OPEN

Idiopathic Ileal Ulceration After Intestinal Transplantation

Elsadig Hussan, MD,[1] Alexander Kroemer, MD,[1,2] Ahmed M. Elsabbagh, MD,[3] Khalid M. Khan, MD,[1,2] Nada A. Yazigi, MD,[1,2] Udeme D. Ekong, MD,[1,2] Sukanya Subramanian, MD,[1,2] Shahira S. Ghobrial, PharmD,[4] Juan-Francisco Guerra, MD,[1,2] Thomas M. Fishbein, MD,[1,2] Cal S. Matsumoto, MD,[1,2] and Stuart S. Kaufman, MD[1,2]

Background. Idiopathic ileal ulceration after intestinal transplantation (ITx) has been discussed infrequently and has an uncertain natural history and relation to graft rejection. Herein, we review our experience with this pathology. **Methods.** We retrospectively reviewed 225 ITx in 217 patients with minimum 1 y graft survival. Routine graft endoscopy was conducted up to twice weekly within the first 90 d after ITx, gradually decreasing to once yearly. Risks for ulceration over time were evaluated using Cox regression. **Results.** Of 93 (41%) patients with ulcers, 50 were found within 90 d after ITx mostly via ileoscopy; delayed healing after biopsy appeared causal in the majority. Of the remaining 43 patients with ulcers found >90 d after ITx, 36 were after ileostomy closure. Multivariable modeling demonstrated within 90-d ulcer associations with increasing patient age (hazard ratio [HR], 1.027; $P < 0.001$) and loop ileostomy (versus Santulli ileostomy; HR, 0.271; $P < 0.001$). For ulcers appearing after ileostomy closure, their sole association was with absence of graft colon (HR, 7.232; $P < 0.001$). For ulcers requiring extended anti-microbial and anti-inflammatory therapy, associations included de novo donor-specific antibodies (HR, 3.222; $P < 0.007$) and nucleotide oligomerization domain mutations (HR, 2.772; $P < 0.016$). Whole-cohort post-ITx ulceration was not associated with either graft rejection ($P = 0.161$) or graft failure ($P = 0.410$). **Conclusions.** Idiopathic ulceration after ITx is relatively common but has little independent influence on outcome; risks include ileostomy construction, colon-free ITx, immunologic mutation, and donor sensitization.

(*Transplantation Direct* 2023;9: e1529; doi: 10.1097/TXD.0000000000001529.)

Allograft rejection is the primary immunologic complication of solid organ transplantation and thereby critically influences graft and patient survival. This relationship is particularly relevant to intestinal transplantation (ITx)[1] because the unusually high graft immune cell load elicits a correspondingly frequent and intense recipient immune response.[2] Another, less appreciated, inflammatory complication of ITx is idiopathic ulceration of the graft ileum in proximity to colon.[3,4] Early in our ITx experience, we periodically observed graft ileal ulcers during protocol surveillance endoscopy, mainly after ileostomy closure. These ulcers seemed to produce few symptoms and consistently spared more proximal graft. Differing from descriptions by other investigators,[5] we rarely found evidence of infection or other typical features of graft rejection.[6]

Although the inflammatory pathway or pathways leading to focal ileal ulceration remain unclear, it is intuitive that idiopathic ileal ulceration should have a connection to allograft rejection as both represent graft-directed inflammatory processes. However, discerning the place of idiopathic ileal ulceration within the spectrum of post-ITx allo-pathologies is complicated by its similarity to auto-inflammatory disorders such as ileum-predominant Crohn's disease (CD)[7] and intestinal ulceration following ileocolonic resection.[8–10] The main objective of this study was to determine the clinical importance of post-ITx ileal ulceration, specifically its impact on graft survival, and, secondarily, its clinical relationship to graft rejection. To fulfill these objectives, we characterized

Received 11 May 2023. Revision received 9 June 2023.

Accepted 19 June 2023.

[1] MedStar Georgetown Transplant Institute, MedStar Georgetown University Hospital, Washington, DC.

[2] Georgetown University School of Medicine, Washington, DC.

[3] Gastroenterology Surgical Center, Department of Surgery, Mansoura University, Mansoura, Egypt.

[4] Pharmacy, MedStar Georgetown University Hospital, Washington, DC.

The authors declare no funding or conflicts of interest.

E.H., A.K., and S.S. participated in research design and writing the article. A.M.E. participated in data analysis and writing the article. K.M.K., N.A.Y., U.D.E., S.S.G., J.F.G., T.M.F., and C.S.M. participated in writing the article. S.S.K. participated in research design, performing the research, data analysis, and writing the article.

Correspondence: Stuart S. Kaufman, MD, MedStar Georgetown Transplant Institute, MedStar Georgetown University Hospital, 2-PHC, 3800 Reservoir Road NW, Washington, DC 20007-2197. (Stuart.S.Kaufman@gunet.georgetown.edu).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001529

idiopathic ileal ulceration comprehensively including risks for occurrence, complications, and efficacy of treatment.

MATERIALS AND METHODS

Study Population

Two hundred and eighty-nine ITx were performed at this center between November 2003 and December 31, 2019; overall 1-, 5-, and 10-y graft survival equaled 82.7%, 65.1%, and 60.3%, respectively. To ensure adequate time for ulcer development, at least 1 y of graft survival was required for inclusion in the study that was closed to further analysis on December 31, 2020; 217 patients receiving 225 ITx met this criterion. ITx were analyzed individually in patients receiving 2 grafts.

Clinical Transplant Practice

ITx was performed for indications using variant procedures as described;[11] a graft colon and ileocecal valve (ICV) were included in case of earlier total or near-total native colectomy until 2009 and routinely thereafter. Graft stoma placement, most typically ileostomy, accompanied all ITx. A Santulli ileostomy consisting of a single barrel with an internal, side to end anastomosis to remnant native colon[12] was mostly used through 2009. Afterward, mostly loop ileostomies were performed. With small bowel-only grafts, the loop distal limb was anastomosed to remnant native colon (no ICV), whereas colon-inclusive grafts incorporated a transverse or descending colon anastomosis to remnant native colon. Loop ileostomies were typically placed 10–20 cm proximal to the graft to native ileocolonic anastomosis or graft ICV. Patients who had undergone total colectomy typically received a graft end-colostomy in place of ileostomy. Induction immunosuppression consisted of either basiliximab or rabbit anti-thymocyte globulin (r-ATG). Maintenance immunosuppression consisted of tacrolimus supplemented with either sirolimus (SIR) or mycophenolate mofetil (MMF) and low-dose steroids as previously detailed.[11]

Graft Evaluation

Protocol surveillance endoscopy and biopsy of the graft via ileostomy or colostomy began 1 wk after ITx, continuing twice weekly for 6 wk and once weekly for 6 more wk (total about 90 d). Thereafter, endoscopic surveillance was conducted once monthly until ileostomy closure, typically 3–5 mo after ITx, then 3 mo after ileostomy closure, and 12, 18, and 24 mo after ITx, and then annually. Once ulcers were detected, additional endoscopies were performed to assess treatment responses. Endoscopic biopsy generally used 1.8 mm forceps in infants and small children and 2.8 mm forceps in older children and adults.

