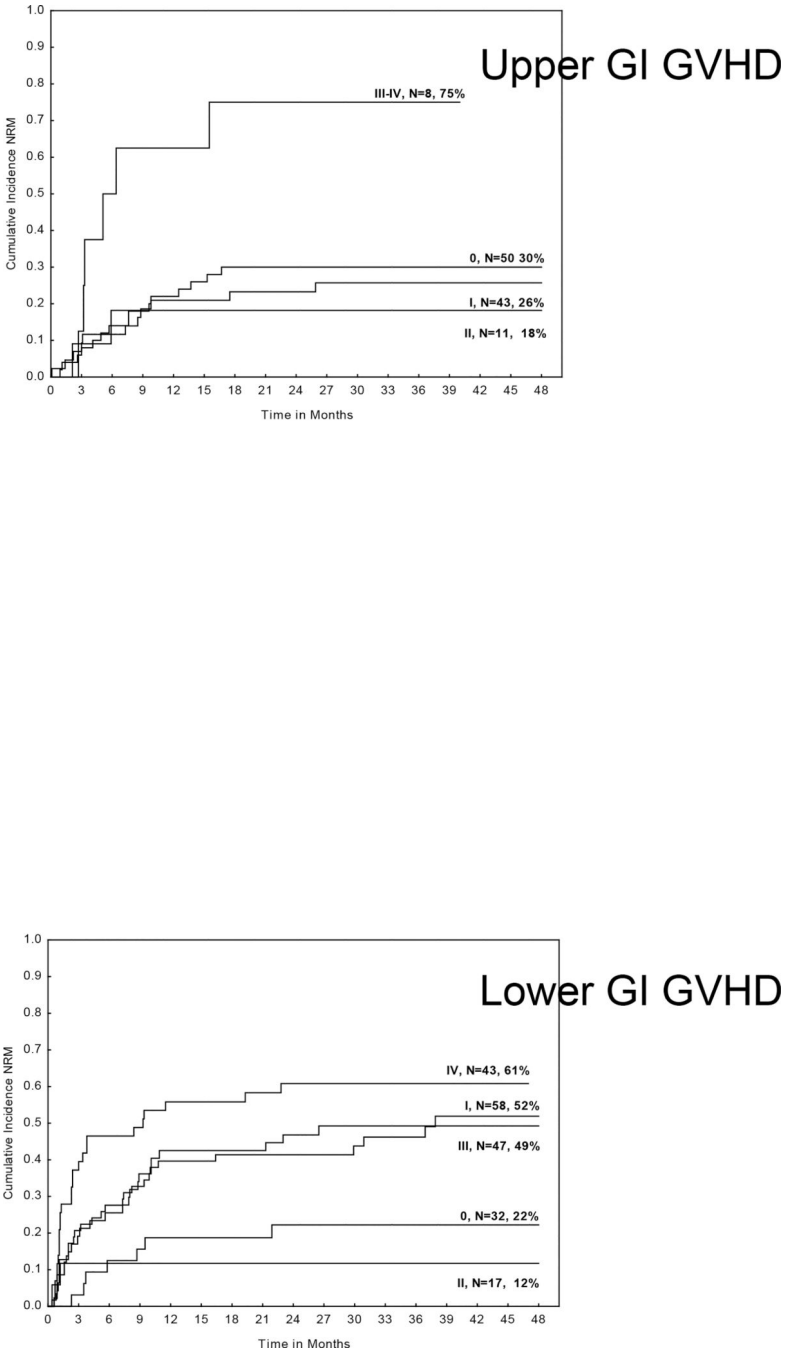

Figure 2.

Non-relapse Mortality of patients with Upper or Lower GI GVHD according to histological grades. While only histologic grade 3–4 was associated with significant increase in NRM in patients with isolated upper GI symptoms, histologic grade 1, 3 and 4 were associated with increased NRM in patients with lower GI symptoms.

