# Rectal biopsy for Hirschsprung's disease: a review of techniques, pathology, and complications

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***Background:*** **Hirschsprung's disease (HD) is one of the most common congenital anomalies of colorectal function, affecting approximately 1 in 5000 live births, with a 4:1 male predominance. HD is characterized by aganglionosis that is most often limited to the rectosigmoid, but can extend proximally along the colon and, in rare instances, reach into the small intestine. A clinical history of delayed passage of meconium beyond 48 hours after birth, physical exam findings of abdominal distention and vomiting, and a contrast enema demonstrating a transition zone are highly suggestive of HD.**

***Data sources:*** **We searched databases including PubMed, Google Scholar, and Scopus for the following key words: Hirschsprung's disease, rectal biopsy, pathology, ganglion cell, nerve trunk hypertrophy, pediatric constipation, and selected publications written in English that were relevant to the scope of this review.**

***Results:*** **Based on the data presented in the literature, we reviewed 1) biopsy techniques for the diagnosis of Hirschsprung's disease, addressed inadequate biopsies, and complications from rectal biopsy, and 2) pathologic and histologic interpretation of biopsy specimens for the diagnosis of Hirschsprung's disease.**

***Conclusion:*** **A well-executed rectal biopsy with expert pathologic evaluation of the specimen remains the gold standard for the diagnosis of Hirschsprung's disease and is the subject of this review.**

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***Key words:*** **ganglion cell; Hirschsprung's disease; nerve trunk hypertrophy; pediatric constipation; rectal biopsy**

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Review article

## Introduction

Hirschsprung's disease (HD) most often presents as functional intestinal obstruction in the neonatal period. The most consistently reported clinical symptom is delayed passage of meconium, and this is seen in up to 90% of patients diagnosed with HD. Classically, neonates with HD are noted to have abdominal distention and forceful passage of gas and meconium following digital rectal examination.[1-4] In 10%-25% of neonates, HD initially presents with serious complications such as Hirschsprung-associated enterocolitis. Less frequently, older children who are eventually found to have HD initially present with chronic, severe, and unexplained constipation. While a diagnosis of HD is unlikely in older children presenting with chief complaint of chronic constipation, if there is any suspicion for HD, a rectal examination and rectal biopsy should be done to evaluate for this disorder with the ultimate goal of not missing the diagnosis.[5-7] The differential diagnosis for a patient presenting with constipation is broad, and the astute pediatrician must consider surgical as well as medical etiologies (Table 1).

When a detailed neonatal history is obtained from parents of children diagnosed with HD, most recall an onset of infrequent stools within the first month of life, regardless of the eventual age at presentation.[8-10] A rectal biopsy should be recommended when a careful history and focused physical examination reveal the classic triad of delayed passage of meconium, abdominal distention, and vomiting, though the presence of all three is not necessary for the evaluation of HD. It should be noted that the diagnosis of HD is a particular challenge at the extremes of the pediatric age group. For example, in premature infants with obstructive symptoms, only 5% are eventually diagnosed with HD.[11] Similarly, older children present a diagnostic challenge as their

parents may not recall a neonatal stooling history, and the incidence of functional constipation in this age group is much higher. Patients presenting to the pediatric surgeon or pediatric gastroenterologist for evaluation of constipation who do not admit to a history of delayed passage of meconium, abdominal distention, or vomiting may not require rectal biopsy to rule out HD, as the incidence of HD would be predicted to be extremely low given the absence of these cardinal symptoms. Any procedure carries risks, and by following these guidelines, Lewis et al[4] estimated that up to 36% of suction rectal biopsies (SRB) could be avoided.

During the evaluation of a neonate with delayed passage of meconium, who is suspected of having HD, diagnostic radiology including a contrast enema is an important adjunct to diagnosis. A plain abdominal X-ray may have been obtained by the newborn nursery or neonatal intensive care unit, and would likely reveal a nonspecific bowel gas pattern or may show signs of distal intestinal obstruction with dilated loops of bowel. Contrast enema may demonstrate a transition zone, or change in caliber, created by distention of the normally innervated bowel proximal to the narrowed and functionally obstructed aganglionic segment. Identification of a clear transition zone in the colon strongly suggests the diagnosis of HD, and can help direct the surgical approach.[2] In cases where there is a clinical suspicion of HD even a normal-appearing contrast enema cannot reliably eliminate the possibility of HD, as a transition zone may not be noted, especially in the setting of ongoing rectal irrigations.[12] HD can be described as very short segment affecting only the distal rectum, classical or short segment affecting the rectosigmoid, or long segment affecting the more proximal bowel. Approximately 60%-85% of patients have short segment HD.[13]