Post-ITx ileal ulceration was defined as given in Table 1. All endoscopy reports were reviewed for photographs showing ulcers and their descriptions, being categorized by adaptation of the Simplified Endoscopic Activity Score for Crohn's Disease (SES-CD) of Daperno et al[13] as summarized in Table 2; predominant histological features were abstracted from accompanying pathology reports. Location and severity of graft rejections were catalogued for each ITx. New rejection events were designated if separated from past occurrences by at least 1 unambiguously normal endoscopy and accompanying biopsies. Acute and chronic rejection, various infective enterocolitises, graft versus host disease, and miscellaneous pathologies were as defined previously.[11] Antibody-mediated rejection was assessed in selected cases of acute rejection with disproportionately severe vascular change and detectable donor-specific antibodies (DSAs) with C4d stain.[14] Although ulcer treatment was often the subject of extensive discussion, treatment decisions were at the discretion of individual practitioners. Grafts were considered to have failed, that is become nonfunctional, if progressive decline in enteral nutrition tolerance resulted in permanent total parenteral nutrition or crystalloid therapy.

TABLE 2.

Adapted SES-CD for categorization of distal ileal graft ulcers[13]

Score components
Criterion 1: Ulcer size—maximal diameter
None = 0
0.1–0.5 cm = 1
0. 5–2 cm = 2
> 2 cm = 3
Criterion 2: Extent of ulcerated surface
None = 0
<10% = 1
10%–30% = 2
>30% = 3
Criterion 3: Extent of affected surface
None = 0
<50% = 1
50%–75% = 2
> 75% = 3
Criterion 4: Presence and type of narrowing
None = 0
Single, can be passed = 1
Multiple, can be passed = 2
Cannot be passed = 3

Four endoscopic criteria listed above are scored from 0 to 3 and summed to a give a total score.
SES-CD, Simplified Endoscopic Activity Score for Crohn's Disease.

TABLE 1.

Graft terminal ileal ulceration after ITx—definition

Definition criteria
Endoscopic aphthous ulceration generally within 10 cm of colon with normal or minimally abnormal intervening mucosa
No typical endoscopic features of acute graft rejection including visible mucosal slough[11] and no increase in crypt apoptosis in inter-ulcer epithelium
No typical endoscopic features of chronic graft rejection including reduced mural compliance with or without stricture, poor motility, and diffuse mucosal atrophy accompanying inflammation with or without frank ulceration[11]
No features of EBV, CMV, or other infection
Limited proximal ileal ulcer extension with visually normal mucosa upstream
With or without anastomotic involvement but not exclusively anastomotic
No involvement of adjacent native or graft colon
Consistent jejunal graft sparing

CMV, cytomegalovirus; EBV, Epstein-Barr virus; ITx, intestinal transplantation.

TABLE 3.

Data obtained from chart review

Data categories
Demographic factors
Age at intestinal transplant
Gender
Underlying disease and classification
Anatomic intestinal failure (short gut syndrome)
Functional intestinal failure (secretory diarrhea and chronic pseudoobstruction)
Nonintestinal failure
Underlying genetic diagnosis
NOD mutation
Previous intestinal transplant
Transplant-related factors
Total panel reactive antibody percentage at transplant
Preformed DSAs
Human leukocyte antigen DR and human leukocyte antigen DQ mismatches
Donor: Recipient age ratio
Donor: Recipient weight ratio
r-ATG to donor
Induction immunosuppression
Type of intestinal transplant
Isolated intestine, liver-inclusive intestine, multivisceral, or modified multivisceral
Graft colon inclusion
Type of colo-colostomy construction
If no graft colon, then manner of ileocolonic anastomotic construction
Graft kidney inclusion
Ileostomy construction including Santulli, loop, or end vs end-colostomy
Cold ischemia time
After transplant factors
Time of ileostomy closure
Serial colonoscopic estimation of colon length
Time of de novo DSAs
Duration SIR and MMF exposure within first 3 mo and first year after transplant
Time of first colonoscopy after ileostomy closure and final colonoscopy before study closure
Time of onset of all acute rejection events
Time of onset of postintestinal transplant ileal ulcer events
Symptoms concurrent with ulcer detection
Ulcer responses to treatment
Status of ileal ulceration at final colonoscopy before either study closure or graft failure
Time of graft failure when before study closure
Time of death when before study closure

DSA, donor-specific antibody; MMF, mycophenolate mofetil; NOD, nucleotide oligomerization domain; r-ATG, rabbit anti-thymocyte globulin; SIR, sirolimus.

Data Extracted From Chart Review

Information collected was as summarized in Table 3.

Analysis

Comparisons of continuous variables were made using Mann-Whitney and Kruskal-Wallis tests, and proportions were compared using Fisher and Fisher-Freeman-Halton tests. Central tendencies were expressed as medians with interquartile range (first quartile–third quartile).

Cox proportional hazards regression was used for outcome modeling with the censoring date designated December 31, 2020. Conformity of predictor variables with the proportional hazards assumption was confirmed by parallelism of log-minus-log plots (categorical variables) and absence of interaction with time (continuous variables).[15,16] Predictor variables were treated as time-independent if established by the day of ITx, for example patient age, indication for and type of ITx, and presence of preformed DSA. Predictor variables were treated as time-dependent if arising during post-ITx follow-up, for example appearance of DSA de novo, episodes of graft rejection, and detection of ulcers themselves.[17] Regression results were expressed as hazard ratios (HRs) with 95% confidence interval. Significance was taken as $P < 0.050$. Calculations were performed using IBM SPSS Statistics for Windows, Version: 28.0 (Armonk, NY: IBM Corp). This study was approved by the Institutional Review Board of Georgetown University (STUDY00006449).

RESULTS

Of the 225 ITx included in this study, ulceration within the distal ileal graft was found at least once in 93 patients (41%) whose median age at ITx was 23.0 y (1.7–43.7 y). In 50 of the 93 (54%), ulcers were first seen within 90 d (median 28 d [19–40 d]) after ITx, the period of once to twice weekly endoscopic graft surveillance. Of these 50, 47 patients had an ileostomy and 3 had an end-colostomy. Of those with ulcers demonstrated via ileostomy, stoma patency was maintained during the entire 90-d period except for 2 patients whose stomas were closed 68 and 89 d post-ITx.

