Diagnostic disagreement between clinical standard histopathological- and retrospective assessment of histopathology-based gastrointestinal graft-versus-host disease in children

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Abstract

Background: No previous paediatric study has evaluated the frequency of diagnostic disagreement between clinical standard histopathological assessment (CSHA) and retrospective, independent, histopathological assessment (RIHA) of gastrointestinal Graft-Versus-Host Disease (GI-GVHD)

Methods: In a retrospective cohort study, based on gastrointestinal biopsies collected from allogeneic HSCT-treated children (<18 years) with symptom-based GI-GVHD, we evaluated; disagreement of histopathology-based GI-GVHD diagnosis in CSHA vs RIHA, and potential clinical consequences of differences between the assessments. The CSHA-based diagnoses were retrieved from histopathology reports. The RIHA was performed by one pathologist, blinded to the CSHA outcomes and based on the minimal criteria for histopathology-based GI-GVHD diagnosis by the NIH 2014.

Results: Seventy children with 92 endoscopic occasions (including 22 re-endoscopies) were enrolled. GI-GVHD was observed in 73% (67/92) of the endoscopies in the RIHA and in 54% (50/92) in the CSHA (P = .014). The RIHA confirmed 94% (47/50) with GI-GVHD and 52% (22/42) with non-GI-GVHD diagnoses, established in the CSHA. Disagreement, that is endoscopic occasions with GI-GVHD solely detected in RIHA or detection of GI-GVHD in CSHA but not in RIHA, was observed in 20/42 (48%) and 3/50 (6%), respectively (McNemar’s test, P = .0008). The risk of a subsequent re-endoscopy was higher in endoscopic occasions with GI-GVHD detected in RIHA but not in CSHA vs if non-GI-GVHD were detected in both readings (P = .005).

Conclusion: Our results suggest that in children with symptom-based GI-GVHD without histopathological confirmation in CSHA, a second, NIH 2014 based histopathological assessment should be considered before performing a re-endoscopy.

Abbreviations: aGI/cGI-GVHD, Acute and chronic graft-versus-host disease in the gastrointestinal tract; CI, Confidence interval; CMV, Cytomegalovirus; CSHA, Clinical standard histopathological assessment; EBV, Epstein-Barr virus; HR, Hazard ratio; HSCT, Allogeneic hematopoietic stem cell transplantation; IQR, Interquartile range; ISD, Immunosuppressive drug; IV, Intravenous; MAGIC, Mount Sinai aGVHD International Consortium; N/A, Not applicable; NIH, National Institutes of Health; PTLD, Post-transplant lymphoproliferative disorder; RIHA, Retrospective, independent, blinded, histopathological assessment; SD, Standard deviation.

Britt Gustafsson and Thomas H. Casswall contributed equally to this work.

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1 | INTRODUCTION

Acute GI-GVHD (aGI-GVHD) is one of the major factors associated with increased risk of transplant-related mortality, following paediatric allogeneic HSCT.\(^1\)\(^-\)\(^3\) In children, approximately every fourth HSCT is complicated by aGI-GVHD.\(^4\)\(^,\)\(^5\) However, paediatric, non-histopathology-based chronic GI-GVHD (cGI-GVHD) is rare.\(^6\)

The diagnosis of aGI-GVHD is primarily based on clinical symptoms.\(^7\)\(^-\)\(^9\) These symptoms are non-specific and include diarrhoea with or without visible blood, weight loss, nausea, vomiting, severe pain and ileus. Furthermore, in children with endoscopy performed due to clinically suspected aGI-GVHD, histopathology reveals GI-GVHD in approximately every second case with normal macroscopic appearance of the gastrointestinal mucosa.\(^10\)\(^,\)\(^11\) Thus, to confirm the GI-GVHD diagnosis, histopathological assessment of endoscopy guided biopsies is recommended.\(^8\)\(^,\)\(^9\)\(^,\)\(^12\)\(^,\)\(^13\) even though consensus regarding minimal histological changes needed for the GI-GVHD diagnosis is lacking.\(^12\)

Every second to third endoscopy in children, performed to confirm symptom-based aGI-GVHD, has previously been reported to be followed by treatment changes, based on the histopathological assessment.\(^14\)\(^,\)\(^15\) Therefore, differences in the assessment of histopathology-based GI-GVHD diagnosis\(^12\)\(^,\)\(^16\) as well as certain drugs or infectious agents with the potential to induce apoptosis, thus mimicking the histopathological hallmark of GI-GVHD,\(^12\)\(^,\)\(^17\)\(^,\)\(^18\) may cause substantial clinical problems.