Anorectal manometry has also been discussed in the literature as a possible noninvasive method to demonstrate a normal anorectal inhibitory reflex in children with functional constipation. Though it may be more complex to set up than diagnostic imaging, in programs that utilize manometry to demonstrate a normal anorectal inhibitory reflex, a rectal biopsy may be avoided in a child with low suspicion for HD based upon manometric data.[14]

**Table 1.** Differential diagnosis of constipation in children

| Variables | Medical | Surgical |
|---|---|---|
| Newborn | Prematurity<br>Functional ileus of prematurity, AXR demonstrates gaseous distention without air fluid levels, usually self limited<br>Infantile botulism<br>Rare but life threatening paralysis from neurotoxin in clostridium botulinum, linked to honey | Hirschsprung's disease<br>Delayed passage of meconium, abdominal distention, and vomiting herald diagnosis, congenital absence of ganglion cells on rectal biopsy, requires pull through<br>Meconium ileus (cystic fibrosis)<br>Contrast enema may be diagnostic and therapeutic, clinical diagnosis of cystic fibrosis confirmed with blood tests for immmunoreactive trypsinogen and DNA analysis for mutations in CFTR<br>Anorectal malformation<br>Prenatal diagnosis on ultrasound possible, positioning of anus is key in physical exam, operative repair to eliminate fistula and preserve sphincter function |
| Toddler and child | Functional constipation<br>Must exclude organic causes, diagnostic symptoms include incontinence, stool withholding, painful bowel movements, fecaloma, large diameter stools<br>Cow's milk intolerance<br>Food protein-induced enteropathy, allergic basis suggested for symptoms<br>Celiac disease<br>Blood tests for IgA anti-tissue transglutaminase and IgG deaminated gliadin peptides or classical biopsy shows duodenal mucosal atrophy, life long gluten free diet<br>Lead poisoning<br>Lead colic, occurring with low levels of ingestion can present with vomiting and abdominal pain as well<br>Hypothyroidism<br>Congenital or acquired hypothyroidism, diagnosed by blood levels of TSH and free T4<br>Intestinal pseudoobstruction<br>Rare congenital or acquired condition caused by neuropathic or myopathic disorders, colonic transit time prolonged<br>Extremely low birth weight<br>Neurodevelopmental delay and neurosensory impairment<br>Neurologic disorders<br>Resulting in intestinal hypotonia<br>Intestinal neuronal dysplasia | Hirschsprung's disease<br>Delayed diagnosis may occur especially in patients with a very short aganglionic segment, consider anorectal manometry and repeat biopsy to avoid missing diagnosis<br>Anorectal malformation<br>Constipation and functional disorders of elimination present long after intiial repair, and remain a challenge for surgeons and gastroenterologists<br>Intestinal pseudoobstruction<br>Surgical involvement may include central venous access for TPN, cecostomy tube placement, or colostomy/ileostomy<br>Neurologic disorders<br>Surgical involvement may include central venous access for TPN, cecostomy tube placement, or colostomy/ileostomy<br>Intestinal neuronal dysplasia<br>Histologic features of hyperganglionosis and abnormal AChE staining, controversial subset of variant Hirschsprung's disease, surgical involvement may include cecostomy tube placement or colostomy/ileostomy<br>Internal anal sphincter achalasia<br>Absent rectosphincteric reflex on anorectal manometry as in Hirschsprung's disease, but ganglion cells present on biopsy |

IgA: immunoglobulin A; IgG: immunoglobulin G; AChE: acetylcholinesterase; AXR: abdominal X-ray; CFTR: cystic fibrosis transmembrane conductance regulator; TSH: thyroid stimulating hormone; TPN: total parenteral nutrition.

