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CORRESPONDENCE

Utility of colon allograft biopsies in surveillance of patients with small intestinal transplantation

To the Editor,

Mortality following small intestinal transplantation remains one of the highest among all solid organ recipients.[1] Inclusion of the right colon and the ileo-caecal valve with the small intestinal graft confers significant clinical and survival advantages, possibly by enhancing gut function through better fluid absorption and uptake of free fatty acids.[2] Therefore, since 2000, there has been a six-fold increase in and including a segment of the colon, and currently 30% of small intestinal grafts include a colonic segment.[1]

Colon allografts are often biopsied, either when the patient is symptomatic, or as part of surveillance endoscopies. However, experience in interpretation of colon allograft biopsies is limited and the significance of histological findings, especially in surveillance biopsies, is uncertain. We performed a systematic review of all colon allograft mucosal biopsies obtained during surveillance endoscopies and correlated histological findings with endoscopic and microbiology data. In particular, we were interested in the significance of apoptosis and cryptitis in these biopsies, and the utility of surveillance biopsies in clinical practice.

Following approval by the Institutional Review Board, the transplantation database was used to identify patients with small intestinal or multi-visceral allografts that included a segment of colon. The pathology database was then queried to identify biopsies of colonic allografts from these patients. All colonic biopsies were reviewed blinded to clinical presentation, endoscopic findings, microbiology studies and clinical outcomes. Biopsies from second transplantations (re-transplantations) were excluded. For each biopsy, we recorded cryptitis, apoptotic activity (<6 or ≥ 6 per 10 consecutive crypts), crypt architectural distortion, and inflammatory infiltrate in the lamina propria. Microbiology data from within a week before or after the biopsy, including results of bacterial and viral cultures, detection of *Clostridioides difficile* toxin and viral loads, were recorded. Endoscopic and microbiological data were correlated with histology findings of each biopsy (Fig. 1).

There were 116 colon biopsies from 28 patients, 17 females and 11 males (Table 1) ranging from 21−62 years of age. The indications for transplantation included mesenteric vein thrombosis (n=8), refractory/fistulising Crohn disease (n=6), abdominal tumors (n=6), and miscellaneous conditions causing insufficient small intestinal function (n=7). Eighty-five were histologically normal (NA. 1), whereas 31 showed histological findings in the form of apoptosis (n=18), focal cryptitis (n=10), chronic inflammation in lamina propria (n=5), crypt architectural distortion (n=4) and ulceration with fibrinopurulent exudate (n=1), which represented an anastomotic ulcer (Table 1). Of the biopsies with apoptosis, all except one showed <6 apoptotic bodies per 10 crypts (Fig. 2). The one biopsy with >6 apoptotic bodies per 10 crypts additionally showed crypt architectural distortion with crypt loss; there was a concurrent urine culture positive for *Escherichia coli*, and blood and urine cultures 2 months earlier had also been positive for *E. coli*. Nineteen paired small intestinal biopsies were present; these either showed findings concurrent with those in the colon, or no pathological abnormality.

Of the biopsies with cryptitis, three were associated with evidence of concurrent infection. One was associated with *C. difficile* toxin in the stool; this biopsy also showed apoptosis (<6 per 10 crypts). The second biopsy with cryptitis had concurrent positive cultures for stenotrophomonas in blood and *Candida glabrata* in urine. The third biopsy was associated with positive urine culture for vancomycin resistant enterococcus. The remaining biopsies with cryptitis did not have concurrent infections or positive cultures.

Twenty-three biopsies were associated with positive cultures, either in blood (n=6), urine (n=14) or wound/peritoneum (n=6). Of these, five were associated with abnormal endoscopies and six with abnormal histology, whereas 10 biopsies had no abnormal endoscopic or histological findings in the colon (Fig. 1). The abnormal endoscopic findings comprised congested or erythematous mucosa in four instances and a mildly altered vascular pattern in the fifth. The histological findings comprised cryptitis in three biopsies, one of which also showed 1−2 apoptosis in 10 crypts. An additional two cases showed mild non-specific crypt architectural distortion and one of these two had <6 apoptotic bodies per 10 crypts (patient with CMV DNA in blood). The sixth biopsy showed >6 apoptotic bodies per 10 crypts and mild crypt architectural distortion.

Of 11 biopsies with abnormal endoscopic findings, (erythema, congestion, ulceration), four biopsies had no pathologic findings, four had <6 apoptotic bodies per 10 crypts, two had focal cryptitis and one confirmed an anastomotic ulcer noted on endoscopy(Fig. 1).