Of the 43 patients who first demonstrated ileal ulcers >90 d (median 571 d [295–1450 d]) after ITx, the diagnosis was made via still-open ileostomy in 6, end-colostomy in 1, and standard colonoscopy after ileostomy closure in 36. Because ileal ulcers tended to segregate into 2 groups defined by time after ITx and ileostomy status, these groups were further evaluated individually.

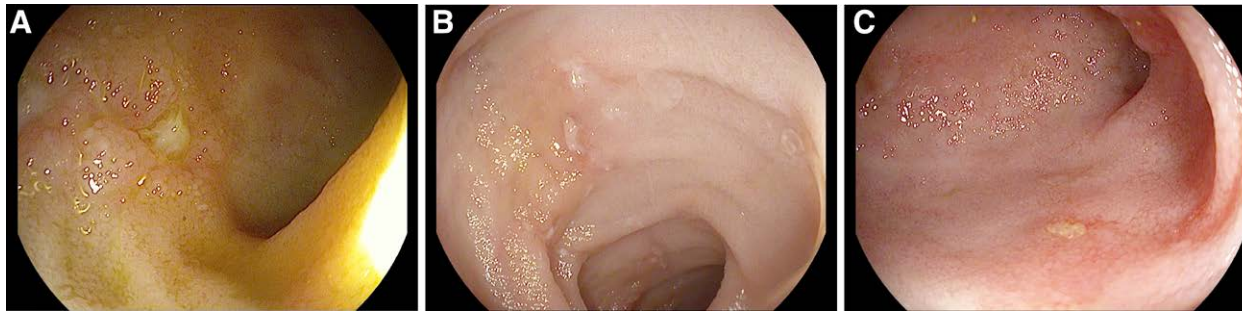

FIGURE 1. Ileal ulceration within 90 d after intestinal transplant. A, First ulcer 58 d after intestinal transplant. Contemporaneous report suggested site of prior biopsy. B, Multiple, shallow first ulcers 33 d after intestinal transplant. Contemporaneous report suggested sites of prior biopsy. C, First ulcer 45 d after intestinal transplant. Contemporaneous report suggested no specific etiology.

Ulcers First Identified Within 90 d After ITx

Of the 50 patients, ulcers were contemporaneously thought to have been sites of previous biopsy in 38 (76%) and without definite etiology in the remainder (Figure 1). In 29 of 33 patients with a loop ileostomy, 17 patients had ulcers exclusively in the afferent, in-continuity limb, 4 exclusively in the efferent, diverted limb, and 8 in both limbs. Median total mucosal SES-CD score in the 50 patients equaled 3 (3–3). Mucosa next to ulcers was visually normal in 49 of the 50 patients, the sole exception demonstrating mild erythema without an identifiable pathogen. Notable microscopic findings were present in 22 of 48 (46%) available biopsy specimens as delineated in Table 4. Exclusion from the analysis of the 3 patients whose ileal biopsies were obtained via endcolostomy did not affect proportions of patients in the various histological categories (P = 1.000). Although cytomegalovirus DNAemia was found contemporaneously in 1 of 31 and Epstein-Barr virus DNAemia in 7 of 32 patients, none had histological evidence of ileal infection.

Of 34 patients with adequate documentation, 9 patients (26%) had symptoms at ulcer detection as detailed in Table 5; all had an ileostomy. Interventions included discontinuation of SIR in 3 (of 12 patients receiving SIR) because of concurrent norovirus infection, postbiopsy bleeding, and spontaneous graft perforation in single patients. A fourth patient with postbiopsy bleeding received local therapy, and a fifth patient received methylprednisolone intravenous for contemporaneous graft versus host disease. Ulcers resolved permanently or temporarily in all 5 patients. No patient initially received other anti-inflammatory or anti-microbial therapy. MMF was not discontinued in any of the 8 patients receiving it at ulcer diagnosis (versus SIR discontinuation; P = 0.24).

Ulcers first found within 90 d of ITx recurred at least once in 27 of the 50 (54%) patients, including 2 of the 5 who had received treatment. By study closure on December 31, 2020, grafts in 23 of the 50 (46%) patients remained functional without ulceration on no treatment. Grafts in 2 (4%) patients remained functional without ulceration under metronidazole or infliximab started after ulcer recurrence, and a single (2%) patient maintained a functional graft despite persistent ulceration on metronidazole. Grafts in the remaining 24 of 50 (48%) failed due refractory acute rejection in 5, chronic rejection in 6, other causes including death in 11, and unknown in 2. Just 1 of the 24 patients whose graft failed because of acute infective respiratory failure had persistent ulceration despite receiving metronidazole, ciprofloxacin, and budesonide at time of death.

Ulcers First Identified >90 d After ITx and After Ileostomy Closure

Of the 36 patients in anatomic continuity at first ulcer detection, median time of first colonoscopy after ileostomy closure was 90 d (48–122 d), and median time of initial ulcer

TABLE 4.

Mucosal pathology adjacent to graft ileal ulcers

	Ulceration within 90 d after ITx	Ulceration after ileostomy closure	*P*
Predominant finding	**N abnormal[a] = 22 of 48 (46%)**	**N abnormal[b] = 26 of 34 (76%)**	**< 0.001**
Acute inflammation	8 (17%)	7 (21%)	0.774
Apoptosis without inflammation[c]	2 (4%)	1 (3%)	1.0
Chronic inflammation	7 (15%)	5 (15%)	1.0
Eosinophilic inflammation	2 (4%)	3 (9%)	0.644
Lymphoid hyperplasia	**3 (6%)**	**10 (29%)**	**0.006**

[a] Forty-eight of 50 patients with adequate data for review.
[b] Thirty-four of 36 patients with adequate data for review.
[c] Apoptosis nondiagnostically increased.
Bold values indicate statistically significant (P < 0.050), associated test results in the same line, particularly for multivariable regression.
ITx, intestinal transplantation.

TABLE 5.

Symptoms at ulcer detection

	Ulceration ≤ 90 d after ITx	Ulceration after ileostomy closure	
	N = 34[a]	**N = 34[b]**	***P***
Feature	**N with symptoms = 9 of 34 (26%)**	**N with symptoms = 15 of 34 (46%)**	**0.204**
Abdominal pain	2 (6%)	6 (18%)	0.258
Fever	3 (9%)	5 (15%)	0.709
Increased stool	**4 (12%)**	**12 (35%)**	**0.043**
Lower gastrointestinal bleeding	2 (6%)	5 (15%)	0.428
Nausea and vomiting	2 (6%)	4 (12%)	0.673
Weight loss	0 (0%)	2 (6%)	0.492

[a] Thirty-four of total 50 patients with adequate data for review. Of the 34, 9 patients experienced 1 or more symptoms.
[b] Thirty-four of 36 total patients with adequate data for review. Of the 34, 15 experienced 1 or more symptoms.
Bold values indicate statistically significant (P < 0.050), associated test results in the same line, particularly for multivariable regression.
ITx, intestinal transplantation.

detection after the first colonoscopy was 307 d (82–1008 d). Ulcers were detected during the first postclosure colonoscopy in 6 of the 36 (17%). Eight other patients in whom ulcers were found within 90 d after ITx demonstrated ulcer recurrence after ileostomy closure including 2 during the first postclosure colonoscopy.