The present study was based on biopsies collected from children to confirm symptom-based GI-GVHD. The main objectives of the study were as follows: (a) to evaluate disagreement between histopathology-based GI-GVHD diagnosis established in clinical standard histopathological assessment (CSHA) and retrospective independent, assessment (RIHA) based on the minimal histological criteria by the NIH 2014,\(^12\) and (b) to assess the potential clinical consequences of mismatch of the GI-GVHD diagnosis between the readings.

2 | PATIENTS AND METHODS

2.1 | Design and inclusion criterion

This was a retrospective cohort study including all paediatric HSCT centres in Sweden (Gothenburg, Lund, Stockholm and Uppsala). The participants were identified via databases for pathology, hospital record databases and local registers of HSCT-treated children. The inclusion criteria were as follows: (a) Age below 18 years at the time of the HSCT, (b) HSCT performed during 2000-2012, (c) endoscopy with biopsy sampling performed to confirm symptom-based GI-GVHD within the first-year post-HSCT and (d) available histopathology reports and biopsy slides.

2.2 | Subgroups

Potential clinical consequences of mismatch of the GI-GVHD diagnosis in CSHA vs RIHA were analysed based on four subgroups; endoscopic occasions with an agreement of histopathology-based GI-GVHD diagnosis in both assessments (+ +); endoscopic occasions without detection of GI-GVHD in the CSHA but detected in the RIHA (− +); GI-GVHD detected in the CSHA but reclassified as non-GI-GVHD in the RIHA (+ −) and; endoscopic occasions with agreement of non-GI-GVHD in both assessments (− −).

In the CSHA and the RIHA, normal histology or finding of a differential diagnosis to GI-GVHD without concurrent detection of GI-GVHD were classified as non-GI-GVHD (−). Detection of GI-GVHD, with or without concurrent detection of a GI-GVHD differential diagnosis were classified as GI-GVHD (+).

2.3 | Primary aims

- Frequency of histopathological disagreement of the GI-GVHD diagnosis, between CSHA and NIH 2014 based RIHA,
- Risk of a subsequent re-endoscopy within one-year post-HSCT, and of death within two-year post-HSCT, in each subgroup (+ +, − +, + −, − −), respectively, and to assess if the risk of these clinical consequences were different between the subgroups.

2.4 | Secondary aim

Differences in histopathology-based disease severity scores of acute and overlap GI-GVHD (features of acute and chronic GVHD observed together) solely detected in the RIHA (− +) vs corresponding cases detected in both evaluations (+ +).

2.5 | Re-endoscopies

The analysis of the risk of a subsequent re-endoscopy was based on subgroup affinity (+ +, − +, + −, − −) of the endoscopic occasion that preceded the re-endoscopy. However, the frequencies of histopathological disagreement of the GI-GVHD diagnosis between the CSHA and the RIHA were based on either A) all endoscopic occasions or B) solely the re-endoscopies.
2.5.1 | Endoscopic occasions

Endoscopic occasion was defined as: any single diagnostic endoscopy procedure, regardless if either upper, lower or combined endoscopies were performed.

2.6 | Data sources and data collection

The study was based on data from hospital records, histopathology reports and results from the RIHAs. Data collection from the hospital records included clinical background and survival data. Information regarding death was sought from the first endoscopic occasion and forward, up to two years post-HSCT.

2.7 | Retrospective independent assessment

The retrospective independent assessment process has been described in a previous study by our group. The RIHA was performed at least 1 year after the CSHA, in a scientific setting, thus without influencing the clinical management of the patients.

The RIHA was performed protocol-based, by one transplant pathologist (A.S), blinded to the results from the CSHA and blinded to clinical background data. Slides with good-quality haematoxylin and eosin and immunohistochemical CMV staining were prerequisites for the retrospective assessment. If this was not met or if the pathologist requested additional staining, new slides were produced from stored formalin-fixed paraffin-embedded biopsies.