There were 59 paired small intestinal biopsies; seven were histologically abnormal, six showed rare apoptosis and one, focal cryptitis. The corresponding colonic biopsies showed similar findings in the form of rare apoptosis and/or focal cryptitis.

Inclusion of a small segment of colon in continuity with small intestinal allograft leads to better outcomes, both in terms of small intestinal function as well as graft survival. In our institution, we receive numerous biopsies of the colon allograft from ‘surveillance’ endoscopies. Most of these are histologically normal, but they are interspersed with biopsies that show mild histological changes, which are of uncertain clinical significance. Because experience of, and literature on colon allograft biopsies is not extensive, we undertook a review of all colon allograft biopsies obtained during surveillance procedures over a 4-year period to document histological findings, and elucidate correlations, if any, with endoscopic and microbiological findings.

The most common findings in this series were apoptosis and cryptitis. In the context of small intestinal transplantation, ≥ 6 apoptotic bodies per 10 crypts, along with inflammatory infiltrate in the lamina propria and crypt regenerative changes, is considered diagnostic of rejection.[3,4] However, apoptosis may also be seen in bacterial and viral infections and are particularly well documented in CMV infection.

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Table 1 Clinicopathological correlation of 31 biopsies with histological findings

Patient ID	Age/Gender	Indication for transplant	Colon allograft biopsy findings	Paired small intestinal biopsy findings	Colonoscopy findings	Microbiology results	Clinical symptoms	Clinical management	Pathology report of subsequent biopsy	Days between biopsies
2	56 F	Mesenteric vein thrombosis	<6 apoptosis per 10 crypts	NA	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change	9
4	38 F	Refractory/ fistulising Crohn's disease	<6 apoptosis per 10 crypts	NA	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change	49
7	23 F	Gastroparesis and intestinal pseudo-obstruction	<6 apoptosis per 10 crypts	<6 apoptosis per 10 crypts	Normal, healthy appearing mucosa	None or negative	Nausea and vomiting and abdominal pain	No change in base line medications	No significant pathological change	13
8	61 M	Abdominal tumour	<6 apoptosis per 10 crypts	<6 apoptosis per 10 crypts	Ulcer in ascending colon	None or negative	Follow-up surveillance	Viral culture was negative, no change in medications	Negative for acute cellular rejection	61
9	37 M	Abdominal tumour	Mild cryptitis, chronic inflammation in lamina propria	<6 apoptosis per 10 crypts	Mild granularity in entire examined colon	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change	46
11	39 M	Abdominal catastrophe following duodenal switch surgery for morbid obesity	<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance, post-operative assessment	More frequent screening, with no change in medications	No significant pathological change	112
12	57 M	Mesenteric vein thrombosis	<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance, post-operative assessment	No change in medications	No significant pathological change	35
13	55 F	Refractory/ fistulising Crohn's disease	Mild cryptitis, <6 apoptosis per 10 crypts, architectural distortion	NA	Normal, healthy appearing mucosa	Stool culture: postive for *C. difficile*	Abdominal pain	Hospitalised, increased dose of immunosuppresors, broad spectrum antibiotics, IV fluids	Crypt apoptosis, indeterminate for acute cellular rejection	18
			Chronic inflammation in lamina propria, architectural distortion	NA	Erythematous mucosa	Blood culture: coagulase- negative staphylococci	Abdominal pain	Further increase in the dose of immunosuppresors	Crypt apoptosis and confluent crypt loss present, viral inclusions	6
15	44 M	Mesenteric vein thrombosis	<6 apoptosis per 10 crypts	<6 apoptosis per 10 crypts	Normal, healthy appearing mucosa	None or negative	Dark brown to black coloured stools for one week	Offered tacrolimus and dose of prednisone was increased, CMV cultures ordered	No significant pathological change	74
			<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No evidence for acute cellular rejection	205
17	21 M	Mesenteric vein thrombosis	Ulceration and fibrinopurulent exudate	No signifcant pathological change	Poor prep, anastomotic site, friable, ulcerated, erythematous	None or negative	Follow-up surveillance	Lost follow up	NA	NA