Because of the implication that ileostomy closure itself might have influenced ulcer development, analysis focused on the 36 patients with no prior ulcer history. These ulcers were typically larger and more complex (median total SES-CD score 4 [3–4]; Figures 2 and 3) than ulcers detected within 90 d of ITx (P = 0.004). Mucosa bordering ulcers was inconsistently erythematous. Biopsies obtained next to ulcers were available for review in 34 of the 36 patients; as summarized in Table 4, only lymphoid hyperplasia was more frequent after ileostomy closure. Concurrent cytomegalovirus DNAemia was found in 1 of 32 patients (3%) and Epstein-Barr virus in 6 of 30 (20%), but no patient had evidence of local infection. Of 34 patients receiving SIR, 6 (18%) developed ulcers of whom 5 continued treatment, and of 33 patients receiving MMF, 6 (18%) developed ulcers all of whom continued treatment.

Notable symptoms were present at first ulcer detection in 15 of 34 (46%) patients with adequate records for review, which did not differ from overall frequency of symptoms present at ulcer diagnosis within 90 d of ITx as detailed in Table 5. However, an increase in stool output over baseline was more commonly observed after ileostomy closure.

Ulcers first appearing after ileostomy closure were treated in 26 of the 36 (72%) patients with various antimicrobial and anti-inflammatory drugs including metronidazole in 21, ciprofloxacin in 14, rifaximin in 5, vancomycin in 2, trimethoprim-sulfamethoxazole in 2, mesalamine in 5, infliximab in 5, and budesonide in 2. Of the 26 patients who received treatment, complete (n = 21; Figure 2) or incomplete (n = 3) response was followed by ulcer recurrence or worsening in 15, giving a combined relapse or recurrence rate of 62%. Of the 10 initially untreated patients, 9 experienced spontaneous ulcer resolution of whom ulcers recurred in 4 (44%; versus treated patients; P = 0.442).

By study closure on December 31, 2020, 31 of the 36 (86%) patients had a functional graft. Of these 31, 6 patients were receiving treatment for persistent ulcers, whereas 11 of the 31 were in ulcer remission but continuing treatment intended to forestall recurrence (Figure 3). Of the 5 (14%) patients whose grafts ultimately failed, 2 had persistent ulcers despite ongoing treatment, whereas 2 others with resolved ulcers were continuing treatment. Causes of graft failure included protracted severe acute rejection in 1, chronic rejection in 3, and death from other causes in 1 (versus 48% all-cause graft failure after ulceration within 90 d of ITx; P = 0.001).

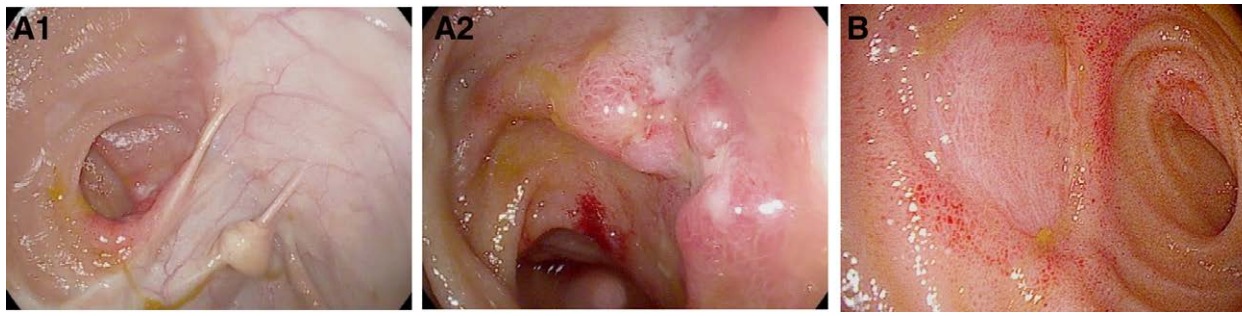

FIGURE 2. Transient ulceration after ileostomy closure. A1, First ulcer 221 d after ileostomy closure. Resolved with metronidazole and ciprofloxacin continuing at study close. A2, Close-up of A1. B, First ulcer 272 d after ileostomy closure. Resolved with metronidazole continuing at study close

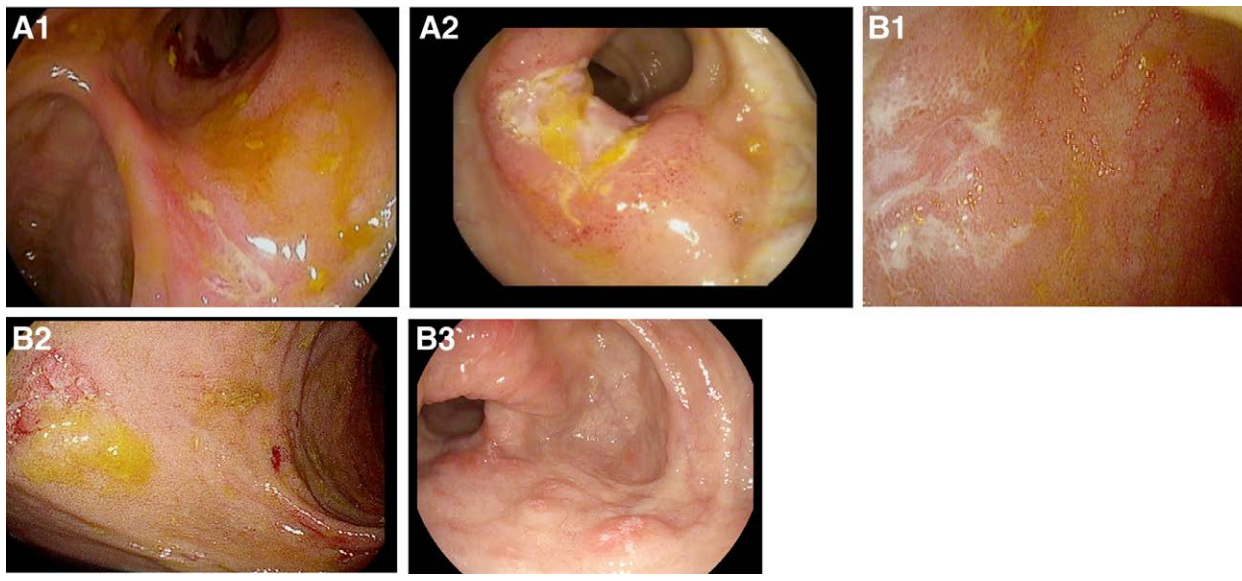

FIGURE 3. Persistent ulceration after ileostomy closure. A1, First ulcer 432 d after ileostomy closure. A2, Persistent ulcer with metronidazole continuing at study close. B1, First ulcer 216 d after ileostomy closure. B2, Persistent ulceration on rifaximin after metronidazole and ciprofloxacin. B3, Improved ulceration on infliximab, metronidazole, and ciprofloxacin.