2.8 | Histopathology-based GI-GVHD diagnosis

2.8.1 | CSHA

Diagnoses established in the CSHA were retrieved from the histopathology reports. All endoscopic occasions with histopathology reports indicating GI-GVHD were classified as GI-GVHD. Thus, phrases such as "suggestive of", "favour", or "minimal" GI-GVHD were judged as GI-GVHD.

2.8.2 | RIHA

Apoptosis was defined in accordance with Krefet al. The histopathological threshold of the GI-GVHD diagnosis was based on the NIH 2014 criteria; detection of at least one apoptotic body in a crypt per biopsy piece. Histopathology-based GI-GVHD diagnosis was classified as acute, overlap or chronic. If acute and overlap GI-GVHD was established in the RIHA, histological severity grading was performed in accordance with Lerner et al. Thus, throughout this article, histological severity grading has solely been based on the RIHA.

GI-GVHD diagnosis based on the RIHA was reported as, possible or likely GI-GVHD. In the present study, only likely GI-GVHD was classified as GI-GVHD. Endoscopic occasions with histological findings indicating GVHD, but other explanations possible, thus possible GI-GVHD, were classified as "normal or non-specific findings".

2.9 | Mimics to histopathology-based GI-GVHD

During the RIHA, all cases were assessed for CMV as a cause of apoptosis. To evaluate the influence of factors other than CMV, that may induce apoptosis, potentially mimicking histopathology-based GI-GVHD, data collection included:

- Results from stool cultures for Salmonella sp, Shigella sp, Yersinia enterocolitica, and Campylobacter jejuni, and results from faecal detection tests for Rotavirus, Norovirus and Clostridium difficile toxin. These data were collected during the 21 days preceding each endoscopy.
- Ongoing treatment with mycophenolate mofetil (MMF), tacrolimus and proton pump inhibitors (PPI) at the time of endoscopy.
- Time span between HSCT and endoscopy to evaluate the potential influence of the conditioning regimen. In this aspect, the endoscopic occasions were divided into; endoscopies performed ≤20 days from HSCT, and those performed thereafter, but within one-year post-HSCT.

2.10 | Number of biopsied regions of the GI tract

To define the extent of the endoscopic procedures, a previous described approach was used to divide the GI tract into maximum 15 different regions.

2.11 | Classification and staging of clinical GI-GVHD

Classification and staging of clinical cGI-GVHD have been adapted to the criteria from NIH 2014, and for symptom-based aGI-GVHD to the MAGIC. The MAGIC staging has been applied as stage I-II as one group and stage III-IV as another group. Cases with cGI-GVHD based on previous definition (time for onset of symptoms >100 days post-HSCT), without fulfilling the NIH 2014 criteria, thus reclassified to aGI-GVHD, were re-staged as follows: mild cGI-GVHD into aGI-GVHD "stage I-II" and moderate to severe cGI-GVHD, into aGI-GVHD "stage III-IV".

2.12 | Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethical Committee, Stockholm, Sweden.
2.13 | Statistics

In patients with more than one endoscopic occasion performed, each occasion was considered independent in the analyses. Categorial variables were summarized and presented as frequencies and percentages, and numerical variables as mean, median, SD and IQR, as appropriate. Fisher’s exact test was used to compare categorial data. Numerical data were analysed with t test for those variables normally distributed, and for non-normally distributed variables, Mann-Whitney U test was performed. In all cases, the calculations were performed with exclusion of missing data. McNemar’s test was used to identify potential disagreement of the GI-GVHD diagnosis between CSHA and the RIHA. For the comparison of overall survival and risk of subsequent re-endoscopy, a stratified proportional cox regression analysis was performed. These analyses were based on endoscopic occasions and stratified over recurring events. Thus, each endoscopic occasion, if serial procedures were performed, contributed to survival function and risk of a subsequent re-endoscopy, until the time point of the next endoscopic occasion, but was thereafter censored. Data analysis was performed using R version 3.4.4, \(^{33}\) and statistical significance was defined as \( P < .05 \).