18	26 F	Mesenteric vein thrombosis	<6 apoptosis per 10 crypts	<6 apoptosis per 10 crypts	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No evidence of acute cellular rejection	65
			Chronic inflammation in lamina propria, architectural distortion	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	Culture was ordered, no change in base line medications	No significant pathological change	91
19	62 F	Short bowel syndrome	<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Chronic diarrhoea	Continue follow-up	No significant pathological change	219
20	43 F	Refractory/ fistulising Crohn's disease	Mild cryptitis, chronic inflammation in lamina propria	NA	Normal, healthy appearing mucosa	Blood culture: *Stenotrophomonas maltophila.* Urine culture: *C. glabrata*	Follow-up surveillance	No change in base line medications, antibiotics for UTI	No significant pathological change	27
			Mild cryptitis	NA	Normal, healthy appearing mucosa	Urine culture: vancomycin-resistant enterococci	Follow-up surveillance	No change in base line medications	Negative for acute rejection, acute inflammation or viral inclusion	29
			<6 apoptosis per 10 crypts	NA	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change	11
			<6 apoptosis per 10 crypts	NA	Areas of mild inflammation	None or negative	Follow-up surveillance	No change in base line medications	Minimal non-specific changes, indeterminate for acute cellular rejection	6
21	23 F	Abdominal tumour	Mild cryptisis	NA	Eythematous, villi suggestive of recovery	None or negative	Abdominal pain and nausea, and one day of blood	Offered tacrolimus and dose of prednisone was increased, CMV cultures ordered, hospitalised and received IV fluids	Granulation tissue with acute and chronic inflammation	18
			Mild cryptitis	NA	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change. No acute rejection or viral inclusions	14
24	43 F	Refractory/ fistulising Crohn's disease	Chronic inflammation in lamina propria	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	Treated with probiotics without any changes to immunosuppression	Severe acute colitis with crypt injury, crypt dropout	88
			Mild acute cryptisis	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	Focal acute colitis	186
			<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Abdominal pain, shingles rash	Offered prograf and cellcept 1000 mg	No significant pathological change	154
			Mild cryptitis	Mild acute enteritis	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance	No change in base line medications	No significant pathological change	31
25	60 M	Mesenteric vein thrombosis	>6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	Blood and urine culture: *E. coli*	Follow-up surveillance, post-operative assessment	Received high dose steroids	Moderate acute cellular rejection	16

Table 1 (continued)

			<6 apoptosis per 10 crypts	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance, post-operative assessment	No change in base line medications		79
26	49 M	Abdominal tumour	Mild cryptisis	No significant pathological change	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance, post-operative assessment	No change in base line medications	Rare crypt apoptosis (1/10 crypts), indeterminate for acute cellular rejection. Negative for viral inclusions No significant pathological change	414
27	23 M	Crush injury	<6 apoptosis per 10 crypts, architectural distortion	NA	Congested mucosa	Positive CMV DNA	Abdominal pain	IV ganciclovir for CMV+ test. No other change in medication	Graft rejection and retransplant	12
28	60 F	Cirrhosis and portal vein thrombosis	Mild cryptitis	NA	Normal, healthy appearing mucosa	None or negative	NA	NA	No significant pathological change	168
			<6 apoptosis per 10 crypts	<6 apoptotis per 10 crypts	Normal, healthy appearing mucosa	None or negative	Follow-up surveillance, post-operative assessment	No change in base line medications	Focal acute colitis	63

CMV, cytomegalovirus; NA, not available; UTI, urinary tract infection.

Most often, there is accompanying acute cryptitis, but this feature may not always be present in an immunocompromised patient. Therefore, apoptosis in intestinal allograft biopsies induces uncertainty, not to mention significant anxiety, especially in colon allograft biopsies for which clear diagnostic guidelines do not exist, and diagnostic criteria are extrapolated from small intestinal allograft pathology.

We found that as in the small intestine, isolated apoptosis in the absence of additional histological findings have no clinical significance; these patients were not treated for rejection. Although not causal, one biopsy with >6 apoptotic bodies in 10 crypts was associated with positive *E. coli* cultures in blood and urine over a few months. The second most common finding of focal cryptitis was not associated with concurrent infection in the majority of patients; rare patients had positive cultures in urine/stool/blood around the time of the biopsy. Conversely, of the 23 events with positive cultures in blood, urine, skin or peritoneal fluid, histological changes (all mild) were seen in only six corresponding biopsies. Concurrent small intestinal biopsies, present in approximately 50% of cases, did not demonstrate discordant findings that altered the clinical management of the patient. Similarly, a study of 51 paired biopsies from 15 patients showed concordance between small intestinal and colonic biopsies; it is uncertain if these biopsies were obtained during surveillance procedures.[5] A study of six colon allograft biopsies from 17 patients with colon grafts showed one case in which apoptotic injury was limited to the colon allograft; progressive injury led to explantation only of the donor colon. An additional patient showed mild acute rejection in a paired small intestinal biopsy without concordant changes in the colon allograft.[6]