Risks for Ileal Ulceration

Ulcer risks were assessed by comparison of the 93 patients who developed ulcers with 132 patients who did not. ITx cases were distributed similarly throughout the study ($P = 0.970$) as was distribution of patients developing and not developing ulcers ($P = 0.137$). Similar proportions of patients who did and did not develop ulcers received an ileostomy with ITx (95.7% versus 92.4%; $P = 0.407$) and underwent ileostomy closure (84.9% versus 86.4%; $P = 0.847$); the 2 groups also underwent initial colonoscopy after ileostomy closure at similar intervals (104 d [48–180 d] versus 87 d [54–138 d]; $P = 0.106$), respectively.

As shown in Table 6, the only factors associated with increased risk of ulceration within the 90-d interval after ITx were increasing age at ITx and a Santulli rather than loop ileostomy, both individually and combined in a multivariable model. There was no interaction between ileostomy type (loop versus Santulli) and graft colon inclusion as predictor variables ($P = 0.893$). Patients developing ulcers within 90 d after ITx were not more likely to have received MMF or SIR in that period ($P = 0.736$ and 0.468, respectively). Furthermore, there was no association between early ulceration and indication for ITx ($P = 0.223$), type of ITx ($P = 0.324$), colon inclusion ($P = 0.091$), cold ischemia time ($P = 0.220$), nucleotide oligomerization domain (NOD) mutation ($P = 0.911$), number of human leukocyte antigen DQ mismatches ($P = 0.189$), number of human leukocyte antigen DR mismatches ($P = 0.121$), preformed DSA ($P = 0.498$), or previous acute graft rejection ($P = 0.300$).

Table 7 summarizes Cox modeling of risks of first ileal ulceration after ileostomy closure. Although immunologic factors including NOD mutation and size of donor-recipient human leukocyte antigen DR mismatch were individually associated with ulceration, the sole factor showing both high and unambiguously independent risk for ulceration in the multivariable model was use of a small bowel graft without colon. Transplantation of a small without large intestinal graft remained the sole risk factor for postileostomy closure ulceration in a model combining ulcers recurring after ileostomy closure and new ulcers (HR, 5.411 [2.507–11.682]; $P < 0.001$).

To illustrate better those hazards associated with development of more clinically important ulcers, patients receiving continuing therapy with or without achieving ulcer remission were compared with all other patients including those never experiencing ulceration and those whose ulcers resolved with no or transient treatment. This analysis was summarized in Table 8. As shown in Table 8, immunologic factors, viz. NOD mutations and DSA acquired de novo, but not anatomic factors were associated with long-lived disease in the multivariable analysis.

Further insight into risk factors for persistent ulceration and/or persistent therapy to maintain ulcer remission was sought by focused follow-up of the 93 patients developing ulcers. As summarized in Table 9, multivariable analysis showed ulcer appearance within 90 d of ITx to be independently associated with a reduced risk of continuing treatment, whereas post-ITx induction with r-ATG was associated with increased risk for treatment-dependence. Neither preformed nor de novo DSA was associated with persistent ulceration ($P = 0.141$ and $P = 0.343$, respectively). Ulcer appearance after ileostomy closure was not in and of itself associated with refractory disease ($P = 0.620$).

Ileal Ulceration and Risks for Rejection and Graft Failure

First ileal ulceration at any time was not associated with acute rejection ($P = 0.161$) or eventual graft failure ($P = 0.410$). However, as shown in Table 10, ileal ulceration within 90 d after ITx was associated with an increased risk of eventual graft failure in the univariable model despite a reduced probability of protracted ileal ulceration per se. Conversely, ulceration appearing after ileostomy closure was possibly but

TABLE 6.

Cox regression modeling of risks for occurrence of ileal ulceration within 90 d after ITx[a]

Explanatory variable	Univariable HR (95% confidence interval)	P	Multivariable HR (95% confidence interval)	P
Age at transplant (y)	1.021 (1.008-1.035)	0.002	1.027 (1.013-1.042)	< 0.001
Loop ileostomy[b] relative to Santulli ileostomy[c]	0.384 (0.189-0.779)	0.008	0.271 (0.129-0.570)	< 0.001

[a] Censor date December 31, 2020.
[b] Including patients with graft terminal ileum in continuity with either graft colon incorporating ileocecal valve or anastomosed native colon.
[c] Graft terminal ileum anastomosed to native colon.
HR, hazard ratio; ICV, ileocecal valve; ITx, intestinal transplantation.

TABLE 7.

Cox regression modeling of risks for occurrence of ileal ulceration after ileostomy closure following ITx[a]

Explanatory variable	Univariable HR (95% confidence interval)	P	Multivariable HR (95%confidence interval)	P
No colon graft	**7.468 (3.101-17.984)**	**< 0.001**	**7.232 (2.773-18.861)**	**< 0.001**
NOD[b] mutation present	2.196 (1.069-4.511)	0.032		
Closed loop ileostomy relative to closed Santulli ileostomy[c]	0.369 (0.172-0.792)	0.010		
Number of DR mismatches	0.536 (0.285-1.008)	0.053		

[a] Censor date December 31, 2020.
[b] Nucleotide-binding oligomerization domain.
[c] Significant interaction between type of ileostomy closure and colon transplantation ($P < 0.001$).
Bold values indicate statistically significant ($P < 0.050$), associated test results in the same line, particularly for multivariable regression.
DR, Human leukocyte antigen DR; HR, hazard ratio; ITx, intestinal transplantation; NOD, nucleotide oligomerization domain.

TABLE 8.