3 | RESULTS

A total of 70 children with 92 endoscopic occasions, performed to confirm symptom-based GI-GVHD and with biopsy slides available for RIHA, were enrolled. Twenty-two endoscopic occasions of these were re-endoscopies. One additional re-endoscopy was included in the analysis of the probability of a subsequent re-endoscopy but was otherwise excluded due to lack of biopsy slides available for RIHA.

In the study population, the sex ratio (girls/boys) was 1:1.6 (27/43) and the mean age at the time of HSCT was 9.1 (SD 5.6) years (Table 1). The median duration from HSCT to endoscopy was 78.5 (IQR 36-148.7) days. Pre-endoscopically, none had diagnostic signs of cGI-GVHD. The median duration from onset of symptoms to endoscopy was 16 days (IQR 7-30), and the most frequent symptoms at the time of endoscopy’s were diarrhoea (83%), followed by nausea or vomiting (60%), and abdominal pain (50%).

3.1 | Histopathology-based diagnoses

Based on the entire study population, the median number of biopsied regions of the GI tract was 6 (IQR 4-10). Immunohistochemical staining for CMV was performed in 87% (80/92) in the CSHAs and in all in the RIHA.

In total, including normal or non-specific histological findings, 100 histopathological diagnoses were established in the CSHA and 102 in the RIHA, based on all 92 endoscopic occasions performed (Table 2).

3.2 | Disagreement of histopathology-based GI-GVHD diagnosis

The RIHA confirmed 94% (47/50) of GI-GVHD and 52% (22/42) of non-GI-GVHD diagnoses established in the CSHA. Disagreement, that is endoscopic occasions with GI-GVHD detected in RIHA but not in the CSHA (− +) or detection of GI-GVHD in the CSHA but reclassified as non-GI-GVHD in the RIHA (+ −), was observed in 48% (20/42) and 6% (3/50), respectively (\( P = .0008 \)), (Figure 1 and Table 3). Furthermore, a significant disagreement of

| TABLE 1 | Descriptive baseline HSCT data of 70 children with 92 endoscopies performed to confirm gastrointestinal GVHD |
| --- | --- | --- |
| Gender | All n = 70 | % |
| Boys | 43 | 61.4 |
| Girls | 27 | 38.6 |
| Age (years) at HSCT Mean (SD) | 9.1 (5.6) |
| Underlying diagnosis for HSCT | | |
| Haematologic malignancies | 52 | 74.3 |
| Haematologic benign diseases | 4 | 5.7 |
| Immunodeficiency diseases | 8 | 11.4 |
| Miscellaneous | 6 | 8.6 |
| Stem cell source | | |
| Bone marrow | 42 | 60.0 |
| Peripheral blood stem cells | 19 | 27.1 |
| Umbilical cord blood | 9 | 12.9 |
| Donor type\(^a\) | | |
| Unrelated | 38 | 54.3 |
| Haploidentical | 10 | 14.6 |
| Sibling | 13 | 18.6 |
| Conditioning regimen | | |
| Myeloablative | 56 | 80.0 |
| Reduced intensity | 12 | 17.1 |
| Missing data | 2 | 2.9 |
| GVHD prophylaxis | | |
| Cyclosporine and methotrexate | 30 | 42.9 |
| Tacrolimus ± other ISD | 21 | 30.0 |
| Mycophenolate mofetil ± other ISD | 9 | 12.9 |
| Cyclosporine and prednisolone | 7 | 10.0 |
| Miscellaneous | 2 | 2.9 |
| Missing data | 1 | 1.4 |

\(^a\)Cord blood transplantations excluded.

GI-GVHD was the most frequent histopathology-based diagnosis, observed in 54% (50/92) in the CSHA and in 73% (67/92) in the RIHA (\( P = .014 \)). The corresponding figures for non-GI-GVHD were 42/92 (46%) in the CSHA and 25/92 (27%) in the RIHA (\( P = .014 \)) (Figure 1).
histopathology-based GI-GVHD diagnosis between the readings was likewise observed based on the 22 re-endoscopies, (P = .013) (Table 3).

