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Authors
Yeh, Joanna
Ngo, Khiet D
Wozniak, Laura J
et al.

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Endoscopy Following Pediatric Intestinal Transplant: A 23 Year Single Center Experience

Joanna Yeh, MD¹, Khiet D. Ngo, DO, MS², Laura J. Wozniak, MD¹, Jorge H. Vargas, MD¹, Elizabeth A. Marcus, MD¹, Sue V. McDiarmid, MD¹,², Douglas G. Farmer, MD³, and Robert S. Venick, MD¹,³

¹Division of Pediatric Gastroenterology, Hepatology, and Nutrition, UCLA
²Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Loma Linda
³Division of Liver and Pancreas Transplantation, David Geffen School of Medicine, UCLA

Abstract

Objectives—Biopsies remain the gold standard in the diagnosis of intestinal transplant (ITx) rejection, and gastrointestinal endoscopy plays a pivotal role in patient management. Herein, we describe a single center 23 year endoscopic experience in pediatric ITx recipients.

Methods—A retrospective review of endoscopy and pathology reports of all ITx recipients <18 years old transplanted between 1991 and 2013 was performed with the aim of describing the procedural indications, findings, and complications.

Results—A total of 1770 endoscopic procedures within 1014 sessions were performed. Combination EGD and ileoscopy was the most common procedure (36%). Increased stool output (35%) and surveillance endoscopy (32%) were the most common indications. 162 episodes of biopsy proven rejection were diagnosed. First episode of rejection occurred at a median of 1 month post-ITx. 45% of histology-proven rejection had normal appearing endoscopies. The rate of procedural complications including but not limited to bleeding and perforation was 1.8%.

Conclusions—Endoscopy with biopsy plays a significant role in the care of ITx recipients. Multiple procedures are required for graft surveillance, diagnosis of rejection, subsequent treatment, and follow-up of therapy. The gross endoscopic appearance, particularly in mild to moderate acute cellular rejection, does not correlate well with histology. Complex anatomy, complication rates which are higher than non-ITx pediatric endoscopy cases, and timely histologic interpretation by experienced pathologists are reasons that these procedures should be performed at centers accustomed to caring for ITx recipients. The field would benefit from the development of a noninvasive biomarker to reliably and efficiently detect rejection.
**Keywords**

pediatric endoscopy; intestinal transplant; enteroscopy; colonoscopy; ileoscopy

**Introduction**

Intestinal transplantation (ITx) is a lifesaving operation for children with intestinal failure who develop advanced intestinal failure associated liver disease (IFALD), loss of central venous access needed for parenteral nutrition, or life-threatening fluid and electrolyte problems.¹ Advancements in organ allocation, surgical techniques, immunosuppression, and post-transplant monitoring have translated into significant improvements in patient and graft survival.²

Timely diagnosis and treatment of graft dysfunction has been an instrumental part of improved outcomes. After ITx, a non-invasive test to determine the etiology of allograft dysfunction, and to differentiate infectious enteritis from acute cellular rejection has yet to be developed. Therefore, serial endoscopies with mucosal biopsies have been the standard invasive tests for allograft surveillance and rejection diagnosis since the inception of ITx. Histologic criteria have been agreed upon to grade acute cellular rejection, thus solidifying the role of post-ITx endoscopy.³ Creation of an ileostomy at the time of ITx allows for direct access to facilitate monitoring of graft function. With early recognition of graft dysfunction, immunosuppression can be tailored accordingly. There are few reports on endoscopy in pediatric ITx patients. The purpose of this study is to characterize and analyze the endoscopic experience at a large pediatric ITx center.

**Methods**

This institutional review board approved analysis included all endoscopies performed by a single ITx center over a 23 year period from 1991 to 2013. A retrospective review of a prospectively maintained database and medical record review included all ITx recipients less than 18 years of age at time of transplant. All endoscopy and pathology reports were also reviewed. Surgical techniques, immunosuppression, and outcomes have been previously described.⁴, ⁵