The gold standard test for the diagnosis of HD is rectal biopsy with careful evaluation of the biopsy specimen by an experienced pathologist (Fig. 1). Tissue obtained from the posterior wall of the rectum 2 cm above the dentate line (Fig. 2) in patients with HD will demonstrate an absence of ganglion cells (Fig. 3) in the submucosal plexus and hypertrophic nerve trunks in the submucosal plexus and myenteric plexus.[1,15] Histochemical staining for acetylcholinesterase (AChE) and immunohistochemical staining for calretinin may aid in the diagnosis, though staining with hematoxylin and eosin (HE) may be sufficient for documenting the presence or absence of ganglion cells (Figs. 4-6).[16]

## Rectal biopsy techniques

Clinical practice varies, but due to the possibility of obtaining insufficient tissue for diagnosis or the possibility of taking a biopsy of the physiologic aganglionic region, most practitioners obtain 2-3 rectal biopsy specimens per procedure.[16] An SRB can be performed at the bedside without the use of general

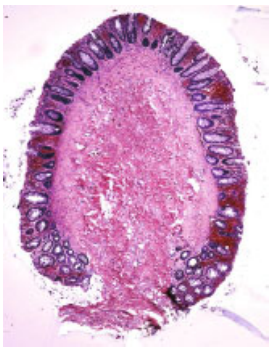

**Fig. 1.** Suction rectal biopsy from a patient with Hirschsprung's disease (hematoxylin and eosin staining, original magnification × 200). At higher power and after examination of 50 sections no ganglion cells were identified and nerve trunk hypertrophy was present (not seen at this magnification).

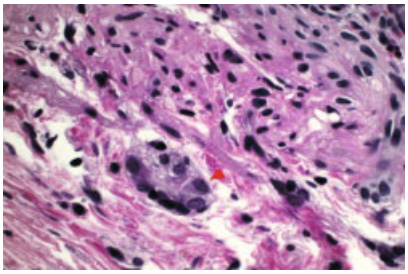

**Fig. 3.** A single normal ganglion cell (indicated above by red arrow) is sufficient to rule out Hirschsprung's disease (original magnification × 400).

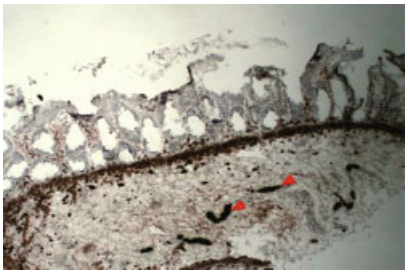

**Fig. 5.** Suction rectal biopsy of a patient with Hirschsprung's disease demonstrates abnormal acetylcholinesterase activity in the muscularis mucosa highlighting hypertrophied nerve trunks in the muscularis mucosa (indicated above by red arrows) (original magnification × 40).

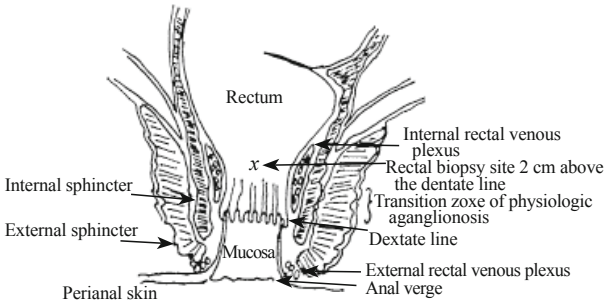


**Fig. 2.** Illustration of the rectum with site of rectal biopsy.

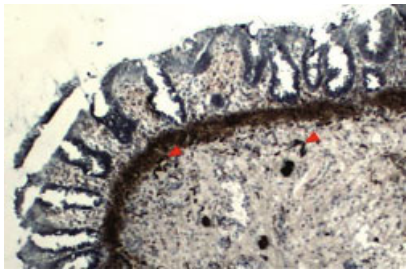

**Fig. 4.** Suction rectal biopsy of a patient without Hirschsprung's disease demonstrates normal acetylcholinesterase activity in the muscularis mucosa staining normal appearing nerve trunks (indicated above by red arrows) (original magnification × 40).