3.3 | Clinical consequences of mismatch of the GI-GVHD diagnosis

The subgroup including endoscopic occasions with GI-GVHD detected in CSHA but not in the RIHA (+ −) was excluded due to low sample size (three endoscopic occasions), (Figure 1). That subgroup did not include any subsequent re-endoscopy and two out of three children in the group were alive two years post-HSCT. Clinical background data of the remaining three subgroups are presented in Table 4.

3.3.1 | Re-endoscopies

In all, twenty-three endoscopic occasions were followed by a re-endoscopy. A subsequent re-endoscopy was observed in nine out of twenty (45%) endoscopic occasions with histopathological detection of GI-GVHD solely in the RIHA (+ −). The corresponding figures of those with GI-GVHD detected in both the CSHA and in the RIHA (+ +) were 13/47 (28%), and for non-GI-GVHD in both readings (− −), 1/22 (5%).

Detection of GI-GVHD in the RIHA but not in the CSHA (− +) was associated with increased risk of a subsequent re-endoscopy, vs endoscopic occasions with detection of non-GI-GVHD in both readings (− −) (HR 10.8; 95% CI 2.1-55.5, P = .005). However, no statistically significant difference in the risk of a subsequent re-endoscopy was found between those with GI-GVHD solely detected in the RIHA (− +) vs endoscopic occasions with an agreement of histopathology-based GI-GVHD diagnosis in CSHA and the RIHA (− +) (HR 2.2; 95% CI 0.9-5.5, P = .08) (Figure 2).

3.3.2 | Survival

The overall 2-year post-HSCT survival in the entire study population was 63% (44/70). No difference in the risk of survival was observed between the subgroup with GI-GVHD solely detected in the RIHA (− +) vs endoscopies with non-GI-GVHD in both readings (− −) (HR 0.7; 95% CI 0.2-2.2, P = .58). Similar result was found comparing GI-GVHD solely detected in the RIHA (− +) vs the subgroup with an agreement of GI-GVHD in CSHA and the RIHA (+ +) (HR 1.3; 95% CI 0.5-3.8, P = .61).

### Table 2

| Histopathological diagnosis | Clinical standard assessment* n = 100 (%) | Retrospective assessment* n = 102 (%) |
|-----------------------------|-----------------------------------------|--------------------------------------|
| Gastrointestinal GVHD       | 50 (50.0)                               | 67 (65.7)                            |
| Acute GI-GVHD               | 46                                      | 62                                   |
| Chronic GI-GVHD             | 3                                       | 2                                    |
| Overlap GI-GVHD             | 1                                       | 3                                    |
| Differential diagnoses (all)| 15 (15.0)                               | 17 (16.7)                            |
| CMV                         | 6                                       | 8                                    |
| PTLD                        | 2                                       | 2                                    |
| EBV infection without lymphoma | 0                                      | 1                                    |
| Aphthous colitis            | 1                                       | 1                                    |
| Enteritis due to Adenovirus | 1                                       | 1                                    |
| Enteritis due fungal infection | 1                                     | 1                                    |
| Pseudomembranous colitis    | 0                                       | 1                                    |
| Esophagitis                 | 2                                       | 1                                    |
| Reactive (chemical) gastritis | 1                                     | 0                                    |
| Bleeding antral polyp       | 1                                       | 1                                    |
| Normal or non-specific findings | 35 (35.0)                | 18 (17.6)                            |

Note: Simultaneous detection of GI-GVHD and a differential diagnosis:
*In 8 cases in the clinical standard assessment (5/8 CMV, 1/8 esophagitis, 1/8 reactive gastritis, 1/8 Adenovirus enteritis) and;
*In 9 cases in the retrospective independent assessment (6/9 CMV, 1/9 CMV + fungal infection, 1/9 esophagitis, 1/9 pseudomembranous colitis).
*Based on immunohistochemical staining.
*Positive Epstein-Barr virus-Encoded RNAs (EBER) in situ hybridization staining.
*Clostridium difficile toxin positive.
3.4 | Histological severity scores of acute and overlap GI-GVHD

An overall lower histological severity grade was observed in endoscopic occasions with acute or overlap GI-GVHD solely detected in the RIHA (− +) vs corresponding cases detected in both the CSHA and the RIHA (+ +) (P = .046). Furthermore, histological severity grade 1 was detected in 14/20 (70%) of the endoscopic occasions with GI-GVHD solely detected in the RIHA (− +) vs 15/45 (33.5%) in those diagnosed with GI-GVHD in both readings (+ +) (P = .008).