**Procedure Protocols and Techniques**

Endoscopy of ITx recipients was performed for two main reasons: surveillance monitoring or allograft dysfunction with suspected rejection. Our post ITx surveillance protocol was weekly for the first four to six weeks, every other week in month two, and monthly in months three through six. Surveillance endoscopy was also performed prior to ostomy takedown and re-establishment of gastrointestinal continuity. During the first month post-ITx, typically only ileoscopy was performed in order to avoid intubation and manipulation of the proximal anastomosis. All ITx recipients had end ileostomy (n=18, 20%), end ileostomy + proximal ileocolostomy (n= 51, 56%), loop ileostomy (n=13, 14%), or proximal end ileostomy + ileo-ileostomy and ileo-colostomy (n=9, 10%) created during the transplant procedure to facilitate surveillance and accurate monitoring of stool output and consistency.
Symptomatic reasons for endoscopy included allograft dysfunction with increased stool output (>30 cc/kg/day), gastrointestinal bleeding, persistent EBV or CMV viremia, or marginal weight gain. For patients who presented acutely with an increase in stool output, our practice evolved into sending a first line of stool studies (which currently includes: stool cells, *Clostridium difficile*, adenovirus, rotavirus, norovirus, viral culture, and early viral antigen). If these stool studies were negative and stool outputs remained elevated for 72–96 hours then we proceeded with second line stool studies (bacterial culture, ova and parasite, cryptosporidium, and giardia) and endoscopy.

Preparation for endoscopy varied significantly depending on clinical indication, age, and clinical status (i.e. hydration status and renal function) of the patient. Patients who underwent routine surveillance were typically placed on a clear liquid diet the day prior to their procedure and were made NPO prior to the procedure according to their age. Patients undergoing upper endoscopy and/or ileoscopy rarely received additional bowel prep. In those patients undergoing colonoscopy, additional bowel preparation including Go-Lytely® or magnesium citrate may have been administered. However, given the frequency of renal insufficiency in this population, bowel preparation was typically administered conservatively.  

Pre procedure labs were performed to ensure a platelet count of ≥ 50,000/uL and INR of ≤ 1.5 to decrease the risk of bleeding. Biopsy forceps used were either Boston Scientific 2.8 mm or 2.0 mm depending on the age and size of the patient. Endoscopes used were Olympus GIF 160, GIF H180, and PCF 160 AL.

EGD (esophagogastroduodenoscopy) and ileoscopy were performed with appropriately sized endoscopes. In cases in which the proximal anastomosis between native and transplanted small bowel was difficult to reach with shorter endoscopes (i.e. greater than 100 cm from mouth), a colonoscope was used. For colonoscopy, typically a PCF 160 AL was utilized, but in younger recipients and in those patients with a shorter length colon, a standard upper endoscope could be used.

In the majority of patients undergoing EGD, 2–3 sites each consisting of 2–3 biopsies were taken every 5–10 cm from the proximal graft, ideally at least 10 cm beyond the anastomosis of the native and transplanted small bowel. For ileoscopies, 2–3 sites each consisting of 2–3 biopsies were also taken every 5–10 cm from the distal graft. Depending on the patient’s anatomy and risks, the distal graft was typically surveyed up to 50–60 cm from the ostomy or ileocolonic anastomosis. 1–2 sites of the native duodenum, jejunum, and/or colon were obtained to help differentiate rejection from infection, since rejection should affect only transplanted bowel.

Due to their complex medical histories and frequent significant sedation requirements, the majority of patients typically required the care of the pediatric anesthesia team. Patients with delayed gastric emptying, a history of aspiration or respiratory issues with procedures often underwent general anesthesia with endotracheal intubation. Nearly all ileoscopies alone were performed with deep sedation without intubation.
The visual appearance of the bowel was assessed by the endoscopists (combination of gastroenterology fellow in training and attending physicians experienced in the care of ITx recipients). Standard descriptors of mucosal appearance included erythematous, friable, ulcerated, denuded, or grossly unremarkable. Assessment for the villous appearance (normal or blunted) and the vascular pattern were also made.

All biopsy samples were processed within 24 hours. Immunohistochemical tissue staining for adenovirus and CMV became our standard practice with the selective use of EBER (EBV) and other hematopathology staining on a case by case basis if there were concerns for post-transplant lymphoproliferative disease (PTLD). Biopsies were reported to have absence of, indeterminate, mild, moderate, or severe acute cellular rejection per criteria established in 2004. Enteritis was also reported.

Biopsy proven mild to moderate acute cellular rejection episodes were treated with high dose intravenous methylprednisolone bolused over 7 days. ATG was generally reserved for episodes of severe rejection. Infectious enteritis was treated with supportive care and in some instances with antibiotics when appropriate. Following diagnosis of either acute rejection or infection, treatment was initiated and patients were observed closely for clinical response. Subsequent follow up endoscopies were performed as frequently as biweekly to monthly to track histologic changes closely.

Results

74 children received 91 ITx during this time period. A total of 1770 endoscopies were performed during 1014 endoscopy sessions in 71 children (Table 1 and 2). Their ages ranged from 9 months to 18 years old at time of transplant. The mean age at time of transplant was 4.7 years ± 4.3 years and median age was 2.8 years (1.3, 7.7 years). 76% of children underwent ostomy takedown at 16 months (11, 24) post-ITx. Overall 1 and 5 year survival among pediatric ITx recipients was 80% and 68% patient, and 68% and 59% graft survival.