Cox regression modeling of risks of posttransplant ileal ulceration under continuing treatment to final colonoscopy before study close[a] or patient drop-out[b]

Explanatory variable	Univariable HR (95% confidence interval)	P	Multivariable HR (95% confidence interval)	P
De novo DSAs	**3.366 (1.518-7.465)**	**0.003**	**3.222 (1.385-7.495)**	**0.007**
NOD[c] mutation present	**3.052 (1.388-6.714)**	**0.006**	**2.772 (1.205-6.380)**	**0.016**
No colon graft	3.262 (1.099-9.684)	0.033		
r-ATG relative to basiliximab[d]	2.628 (1.097-6.299)	0.030		
Side-to-end relative to side-to-side ileocolonic anastomosis[e]	0.340 (0.115-1.003)	0.051		

[a] December 31, 2020.
[b] Generally because of graft failure because of graft rejection or patient death.
[c] Nucleotide-binding oligomerization domain.
[d] As immunosuppression induction.
[e] In the absence of graft colon and graft ileocecal valve.
Bold values indicate statistically significant ($P < 0.050$), associated test results in the same line, particularly for multivariable regression.
DSA, donor-specific antibody; HR, hazrd ratio; ICV, ileocecal valve; NOD, nucleotide oligomerization domain; r-ATG, rabbit anti-thymocyte globulin.

TABLE 9.

Cox regression modeling of risks for continuing treatment of diagnosed ileal ulcers at last colonoscopy before study close[a] or patient drop-out[b]

Explanatory variable	Univariable HR (95% confidence interval)	P	Multivariable HR (95% confidence interval)	P
r-ATG relative to basiliximab[c]	**3.064 (1.275-7.363)**	**0.012**	**3.123 (1.263-7.722)**	**0.014**
Ileal ulceration within 90 d of ITx[d]	**0.219 (0.071-0.677)**	**0.008**	**0.251 (0.080-0.789)**	**0.018**
NOD[e] mutation present	2.513 (1.132-5.582)	0.024		

[a] December 31, 2020.
[b] Mainly because of failure because of graft rejection or patient death.
[c] As immunosuppression induction.
[d] ITx.
[e] Nucleotide-binding oligomerization domain.
Bold values indicate statistically significant ($P < 0.050$), associated test results in the same line, particularly for multivariable regression.
HR, hazard ratio; ITx, intestinal transplantation; NOD, nucleotide oligomerization domain; r-ATG, rabbit anti-thymocyte globulin.

TABLE 10.

Cox regression modeling of risks of graft failure after ITx through study close[a]

Explanatory variable	Univariable HR (95% confidence interval)	P	Multivariable HR (95% confidence interval)	P
Third rejection, any severity	**8.144 (4.252-15.595)**	**<0.001**	**4.582 (1.912-10.982)**	**<0.001**
First rejection, any severity	**3.120 (1.902-5.119)**	**<0.001**	**2.493 (1.272-4.890)**	**0.008**
Isolated intestine transplant[b]	**2.466 (1.306-4.654)**	**0.005**	**2.315 (1.048-5.117)**	**0.038**
Age at ITx[c]	1.017 (1.006-1.029)	0.002		
Ileal ulceration within 90 d of ITx[c]	1.812 (1.099-2.988)	0.020		
r-ATG relative to basiliximab[d]	1.765 (1.043-2.986)	0.034		
De novo DSAs	1.961 (1.035-3.716)	0.039		

[a] December 31, 2020.
[b] Either with or without colon graft compared with liver-inclusive graft.
[c] ITx.
[d] As immunosuppression induction.
Bold values indicate statistically significant ($P < 0.050$), associated test results in the same line, particularly for multivariable regression.
DSA, donor-specific antibody; HR, hazard ratio; ITx, intestinal transplantation; r-ATG, rabbit anti-thymocyte globulin.

not definitively associated with a reduced risk of graft failure (HR, 0.358 [0.122–1.046]; $P = 0.060$).

To put influences of ileal ulceration on graft survival into perspective, graft rejection and other factors derived from univariable Cox modeling were incorporated into a multivariable model also as shown in Table 10; only acute rejection and type of ITx transplant performed, specifically ITx without a liver graft, were independently associated with risk of graft loss in this model.

DISCUSSION

Results of this study show that ileal ulceration after ITx is relatively common, occurring in 41% of ITx recipients

evaluated. Surprisingly, a substantial percentage of ulcers seen within the 90-d period of frequent endoscopic surveillance mainly via ileostomy appeared to arise from delayed biopsy site healing. This finding contrasts with past reports that identified postbiopsy bleeding and perforation but not ulceration as primary complications of post-ITx endoscopy, principally ileoscopy.[18,19] Past lack of ulcer recognition may have been related to comparative infrequency of obvious symptoms and complications, and we, too, largely ignored these ulcers if concurrent biopsies were nondiagnostic.

Early post-ITx ulceration may be evidence of intestinal mucosal fragility resulting from greater abundance of luminal proinflammatory, facultative Proteobacteria including Enterobacteriaceae after ileostomy creation.[20,21] Greater ulcer risk associated with Santulli compared with loop ileostomy likely further emphasizes the microbiome as a determinant of mucosal health, because the Santulli maintains partial ileocolonic continuity[12] expected to maximize ileal bacterial density.[22] Use of larger biopsy forceps may explain ulcer association with increasing age. Increased ulcer frequency in functional stoma segments (Santulli and loop afferent limb) compared with diverted segments (loop efferent limb) suggests that luminal toxins potentially including SIR and MMF[23,24] might further increase ulcer risk. Although we found no compelling link between either SIR or MMF and mucosal injury, an association may have been hidden by our focus on the distal ileal graft rather than entire alimentary tract. Like luminal mucosal toxins, perioperative ischemia/reperfusion injury could theoretically exaggerate tissue injury from biopsy to delay healing.[25] This explanation is undermined by our failure to identify ischemic events associated with ulceration and reported transience of visual and microscopic intestinal mucosal injury due to ischemia/reperfusion.[26,27] In any case, the suggestion that ulceration occurring soon after ITx might increase the hazard of eventual graft failure implies that adverse perioperative events have long-lasting consequences for the graft.[28]

Importance of ulcer risk from partial ileocolonic continuity accompanying Santulli ileostomy was reinforced by repeated detection of de novo and recurrent ulcers after ileostomy closure, sometimes within months of the procedure, and presumably after microbiome transition to strict anaerobic predominance.[20] Inconsistent ulcer symptoms and signs including rarity of overt lower gastrointestinal tract bleeding may have been related to early detection through protocol colonoscopic graft surveillance. Most important, independently increased risk of ileal ulceration after ileostomy closure was confined to small intestinal grafts connected to native colon without intervening ICV, thereby allowing greater ileal bacterial colonization than would occur in presence of a normally functioning ICV.[29] Increased bacterial density in conjunction with immunosuppression[30] might also contribute to periulcerative lymphoid hyperplasia and larger ulcer size after ileostomy closure. Predicted, strict anaerobic bacterial predominance supplies a rationale for metronidazole therapy in this setting.[20,31] Although we found no association between ulcers and other environmental factors such as infection, experience derived from inflammatory bowel disease suggests that other unidentified factors contributed to variability in time of ulcer onset and response to therapy.[32,33]

Although anastomosis of ileal graft directly to native colon was the sole independent risk for any ulceration appearing after ileostomy closure, NOD mutations and de novo DSA were independently associated with those ulcers destined to receive most protracted treatment. These findings imply that immunologic factors are more important to long-term ulcer prognosis than unfavorable anatomy. However, because the impact of de novo DSA on ITx rejection is still somewhat ambiguous,[34,35] by analogy, the association of de novo DSA with post-ITx ulceration may also lack direct causation. R-ATG as induction immunosuppression was associated with prolonged treatment of established ulcers irrespective of the interval separating these events, again highlighting the apparent, long-lasting impact of inflammation and other immunologic processes originating in the early post-ITx period.