3.5 | Histological mimics

No statistically significant difference was found in the distribution of potential histological mimics between endoscopic occasions with GI-GVHD solely detected in the RIHA (− +) vs endoscopies with an agreement of the GI-GVHD diagnosis in the CSHA and the RIHA (+ +) (Table 5).

4 | DISCUSSION

In the present paediatric histopathology-based study, GI-GVHD was more often detected in RIHA than CSHA. Importantly, that

| Retrospective assessment | GI-GVHD | Non-GI-GVHD | Total |
|--------------------------|---------|-------------|-------|
| Clinical standard assessment |         |             |       |
| Non-GI-GVHD | 22 (3) | 20 (8) | 42 (11) |
| GI-GVHD | 3 (0) | 47 (11) | 50 (11) |
| Total | 25 (3) | 67 (19) | 92 (22) |

Note: (): Re-endoscopies. White squares—disagreement, grey squares—agreement between the assessment methods.
| TABLE 4  Clinical and endoscopical background data in three histological subgroups based on results from clinical standard histopathological assessment and retrospective independent blinded assessment*

|                  | GI-GVHD only in retrospective assessment (+) n = 20 (%) | GI-GVHD in both readings (+) n = 47 (%) | Non-GI-GVHD in both readings (−) n = 22 (%) | P-value − vs + | P-value − vs − |
|------------------|--------------------------------------------------------|------------------------------------------|---------------------------------------------|---------------|---------------|
| Endoscopy performed – days post-HSCT | Median (IQR) 121.5 (73.5-180.7) | 55.0 (30.0-138.0) | 74.5 (41.2-117.5) | .014 | .082 |
| Extent of the endoscopies | | | | | |
| Colonoscopy ± EGD or sigmoido-/extended sigmoidoscopy + EGD | 16 (80.0) | 40 (85.1) | 16 (72.7) | .721 | .723 |
| Sigmoido-/extended sigmoidoscopy or EGD | 4 (20.0) | 7 (14.9) | 6 (27.3) | |
| Number of biopsied regions of the GI tract | Median (IQR) 6 (3.7-8.2) | 6 (4.0-11.0) | 4.0 (3.0-8.0) | .520 | .391 |
| Days from onset of symptoms to endoscopy | Median (IQR) 28.5 (17.0-74.7) | 12.0 (6.5-29.5) | 14.0 (6.0-28.0) | .076 | .096 |
| Intensity of GI-GVHD symptoms at time of Endoscopy | | | | | |
| Stage I-II | 13 (65.0) | 26 (55.3) | 17 (77.3) | .591 | .499 |
| Stage III-IV | 7 (35.0) | 21 (44.7) | 5 (22.7) | |
| Diarrhoea at time of endoscopy | | | | | |
| Yes | 16 (80.0) | 40 (85.1) | 19 (86.4) | .721 | .691 |
| With grossly bloody stool | 2 (10.0) | 10 (21.3) | 4 (18.2) | .487 | .665 |
| No (= solely upper GI tract symptoms) | 4 (20.0) | 7 (14.9) | 3 (13.6) | .721 | .691 |
| Anti–GI-GVHD treatment at time of endoscopyb | | | | | |
| Yes | 15 (75.0) | 37 (78.7) | 13 (59.1) | .756 | .338 |
| Median number of days (IQR) | 30.0 (7.0-60.0) | 10 (4.0-26.0) | 19.0 (7.5-32.2) | .100 | .264 |
| Total parenteral nutrition during ≥ 3 consecutive daysc | | | | | 1.000 | 1.000 |
| Yes | 6 (30.0) | 16 (34.0) | 6 (27.3) | |
| No | 14 (70.0) | 31 (66.0) | 16 (72.7) | 1.000 | 1.000 |
| IV antibiotics during ≥ 3 consecutive daysc | | | | | 1.000 | 1.000 |
| Yes | 12 (60.0) | 27 (57.4) | 13 (59.1) | |
| No | 8 (40.0) | 20 (42.6) | 9 (40.9) | |

Abbreviations: EGD, Esophagogastroduodenoscopy.