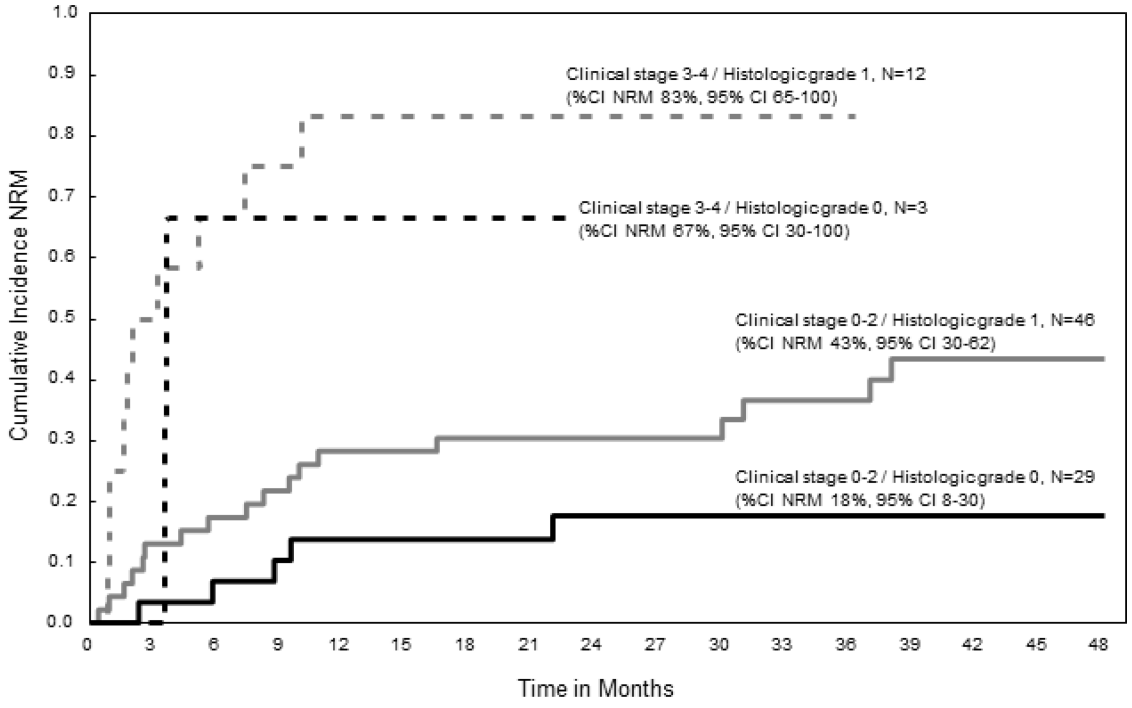

Figure 3.

Non-relapse Mortality of histologic grade 0 or 1 in patients with lower GI symptoms stratified by clinical stage 0–2 or 3–4. Histologic grade 1 was associated with increased NRM comapred to histologic grade 0 in both subgroups, and having negative biopsy (histologic grade 0) did not lessen already poor clinical outcome of patients with advanced clinical stage 3–4 with NRM 43%.

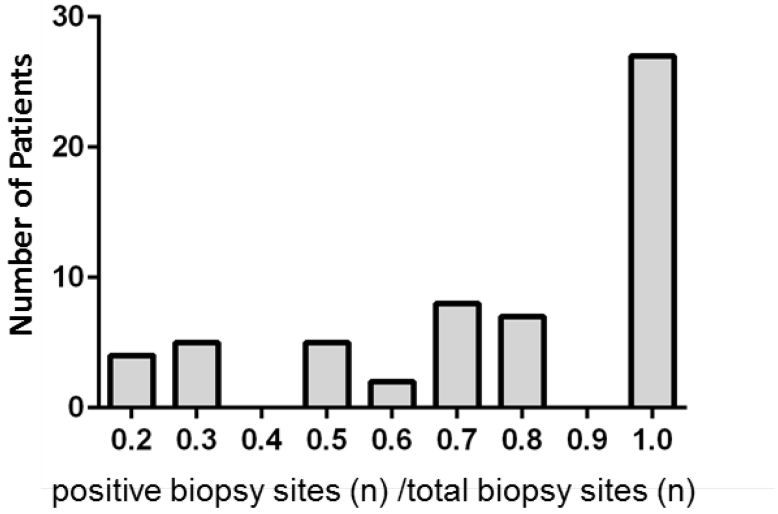

Figure 4.

Distribution of the ratio of number of biopsy sites positve over total number biopsy sites taken in patients with histologic grade 1 lower GI GVHD (N=58).

Table 1:

Patient Characteristics

Characteristic	N= 309 patients
Age, Median in Years (Range)	52 (2–75)
Gender, n (%)	
Female	150 (48%)
Male	159 (52%)
Disease, n (%)	
Acute Myeloid Leukemia/ Myelodysplastic syndrome	1567 (501%)
Acute Lymphoblastic Leukemia	46 (15%)
Lymphoma	48 (15%)
Chronic Lymphocytic Leukemia	310 (10%)
Chronic Myeloid Leukemia/ Mye**l**oproliferative disease	143 (54%)
Myeloma	9 (3%)
Other	5 (2%)
Donor, n (%)	
Matched Related **Donor**	89 (28%)
Matched Unrelated **Donor**	158 (51%)
Alternative Donor **(haploidentical or umbilical cord)**	62 (20%)
Graft Source, n (%)	
Peripheral blood	177 (57%)
Bone marrow	92 (29%)
Umbilical cord	40 (12%)
Conditioning, n (%)	
Myeloablative	209 (67.6%)
Reduced Intensity	100 (32.4%)
Onset of GI GHVD in days (range)	63 (12–178)
GVHD prophylaxis, n (%)	
Tacrolimus	290 (93.9%)
Methotrexate	212 (68.9%)
MMF	78 (25.2%)
Cytoxan	70 (23.7%)
other	1 (0.3%)
Clinical stage at the time of Endoscopy	
Isolated Upper GI	112 (36%)
Lower GI Stage 0 (diarrhea <500 ml per day)	33 (11%)
Lower GI Stage 1 (stool >500 to 1000 ml per day)	77 (24%)
Lower GI Stage 2 (>=1,000 but < 1500 ml per day)	35 (11%)