The procedures included 708 ileoscopies, 725 EGDs (upper enteroscopies), and 337 colonoscopies. The most common type of endoscopy session was EGD + ileoscopy (36% of the endoscopy sessions).

The most common indications for endoscopy were increased stool output, accounting for 35% of the endoscopy sessions, and surveillance (32%). The remaining indications included follow up of allograft from recent rejection episodes, gastrointestinal bleeding, obstructive symptoms, and other indications which can be found in Table 2.

General endotracheal anesthesia was the most commonly used method for sedation, accounting for approximately two thirds of cases compared to conscious or deep sedation.

162 episodes of biopsy proven rejection were detected among 1770 endoscopies (9%). 45% of these had a normal gross appearance to the endoscopists. A total of 7 cases of PTLD involving the GI tract were diagnosed via endoscopy.
32 serious complications were documented (Table 2). The most frequent complications included GI bleeding (13 cases) and perforation (11 cases). The serious complication rate overall was 1.8% (32/1770). No deaths and one graft loss resulted from these complications.

Among patients who sustained perforations, the age at the time of complication ranged from 1 to 12 years old. Overall, most perforations occurred within the first 6 months post-ITx however there were three perforations which occurred 2 to 4 years out from ITx. The majority of perforations occurred during ileoscopies (9 out of 11) while two were during colonoscopies. Of the 11 perforations, 8 underwent exploratory laparotomy and 3 were medically managed with bowel rest and broad spectrum antibiotics. Among the 8 surgically managed, 6 required resection of small bowel (range 1–12 cm) and ultimately underwent primary re-anastomosis or placement of a diverting ostomy. The remaining 2 exploratory laparotomies underwent peritoneal washout with the inability to identify the perforation site, presumably because the site had already sealed off. One patient did lose their entire graft due to delayed recognition of perforation. This child developed peritonitis and subsequent sepsis with hypotension, contributing to the ischemic necrosis of the transplanted bowel.

Discussion
The majority of early post ITx care is performed at specialized centers. However, the community gastroenterologist should be aware of the unique needs of ITx patients when they return back home to their local community. Potential complications from endoscopy, comorbid medical conditions, and complex anatomy are factors that differentiate post ITx patients from the general pediatric GI patient.

Indications for Endoscopy
A significant number of post-ITx endoscopies are performed as surveillance. In an asymptomatic patient, particularly in the early post-transplant time period, surveillance endoscopy is routinely utilized as the gold standard at ITx centers to allow for early detection and effective treatment of rejection. Frequent and early endoscopies are performed given the high prevalence of acute cellular rejection in the early post-transplant period. Indeed this has been the case at our institution where the median time to the first episode of ACR is 35 days.

While stool output is closely measured and recorded daily in the early post-ITx period, waiting for stool outputs to rise prior to performing endoscopy early on post-ITx would be synonymous with waiting for a liver transplant recipient to become jaundiced prior to performing a liver biopsy to delineate their cause of graft dysfunction. Such an approach potentially delays early diagnosis and treatment and may significantly reduce the chances for successful treatment.

The field of ITx lacks a consistent biomarker to screen for and diagnose acute cellular rejection. Unlike liver or kidney transplantation, which rely on changes in transaminases or serum creatinine, such a marker is lacking in ITx. An acute increase in ostomy outputs often raises concern in ITx patients, but this is a nonspecific finding which can be secondary to variation in enteral intake, infectious enteritis, or rejection. Stool calprotectin has been
heralded as a potential biomarker, but it has significant interpatient variability and is unable to differentiate between infection and rejection. Serum citrulline has also been considered, however, it has not been shown to predict asymptomatic rejection and also has significant variability between patients. Immune cell function assay (Cylex ImmuKnow – Viracor IBT Laboratories) has been investigated, and while it may be used as an adjunctive diagnostic tool, it cannot replace endoscopy with biopsy. Finally, the newly FDA-approved Pleximmune™ test (Plexision, Pittsburgh, PA) is designed to predict the risk of acute cellular rejection after pediatric liver or intestine transplantation, but has yet to be utilized in a large multi-center study.

Beyond surveillance, other indications for endoscopy include symptoms such as diarrhea, fever, vomiting, abdominal pain, gastrointestinal bleeding, and abdominal distension. In such cases, endoscopy with biopsies can help in evaluating for PTLD, tissue invasive CMV infection, and rejection. Endoscopy may also be indicated for GJ tube replacement, the evaluation of the health of the graft prior to ostomy takedown, or surveillance following the treatment of rejection or PTLD.