aThe subgroup with GI-GVHD detected in clinical standard histopathological assessment but not in the retrospective independent assessment (+−) was excluded due to low sample size (three endoscopic occasions).

bInitiated ≥ 1-day pre-endoscopically.

cWithin 21 d preceding the endoscopy.
result was not influenced by drugs or infectious agents, potentially mimicking the histopathological pattern of GI-GVHD. Furthermore, we observed that GI-GVHD detected in RIHA, but not in the CSHA (− +), was associated with an increased risk of a subsequent re-endoscopy. Death as competing event for the risk of a subsequent re-endoscopy did not affect that outcome (data not shown). Finally, non-detected GI-GVHD in the CSHA but detected in the RIHA (− +) did not influence the probability of two-year post-HSCT survival.

The NIH 2014 recommend, finding of at least one apoptotic body in a crypt per biopsy piece, as threshold of histopathology-based GI-GVHD diagnosis. However, higher number of crypt-apoptosis has been proposed as minimal criteria, based on the intention to increase diagnostic specificity. Higher number of crypt-apoptosis needed for the GI-GVHD diagnosis may, however, due the trade-off between diagnostic sensitivity and specificity, decrease the sensitivity.

In the present study, we used the cut-off level by the NIH 2014 as minimal criteria of histopathology-based GI-GVHD diagnosis together with evaluation of histopathological mimics to the GI-GVHD diagnosis, that is factors that may cause false-positive results. Furthermore, we used the GI-GVHD histological severity scoring system by Lerner et al. In that scoring system, grade 1 solely includes apoptotic bodies. For grades 2-4, additional histological findings are needed.

We found that histological severity scoring grade 1 was significantly more frequent among those with GI-GVHD solely detected in the RIHA (− +) as compared to endoscopic occasions with detection of GI-GVHD in both readings (+ +). This result may represent differences in the threshold of the GI-GVHD diagnosis, with higher number of crypt-apoptosis needed, by some pathologists performing the CSHAs, compared with the NIH 2014 based RIHA.

In a previous adult patient study, with a design similar to our study, 64% of the endoscopies with histopathology-based non-GI-GVHD diagnosis in CSHA were reclassified as GI-GVHD in a re-evaluation. The corresponding result in our study was 48%. Furthermore, we found that GI-GVHD established in the CSHA was confirmed in the RIHA in 94%. These findings indicate that in children with symptom-based GI-GVHD but without histopathological confirmation in CSHA, a second, NIH 2014 based histopathological assessment, may be of clinical value.

In children, contrary to adults, gastrointestinal endoscopy is recommended to be performed under general anaesthesia, or if not available, under deep sedation. The general anaesthesia/deep sedation may add short- and long-term risks to the endoscopic procedure. In a previous paediatric study, including long-time survivors of childhood acute lymphoblastic leukaemia, a repeatedly exposure to general anaesthesia was associated with neurocognitive impairments and neuroimaging abnormalities. Furthermore, intraduodenal haematoma related to the biopsy sampling have been reported more frequently in children if the indication for endoscopy is suspected GI-GVHD as compared to other medical indications. Thus, an overall avoidance of general anaesthesia and avoidance of potentially unnecessary endoscopies during the post-HSCT period is of clinical importance, in children.