Results of this study support the impression that ulceration after ITx has clinical similarities to CD[7,36,37] and perianastomotic ulceration after intestinal resection[8,38,39] that include a propensity for terminal ileal involvement, frequent recurrence and relapse, and often less than satisfactory response to medical and surgical treatments. Furthermore, results of this study imply some commonality of pathophysiology of post-ITx ulceration and graft rejection despite absence of clinical connection, viz. an exaggerated inflammatory response promoted by the local microbiome and mucosal innate immune system mutations including NOD.[21,40–42] This potential relationship notwithstanding, post-ITx ulceration overall was not definitively associated with graft failure when simultaneously assessed with graft rejection and another established survival hazard, ITx with no liver graft.[43–45]

Antimicrobials given with the intention of suppressing a dysfunctional microbiome have been the mainstay of post-ITx ulcer therapy in our program, especially after ileostomy closure. Similarities to inflammatory bowel disease have more recently motivated use of biologicals such as the anti-tumor necrosis factor agent infliximab by us and others, particularly when post-ITx ulcers have been refractory to previous therapies.[3,4,6] Anti-tumor necrosis factor agents have demonstrated efficacy against graft rejection[3,46] and postintestinal resection ulceration,[8,10] further suggesting a mechanistic relationship between these entities.

The historically uncontrolled nature of our post-ITx ulcer management precludes new, specific recommendations based on this study. However, it seems likely that in the future, several classes of biologicals will be used earlier in disease course together with conventional antimicrobials.

CONCLUSIONS

Idiopathic post-ITx ulceration not connected with rejection is relatively common in certain high-risk anatomic settings. Pathophysiology shared with other types of inflammation, most notably ileal CD, appears to include interaction with luminal bacteria facilitated by ITx surgery itself and mutation-associated variations in innate immune function. However, prognosis is generally favorable, and risk of graft loss is low compared with traditional hazards for poor outcome.

FUTURE CONSIDERATIONS

The high incidence of ulceration attributable to endoscopic biopsy may contribute to ongoing discussions about benefits and risks of protocol graft surveillance after ITx.[47] Current ITx practice generally includes graft colon with ICV, thereby reducing immediate clinical relevance of our findings related to ileostomy closure. However, the extent to which idiopathic

ulceration, graft rejection, and CD share patterns of microbial dysbiosis and inflammation is not established. Should future ITx be conducted without a grafted colon and complicated by idiopathic ileal ulceration, it shall be particularly desirable to investigate immune cell phenotypes, cytokine profiles, and local microbiome. Such investigations may both characterize extent of linkage to other inflammatory bowel diseases and lead to more rationally targeted therapies for various forms of inflammation after ITx.