Infectious enteritis is a common complication in immunosuppressed ITx patients and endoscopy can help to differentiate rejection and infection, especially when initial stool studies are negative yet patients continue to have elevated stool outputs. Infectious enteritis, in particular adenovirus, can also present concomitantly with a rejection episode.

Finally, graft versus host disease (GVHD) of the GI tract is a very rare but serious complication that can occur when donor immune cells in the transplanted bowel attack the native remnant bowel. Histologic evaluation is essential in diagnosing GVHD.

**Type of Endoscopy**

Based on clinical experience and previously published work, it is known that the pattern of intestinal transplant rejection may be patchy. Therefore, sampling of both suspicious and normal appearing bowel in multiple graft locations is necessary to identify histologic evidence of rejection. Studies have shown that the ileum is most reliable in the detection of rejection whereas jejunal sampling alone may miss rejection episodes. Our ability to broadly survey the transplanted allograft is limited to the more proximal and distal portions of the graft with the majority of the middle portion of the graft unreachable. This represents a major limitation of endoscopy. In our experience, the most common type of procedure performed was EGD + ileoscopy, thereby allowing for surveillance of both proximal (jejunal) and distal (ileal) graft. Diagnostic yield from endoscopy can be increased by performing at least 2–3 biopsies at multiple locations typically separated by 5–10 cm. It is also important to evaluate and biopsy the native bowel to allow for adequate evaluation of an infectious process, PTLD, or medication side effects (i.e. mycophenolate associated enteritis). This is an extremely important point as the differential diagnosis of pathology affecting the transplanted allograft alone as compared to both the native bowel and transplanted allograft are unique. Capsule endoscopy to visualize all of the allograft is a consideration, but given the relatively young age of this cohort of patients, their propensity to dysmotility and their high number of prior abdominal surgeries, this must be approached with caution.
**Rejection**

Histology is critical in the diagnosis of acute cellular rejection as gross endoscopic findings can be misleading. Advancements including zoom magnification endoscopy have not replaced histologic examination for the diagnosis of rejection.\textsuperscript{13} Gross findings, including erythema, nodularity, pallor, and edema, are non-specific. Even frank ulcerations, while suspicious for rejection, may be consistent with other diagnoses including infection. Sigurdsson et al reported that 37\% of cases of acute cellular rejection would have been missed without biopsies.\textsuperscript{14} This was consistent with previous findings that mucosal visualization alone was not sensitive enough to establish a diagnosis, and would miss patients with mild acute cellular rejection.\textsuperscript{15, 16, 17} Our experience is congruent with these previous findings in that nearly half of biopsy proven rejection episodes had grossly normal appearing endoscopies.

While gross findings may be unreliable in cases of mild or moderate acute cellular rejection, in cases of severe exfoliative acute cellular rejection, endoscopists will often have a strong sense of the clinical problem and may even elect to initiate treatment prior to the biopsy results being reported. Despite being the gold standard for rejection, there are limitations to endoscopy with biopsy. Rejection of the graft can be patchy in nature and endoscopy is unable to survey the entire transplanted bowel. As reported by Pasternak, et al, in cases of mild and moderate rejection, histologic findings are absent in approximately 20\% of tissue samples.\textsuperscript{18} Furthermore, while useful for diagnosing acute cellular rejection, endoscopic biopsies do not yield the full thickness biopsies needed to diagnose chronic rejection. Finally, while the field of knowledge of antibody mediated rejection including C4d staining has grown, this diagnostic tool has not been validated in ITx recipients.

**PTLD**

Post-transplant lymphoproliferative disorder (PTLD) is an uncommon but potentially fatal complication of ITx. The prevalence of PTLD in our pediatric ITx population is 16\%. The median time to diagnosis of PTLD following ITx is 20 months with 31\% diagnosed in the first year. Presentation and clinical symptoms associated with PTLD vary greatly but can include fever, weight loss, hematochezia, abdominal distension, obstruction, and diarrhea. In our experience, seven patients over 23 years developed PTLD involving the GI tract that was diagnosed histologically via endoscopic biopsies. These GI presentations account for 58\% of the PTLD which we have observed. Grossly, most PTLD lesions were found incidentally on biopsy in that they did not stand out dramatically to the endoscopists. Five of these seven patients had PTLD involving the transplanted ileum while two had PTLD in their native colon. Time to diagnosis ranged from 1.7 to 107 months post-Itx.