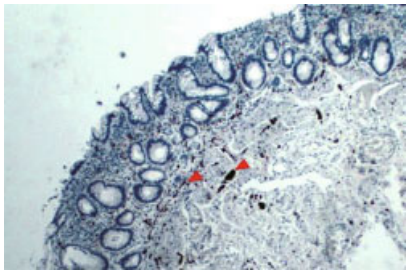

**Fig. 6.** Suction rectal biopsy of a patient without Hirschsprung's disease demonstrates normal calretinin activity in the lamina propria with normal appearing nerve trunks in the muscularis mucosa (indicated above by red arrows) (original magnification × 40) . Rectal biopsy in a patient with Hirschsprung's disease would show an absence of calretinin staining (not shown).

anesthesia, though it often results in smaller tissue specimens compared with full thickness biopsy (FTB). Though larger tissue specimens may be obtained, FTB requires general anesthesia in the operating room and suture closure of the biopsy site (Table 2).

**Suction rectal biopsy**

A myriad of techniques and devices to obtain a SRB have been described. A simplified procedure is as follows: position the patient in lithotomy position, insert the lubricated biopsy instrument along the posterior wall of the rectum, position the opening for the cutting blade 2 cm above the dentate line and aim the blade posteriorly, apply suction, and trigger the cutting blade to obtain tissue specimen (Table 2). Different instrumentation sets recommend different amounts of suction be applied to obtain a biopsy. Our particular instrument, the rbi2 (Aus Systems, Australia), recommends calibration to 300 mm $H_2O$ prior to use, and then withdrawing the plunger to 3-5 mL (using a 10 mL syringe), or ideally 150 cm $H_2O$ when using the manometer.[17] This is comparatively less pressure than previously required by initial devices that classically requires 20 mL of pull back in a 60 mL syringe, due to its completely air tight system that is able to produce biopsy specimens at a lower, more uniform pressure.[17,18] Although practices vary by institution and physician, typically 2-3 specimens are obtained at each session. Suction may be applied manually via syringe (preferred) or mechanically via wall suction, although caution should be taken not to exceed manufacturers recommended suction pressure.

Mechanical biopsy devices have the advantage of reproducibly obtaining a standard volume of tissue sample. Though historically plagued with being difficult to operate triggers and sometimes challenging assembly, the modern devices have become simple to operate and maintain. Some offer multiple capsules of varying sizes to accommodate larger children.[19] Most SRB devices still require two people to execute the procedure: one to position the opening of the device 2 cm proximal to the dentate line, apply light pressure toward the posterior rectal wall, and trigger the cutting edge after suction is applied by the first assistant. Solo models have been developed that apply suction prior to releasing the cutting blade by depression of a trigger, and can be used by one operator, but these are less commonly available.[20] SRB devices can either be disassembled for sterilization or disposable single-use instruments.[19]

**Endoscopic rectal biopsy**

Rectal biopsy obtained endoscopically with biopsy forceps has been described as an alternative to SRB, with reported rates of diagnostic biopsy exceeding those published for SRB at 93% using jumbo biopsy forceps. Endoscopic procedures require at least procedural sedation, though for short procedures inhalational anesthetic may be sufficient, and offer no real advantage to traditional open biopsy in this regard (Table 2). Though smaller, endoscopically-collected rectal biopsy specimens that were inadequate by pathologic standards due to absence of submucosa, were, for all practical purposes, considered diagnostic when ganglion cells adherent to the mucosa were seen, allowing the diagnosis of HD to be eliminated.[21] It is unclear whether these biopsy specimens obtained by standard endoscopic methods would yield tissue of adequate quality to make a positive diagnosis of HD.

**Open rectal biopsy**

Open rectal biopsy techniques, necessitating surgical excision of a segment of the rectal wall, require the patient to be placed under general anesthesia for placement of an

**Table 2.** Major rectal biopsy techniques for comparison

| Biopsy technique | Anesthesia | Procedure location | Bopsies performed by | Additional |
|---|---|---|---|---|
| Suction | No | Bedside | Surgery or gastroenterology | May require wall suction |
| Endoscope | Procedural sedation | GI suite | Surgery or gastroenterology | Endoscopy cart |
| Open | General anesthesia | OR | Surgery | Anorectal surgical instruments and suture |

GI: gastrointestinal; OR: operating room.