Characteristic	N= 309 patients
Lower GI Stage 3 (>=1.5 liters per day)	17 (6%)
Lower GI Stage 4 (>=2.0 liters per day ± ileus ± persistent pain)	35 (11%)
Maximum histologic grade	
Grade 0	82 (26%)
Grade 1	101 (33%)
Grade 2	28 (9%)
Grade 3	53 (17%)
Grade 4	45 (15%)

Table 2:

Hazard Ratio (HR) for Non-Relapse Mortality (NRM) at 4 years according to histologic grades in GI GVHD

Characteristics	N	%CI NRM (95% CI)	HR (95% CI)	P
Upper GI GVHD				
Grade 0 **(reference)**	50	30% (20–46)	Ref	
Grade 1	43	26% (15–43)	0.8 (0.4–1.8)	0.6
Grade 2	11	18% (5–66)	0.6 (0.1–2.7)	0.5
Grade 3 or 4	8	75% (50–100)	3.7 (1.5–9.1)	0.005
Lower GI GVHD				
Grade 0 **(reference)**	32	22% (11–43)	Ref	
Grade 1	58	52% (40–68)	2.6 (1.2–5.9)	0.02
Grade 2	17	12% (3–43)	0.5 (0.1–2.7)	0.5
Grade 3	47	49% (37–66)	2.7 (1.2–6.1)	0.02
Grade 4	43	61% (48–77)	4.1 (1.8–9.3)	0.001

Table 3:

Univariate analysis of risk factors for increased NRM at 4 years in patients with maximum histologic grade 0 or 1 and lower GI symptoms (N90)

Characteristics	N (90)	HR (95% CI)	P
Histologic Grade			
Grade 0	32	Ref	
Grade 1	58	2.6 (1.2–5.9)	0.02
Clinical Stage			
Stage 0	21	Ref	
Stage 1	43	2.9 (0.8–10.5)	0.1
Stage 2	11	1.9 (0.4–9.6)	0.4
Stage 3 or 4	15	12 (2.9–49)	0.001
Organs involved			
GI only	50	Ref	
GI + skin	24	1 (0.4–2.4)	0.9
GI + liver	14	2.4 (1.1–5.2)	0.02
GI + liver + skin	2	6.7 (2.6–17)	<0.001
GI + liver + skin	16	2.8 (1.4–5.6)	0.005
Donor type			
Matched related donor	26	Ref	
Matched unrelated donor	47	1.04 (0.5–2.3)	0.9
Alternative donor (Haploidentical & CBT)	17	2.1 (1.0–4.4)	0.05
Cell type			
Bone marrow	27	Ref	
Peripheral blood	52	1.8 (0.8–4.3)	0.2
Cord blood	11	3.3 (1.2–9.1)	0.02

Note: Age, time to diagnosis, sex mismatch, donor/recipient CMV status, conditioning regimen disease status were not significant risk factors.

Table 4:

Multivariate Analysis of risk factors for increased NRM at 4 years in patients with maximum histologic grade 0 or 1 and lower GI symptoms (N90)

Characteristics	HR (95% CI)	p
Clinical Stage 3–4	9.6 (3.3–28.0)	<0.001
Clinical Stage 0–2 + Histologic grade 1	2.7 (1.0–7.1)	0.044
Multi-organ GVHD (GI+ liver ± skin)	2.5 (1.2–5.0)	0.013

Table 5:

Non-Relapse in Patients with maximum histologic grade 1 identified in ≤ 1/3 of the biopsy sites versus those with more than 1/3 sites positive (reference histologic grade 0= all sites negative) in patients with lower GI symptoms (N=90)

Maximum Histologic Grade	Ratio: Positive Biopsy Sites to Total	N (total)	NRM (N)	HR (95% CI)	p-value
0	n/a	32	7	Reference	
1	≤ 1/3	9	2	1.1 (0.2–5.5)	0.9
1	> 1/3	49	26	3.3 (1.3–6.8)	0.009