In summary, our large cohort of colon biopsies performed for surveillance of small intestinal allografts demonstrates that almost three-quarters of these biopsies show no histological abnormality. The most common histological features observed are apoptosis and cryptitis, which could potentially raise concerns for rejection and infection, respectively. However, in all our biopsies, the apoptosis was <6 per 10 crypts and not accompanied by inflammation or crypt reactive changes, thus not supporting a diagnosis of rejection. The corresponding small intestinal biopsies, when present, did not show evidence of rejection either. However, a handful of cases were associated with concurrent infections. Cryptitis was associated with evidence of infection in blood or urine cultures in a minority of cases but was cryptogenic in the majority. It is pertinent to note that both apoptosis and cryptitis in this series of biopsies were very focal and/or mild. The presence of mild changes might further explain, along with sampling error, operator experience and patchiness of pathology, the observed discordance between endoscopic and microscopic findings. The advantage of obtaining multiple biopsies rather than a single biopsy remains to be determined. Nevertheless, in spite of the discordance, complete concordance with small intestinal biopsies suggests that colonic biopsies represent a viable modality for surveillance of small intestinal allografts. However, this statement is made with caution due to the retrospective nature of this study and lack of corresponding small intestinal biopsies in almost half of our cases.

Crypt architectural distortion is an interesting finding in this series, which we have also observed in non-surveillance biopsies of the colon allograft. However, the number of cases

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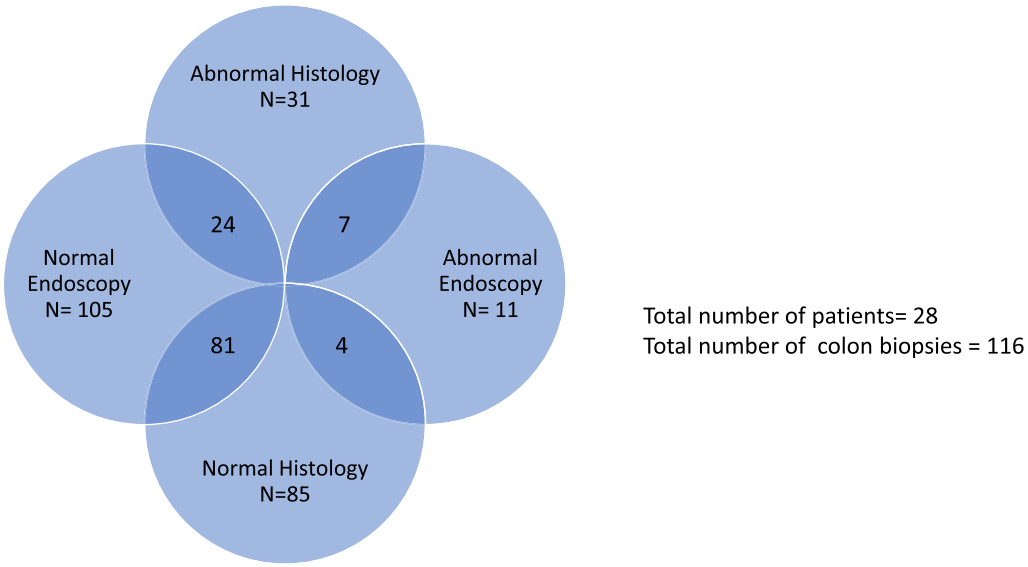

Fig. 1 Venn diagram showing the correlation between normal and abnormal histological with normal and abnormal endoscopic findings.

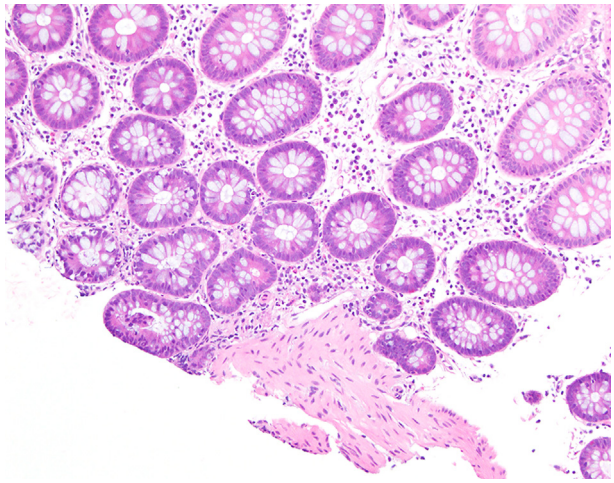

Fig. 2 Colonic mucosa showing presence of apoptotic bodies in the absence of inflammatory infiltrate or reactive crypt epithelial changes.

is too small to allow optimal investigation or draw meaningful conclusions. This observation is worthy of detailed study as significant crypt architectural distortion, observed not infrequently in small intestinal and colon allografts, might potentially affect intestinal absorptive function.

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