No gold standard for diagnosing GI-GVHD has so far been established. Symptom-based diagnosis, which is used most often, is less reliable due to its limited specificity. However, the MAGIC consensus GVHD research guidelines assign histopathological verification of GI-GVHD as the most solid diagnostic test for the attribution of gastrointestinal symptoms to GI-GVHD. Our study design precludes an assessment of whether one of the two histopathological assessments method is superior to the other. However, and in line with the MAGIC guidelines, we believe that it is reasonable to interpret histopathology finding of GI-GVHD, as true GI-GVHD, regardless which
| Histopathological mimic | GI-GVHD detected in both readings \(+ +\) | GI-GVHD only in retrospective assessment \(\rightarrow\) | \(P\) |
|--------------------------|---------------------------------|---------------------------------|-----|
| CMV immunohistochemistry \(i, h\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 6 (12.8) | 1 (5.0) | .665 |
| No | 41 (87.2) | 19 (95.0) | |
| Salmonella, Shigella, Yersinia, Campylobacter spp. \(d, i\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 0 | 0 | -- |
| No | 29 (65.9) | 16 (94.1) | |
| Missing data | 15 (34.1) | 1 (5.9) | |
| N/A \(b\) | 3 | 3 | |
| Norovirus \(a, i\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 3 (10.0) | 2 (22.2) | .270 |
| No | 16 (53.3) | 3 (33.3) | |
| Missing data | 11 (36.7) | 4 (44.4) | |
| N/A \(b\) | 17 | 11 | |
| Rotavirus \(a, i\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 2 (6.7) | 0 | 1.000 |
| No | 21 (70.0) | 4 (44.4) | |
| Missing data | 7 (23.3) | 5 (55.6) | |
| N/A \(b\) | 17 | 11 | |
| Clostridium difficile \(i, j\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 5 (11.6) | 3 (18.7) | .361 |
| No | 32 (74.4) | 8 (50.0) | |
| Missing data | 6 (13.9) | 5 (31.2) | |
| N/A \(b\) | 4 | 4 | |
| Endoscopy within 20 d from HSCT \(c\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 4 (8.5) | 0 | .309 |
| No | 43 (91.5) | 20 (100.0) | |
| Mycophenolate mofetil at time of endoscopy \(c\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 5 (10.6) | 5 (25.0) | .136 |
| No | 42 (89.4) | 14 (70.0) | |
| Missing data | 0 | 1 (5.0) | |
| Tacrolimus at time of endoscopy \(c\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 11 (23.4) | 7 (35.0) | .364 |
| No | 35 (74.5) | 12 (60.0) | |
| Missing data | 1 (2.1) | 1 (5.0) | |
| Proton pump inhibitor at time of endoscopy \(d\) | \(n = 47\) (%) | \(n = 20\) (%) | \(P\) |
| Yes | 16 (76.2) | 4 (80.0) | 1.000 |
| No | 5 (23.8) | 1 (20.0) | |
| N/A \(b\) | 26 | 15 | |

\(a\) GI-GVHD detected in clinical standard histopathological assessment and retrospective independent blinded assessment.

\(b\) Endoscopic occasions were classified as N/A if biopsy sampling was not performed within the "main target region" of the mimic, or if the GI-GVHD diagnosis were solely detected in regions of the GI tract outside the "main target region" of a mimic. Main target regions:

\(c\) Rectum to the terminal ileum and duodenum to oesophagus,

\(d\) Rectum to the terminal ileum, the terminal ileum and the duodenum,

\(e\) Rectum to caecum,

\(f\) Gastric antrum and corpus.

\(i\) Based on retrospective independent assessment.

\(j\) Data collected during 21 days preceding each endoscopy.

\(k\) Clostridium difficile toxin positive.
assessment method that detects it, in our cohort of symptom-based GI-GVHD children.

The results in the present study should be interpreted in the context of its limitations. The clinical consequences of mismatch of the GI-GVHD diagnosis between RIHA and CSHA may have been influenced by the limited sample size. Furthermore, differences in the prerequisites for the RIHA vs the CSHA may have had an impact on the results. The RIHAs were performed in a scientific setting, blinded, protocol-based, by one pathologist using a uniform definition of the histopathology-based GI-GVHD diagnosis. The CSHAs, on the other hand, were performed with access to some clinical data. Furthermore, the CSHAs were performed by different pathologists at different HSCT centres, thus potentially influenced by inter-individual and inter-institutional variations for the threshold of the GI-GVHD diagnosis.

In summary, we have found that GI-GVHD in children with low histological severity grade is at risk to evade detection in CSHA. Furthermore, non-detected GI-GVHD in the CSHA but detected in the RIHA (−→) was associated with increased risk of a subsequent re-endoscopy vs endoscopies with an agreement of non-GI-GVHD in both readings (−−). In conclusion, our results suggest that in children with symptom-based GI-GVHD without histopathological confirmation in CSHA, a second, NIH 2014 based histopathological assessment should be considered before performing a re-endoscopy.

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All authors declare no competing financial interests in relation to the present study.

AUTHORS’ CONTRIBUTION
All authors meet the authorship criteria defined by ICMJE.

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