**Complications**

There is a paucity of integrated, consistent data on the incidence of complications in non-ITx pediatric endoscopy. In non-ITx patients, the EGD complication rate is reported as 2.3\%, most of which were hypoxia related (66\%) and reversible.\textsuperscript{19} In this same study of EGDs, the bleeding rate was 0.3\% (28 episodes out of 10,236 procedures) and there were no perforations.\textsuperscript{19} The overall rate of serious or life-threatening complications in children undergoing upper or lower endoscopy is estimated to be <1\%.\textsuperscript{20}
In non ITx adults, the overall endoscopic complication rate has been estimated at 1.9% including serious and non-serious complications, with a perforation rate of 0.09%. Serious upper endoscopy complication rate is estimated at 0.15% while serious colonoscopy complication rate is 0.2%. Rate of perforation in screening colonoscopies for adults ranged from 0.01% to 0.1%. A recent large single-center pediatric study of roughly 30,000 procedures revealed a perforation rate of 0.014% for EGD and 0.028% for colonoscopy.

Our overall complication rate was 1.8%, with a rate of 0.6% for perforation and 0.7% for bleeding. The remaining complications (0.5%) included hematoma (n=6), gastric mucosa avulsion (n=1), and distension from retained air causing respiratory issues and early termination of the endoscopy (n=1). A limitation of this study is that our database was not designed to include cardiopulmonary complications including hypoxia, wheezing, bradycardia, or arrhythmias as our focus has been on gastrointestinal related complications. One potentially life threatening complication of endoscopy is perforation. In our experience, there is a higher risk of perforation in ITx patients compared to other populations. This is likely multifactorial given the multiple surgical anastomoses, atypical anatomy, and immunosuppressed state of these patients. While the majority of the perforations necessitated surgical exploration, overall patient recovery was good with prompt medical and surgical care. Given the higher rate of complications, atypical anatomy, and need for timely histologic diagnosis, we recommend that endoscopy of ITx patients be performed by teams at ITx centers in an attempt to minimize the risk of complications. The endoscopists need to be familiar with the patients’ anatomy and there must be surgical expertise present to take these children promptly to the operating room in the event of perforation. Continuity of care is also important and a limited number of designated endoscopists are best able to follow endoscopic changes over time. Patients should be monitored especially closely after endoscopy. Abdominal pain, distension, or abnormal vitals ought to prompt evaluation with abdominal radiographs (cross table lateral and plain abdominal film), blood tests, and early notification to the surgical transplant team. While hemostasis is observed directly after each biopsy, bleeding following endoscopy may still occur, may not present immediately, and requires timely recognition by caregivers and the transplant team. Finally, biopsies should be read by pathologists with ITx expertise and experience to allow for prompt and accurate diagnosis and treatment.

**Conclusion**

Endoscopy with biopsy remains the gold standard for surveillance of the graft and detection of rejection in ITx patients. While the complication rate is higher in this specialized population compared to the general population, rates remain acceptable given the benefit and knowledge afforded from the diagnostic procedure. Regardless, ongoing research is necessary to develop reliable, noninvasive biomarkers which can successfully differentiate infectious enteritis and rejection. Given the higher complication rate, endoscopy with biopsy in ITx patients should be performed at a specialized center with multi-disciplinary teams who are intimately familiar with these children.
Acknowledgments

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References


Summary Box

What is known

- Endoscopy with biopsies remains the gold standard for diagnosis of intestinal transplant rejection.
- The rate of serious or life threatening complications in children undergoing endoscopy is estimated <1%; the rate of serious endoscopic complications for pediatric intestinal transplant recipients is not well described.

What is new

- An endoscopic complication rate of 1.8% was observed over a two decade experience at a large pediatric intestinal transplant center.
- Increased awareness of the higher risks and specific nuances in the care of these patients is essential as is the need to develop a noninvasive biomarker to reliably detect rejection.
Figure 1.
Endoscopic view of severe acute rejection with nodularity, loss of normal vascular pattern, and loss of normal appearance of villi (A). Endoscopic view of typical surgical anastomosis site with afferent and efferent limbs “owl’s eye” (B).
Table 1

Demographics

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<tr>
<td>Other n=1</td>
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<tr>
<td>Median Age at Transplant (25%, 75% quartiles)</td>
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<tr>
<td>Mean age at Transplant (+/- s.d.)</td>
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<tr>
<td>Median Follow Up Time (25%, 75% quartiles)</td>
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## Table 2

### Endoscopic and Histologic Results

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<td>Other</td>
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<td>Distension from Retained Air</td>
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‡EBV, CMV, pre-operative ostomy takedown, fever, abdominal pain, PTLD follow up, adenovirus