REFERENCES

1. Elsabbagh AM, Hawksworth J, Khan KM, et al. Long-term survival in visceral transplant recipients in the new era: a single-center experience. *Am J Transplant*. 2019;19:2077–2091.
2. Takahashi H, Kato T, Delacruz V, et al. Analysis of acute and chronic rejection in multiple organ allografts from retransplantation and autopsy cases of multivisceral transplantation. *Transplantation*. 2008;85:1610–1616.
3. Gerlach UA, Koch M, Müller HP, et al. Tumor necrosis factor alpha inhibitors as immunomodulatory antirejection agents after intestinal transplantation. *Am J Transplant*. 2011;11:1041–1050.
4. Fujimura T, Yamada Y, Umeyama T, et al. Maintenance treatment with infliximab for ulcerative ileitis after intestinal transplantation: a case report. *World J Clin Cases*. 2021;9:5270–5279.
5. Sarkar S, Selvaggi G, Mittal N, et al. Gastrointestinal tract ulcers in pediatric intestinal transplantation patients: etiology and management. *Pediatr Transplant*. 2006;10:162–167.
6. Matsumoto CS, Zasloff MA, Fishbein TM. Chronic mucosal inflammation/inflammatory bowel disease-like inflammation after intestinal transplantation: where are we now?. *Curr Opin Organ Transplant*. 2014;19:276–280.
7. Wehkamp J, Koslowski M, Wang G, et al. Barrier dysfunction due to distinct defensin deficiencies in small intestinal and colonic Crohn's disease. *Mucosal Immunol*. 2008;1(Suppl1):S67–S74.
8. Charbit-Henrion F, Chardot C, Ruemmele F, et al. Anastomotic ulcerations after intestinal resection in infancy. *J Pediatr Gastroenterol Nutr*. 2014;59:531–536.
9. Ceylan H, Puntis JW, Abbott C, et al. Recurrent perianastomotic ileo/jejuno-colic ulceration. *J Pediatr Gastroenterol Nutr*. 2000;30:450–452.
10. Freeman JJ, Rabah R, Hirschl RB, et al. Anti-TNF-α treatment for postanastomotic ulcers and inflammatory bowel disease with Crohn's-like pathologic changes following intestinal surgery in pediatric patients. *Pediatr Surg Int*. 2015;31:77–82.
11. Kaufman SS, Hussan E, Kroemer A, et al. Graft versus host disease after intestinal transplantation: a single-center experience. *Transplant Direct*. 2021;7:e731.
12. Vinit N, Rousseau V, Broch A, et al. Santulli procedure revisited in congenital intestinal malformations and postnatal intestinal injuries: preliminary report of experience. *Children (Basel)*. 2022;9:84.
13. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, Simplified Endoscopic Activity Score for Crohn's Disease: the SES-CD. *Gastrointest Endosc*. 2004;60:505–512.
14. Hawksworth JS, Matsumoto CS. Donor-specific antibody management in intestine transplantation: hope for improving the long-term durability of the intestine allograft? *Curr Opin Organ Transplant*. 2019;24:212–218.
15. Kleinbaum DG, Klein M. Evaluating the proportional hazards assumption. In: Statistics for Biology and Health; Gail M, Samet JM, editors. Survival Analysis: A Self-Learning Text, Third Edition. Springer; 2012:161–200.
16. Kuitunen I, Ponkilainen VT, Uimonen MM, et al. Testing the proportional hazards assumption in Cox regression and dealing with possible non-proportionality in total joint arthroplasty research: methodological perspectives and review. *BMC Musculoskelet Disord*. 2021;22:489.
17. Fisher LD, Lin DY. Time-dependent covariates in the Cox proportional hazards regression model. *Annu Rev Public Health*. 1999;20:145–157.
18. O'Keefe SJ, El Hajj II, Wu T, et al. Endoscopic evaluation of small intestine transplant grafts. *Transplantation*. 2012;94:757–762.
19. Yeh J, Ngo KD, Wozniak LJ, et al. Endoscopy following pediatric intestinal transplant. *J Pediatr Gastroenterol Nutr*. 2015;61:636–640.
20. Hartman AL, Lough DM, Barupal DK, et al. Human gut microbiome adopts an alternative state following small bowel transplantation. *Proc Natl Acad Sci U S A*. 2009;106:17187–17192.
21. Wang J, Zhang W, Wang M, et al. Perioperative alterations in the intestinal microbiota and functional changes mediate innate immune activation after small bowel transplantation. *Life Sci*. 2021;277:119468.
22. Losurdo G, Salvatore D'Abramo F, Indellicati G, et al. The influence of small intestinal bacterial overgrowth in digestive and extra-intestinal disorders. *Int J Mol Sci*. 2020;21:3531.
23. Guo YW, Gu HY, Abassa KK, et al. Successful treatment of ileal ulcers caused by immunosuppressants in two organ transplant recipients. *World J Gastroenterol*. 2016;22:5616–5622.
24. Motté E, Pipeleers L, Wilgenhof K, et al. Terminal ileitis after kidney transplantation: Crohn's disease or other? Case reports and literature review. *Acta Gastroenterol Belg*. 2019;82:63–66.
25. Lenaerts K, Ceulemans LJ, Hundscheid IH, et al. New insights in intestinal ischemia-reperfusion injury: implications for intestinal transplantation. *Curr Opin Organ Transplant*. 2013;18:298–303.
26. Galvão FH, Farias AQ, Pompeu E, et al. Endoscopic features in a model of multivisceral xenotransplantation. *Xenotransplantation*. 2010;17:423–428.
27. Grootjans J, Hameeteman W, Masclee AA, et al. Real-time in vivo imaging of early mucosal changes during ischemia-reperfusion in human jejunum. *PLoS One*. 2012;7:e39638.
28. Yandza T, Tauc M, Saint-Paul MC, et al. The pig as a preclinical model for intestinal ischemia-reperfusion and transplantation studies. *J Surg Res*. 2012;178:807–819.
29. Chander Roland B, Mullin GE, Passi M, et al. A prospective evaluation of ileocecal valve dysfunction and intestinal motility derangements in small intestinal bacterial overgrowth. *Dig Dis Sci*. 2017;62:3525–3535.
30. Rubio-Tapia A, Hernández-Calleros J, Trinidad-Hernández S, et al. Clinical characteristics of a group of adults with nodular lymphoid hyperplasia: a single center experience. *World J Gastroenterol*. 2006;12:1945–1948.
31. Strati F, Pujolassos M, Burrello C, et al. Antibiotic-associated dysbiosis affects the ability of the gut microbiota to control intestinal inflammation upon fecal microbiota transplantation in experimental colitis models. *Microbiome*. 2021;9:39.
32. Cadwell K, Patel KK, Maloney NS, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell*. 2010;141:1135–1145.
33. Ni J, Wu GD, Albenberg L, et al. Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol*. 2017;14:573–584.
34. Tsai HL, Island ER, Chang JW, et al. Association between donor-specific antibodies and acute rejection and resolution in small bowel and multivisceral transplantation. *Transplantation*. 2011;92:709–715.
35. Kubal C, Mangus R, Saxena R, et al. Prospective monitoring of donor-specific anti-HLA antibodies after intestine/multivisceral transplantation: significance of de novo antibodies. *Transplantation*. 2015;99:e49–e56.
36. Oostenbrug LE, van Dullemen HM, te Meerman GJ, et al. Clinical outcome of Crohn's disease according to the Vienna classification: disease location is a useful predictor of disease course. *Eur J Gastroenterol Hepatol.* 2006;18:255–261.
37. Kim JY, Park SH, Park JC, et al. The clinical significance of anastomotic ulcers after ileocolic resection to predict postoperative recurrence of Crohn's disease. *Dig Dis Sci*. 2021;66:3132–3140.
38. Fusaro F, Tambucci R, Romeo E, et al. Anastomotic ulcers in short bowel syndrome: new suggestions from a multidisciplinary approach. *J Pediatr Surg*. 2018;53:483–488.
39. Madre C, Mašić M, Prlenda-Touilleux D, et al; pediatric GETAID group and the ESPGHAN IBD Porto group. A European survey on digestive perianastomotic ulcerations, a rare Crohn-like disorder occurring in children and young adults. *J Pediatr Gastroenterol Nutr*. 2021;73:333–337.
40. Kroemer A, Elsabbagh AM, Matsumoto CS, et al. The microbiome and its implications in intestinal transplantation. *Curr Opin Organ Transplant*. 2016;21:135–139.
41. Quraishi MN, Shaheen W, Oo YH, et al. Immunological mechanisms underpinning faecal microbiota transplantation for the treatment of inflammatory bowel disease. *Clin Exp Immunol*. 2020;199:24–38.

42. Campbell C, Kandalgaonkar MR, Golonka RM, et al. Crosstalk between gut microbiota and host immunity: impact on inflammation and immunotherapy. *Biomedicines*. 2023;11:294.
43. Abu-Elmagd KM, Costa G, Bond GJ, et al. Five hundred intestinal and multivisceral transplantations at a single center: major advances with new challenges. *Ann Surg*. 2009;250:567–581.
44. Raghu VK, Beaumont JL, Everly MJ, et al. Pediatric intestinal transplantation: analysis of the intestinal transplant registry. *Pediatr Transplant*. 2019;23:e13580.
45. Clarysse M, Canovai E, Vanuytsel T, et al. Current state of adult intestinal transplantation in Europe. *Curr Opin Organ Transplant*. 2020;25:176–182.
46. De Greef E, Avitzur Y, Grant D, et al. Infliximab as salvage therapy in paediatric intestinal transplant with steroid- and thymoglobulin-resistant late acute rejection. *J Pediatr Gastroenterol*. 2012;54:565–567.
47. Venick RS. Grant monitoring after intestinal transplantation. *Review Curr Opin Organ Transplant*. 2021;26:234–239.