**Table 3.** Pathological staining for rectal biopsy

| Staining | Tissue specimen | Positive findings in HD | Additional |
|---|---|---|---|
| Hematoxylin and eosin | Fixed | Absence of ganglion cells, nerve trunk hypertrophy | Gold standard, universally utilized |
| Acetylcholinesterase histochemistry | Frozen | Nerve trunk hypertrophy | Disadvantage: requires frozen tissue, interobserver variability |
| Calretinin immunohistochemistry | Fixed | Absence of calretinin staining | Disadvantage: CRir positive fibers extend up to 2 cm into aganglionic segment, difficult to interpret |

HD: Hirschsprung's disease; CRir: calretinin immunoreactivity.

anoscope or speculum, positioning of the patient for direct vision of the biopsy site, and suture closure of the biopsy defect for hemostasis (Table 2). This technique aims to obtain a larger volume of tissue from a well-controlled location. Open biopsies are often undertaken when previous biopsies were inadequate or in older children.[22,23]

## Inadequate rectal biopsy

An adequate rectal biopsy has been defined as measuring at least 3 mm in diameter with at least one-third of its thickness being submucosa, ideally one-half submucosa.[16,23,24] Biopsy of the submucosal plexus reflects the same innervation in the myenteric plexus as well, obviating the need for traditional full thickness biopsy in all but the most complicated cases (variant HD, such as intestinal neuronal dysplasia), which remains controversial.[6,25,26] Rectal biopsy should be obtained 2 cm above the dentate line, as the distal 1-2 cm of the rectum is physiologically hypoganglionic (Fig. 2). Also, biopsies taken too high may miss very short segment HD limited to the distal rectum.[16,27,28]

Inadequate rectal biopsies with insufficient submucosa for analysis occur in 8%-26% of specimens obtained, often leading to repeat biopsy to make a diagnosis.[7,15,22,27,29] Inadequate biopsies result in diagnostic delay due to need for repeat biopsy, sometimes at markedly increased cost (inpatient versus outpatient) and prolonged parental anxiety.[27,30] Trainees performing SRB are less likely to obtain adequate tissue specimens for diagnosis compared with attending staff probably due to improper technique and inexperience with positioning of the apparatus.[22] Insufficient suction applied by traditional 60 mL syringe is clumsy and challenging to manage while attempting to provide 20 mL of suction, and it has been suggested that wall suction provides a more constant negative pressure to draw in adequate submucosa for definitive pathologic assessment and diagnosis, though pressures should not exceed manufacturers' recommendations for the biopsy device.[18,27,30]

### Implications of increased age at time of biopsy

It is a widely held belief that SRB in older children may not yield adequate tissue specimens for pathologic analysis. This may be due to over distention of the rectal wall, mucosal edema, or increased fibrous tissue, making it difficult for the suction device to draw in a sufficient volume of submucosa for diagnosis. Some evidence suggests that this may be true even for infants over 6 months of age.[7,22] Older children may present for evaluation of chronic constipation even when symptoms may have begun shortly after birth, and advocating for SRB for all children remains controversial because there is a low likelihood of making a diagnosis of HD in an older child.[31]

Furthermore, there is an inverse relationship with age and density of ganglion cells, suggesting that to rule out HD in an older child would be more challenging, and more sections may need to be examined.[26,32] Forceps with a larger hole through which tissue is pulled result in a generally larger volume biopsy specimen; however as long as sufficient submucosa is present, larger specimens may provide no diagnostic advantage.[33]

## Complications from rectal biopsy

Major morbidity from rectal biopsy occurs in up to 2% of patients. Regardless of the technique used for biopsy, significant bleeding from the biopsy site, perforation, and pelvic sepsis are rare but serious complications.[22,34] Suction, if applied at too high a pressure, can unintentionally lead to larger biopsy specimens including the muscle layer while increasing the likelihood of bleeding and perforation. This is more likely in patients under one year of age due to thinner more pliable tissues.[35] Most bleeding following SRB is self-limited and not concerning. However massive bleeding after SRB, though uncommon, usually occurs immediately after the procedure, but has been reported in one instance to occur 4 days later, highlighting the need to inform patients and parents of this unlikely but life-threatening possibility.[36] Though major morbidity from rectal biopsy is rare, pediatric surgeons and pediatric gastroenterologists must be aware of the possibility, especially since this procedure is frequently performed in the outpatient setting without extended post-procedure observation.

## Pathology

HE staining of rectal biopsies can demonstrate features consistent with HD including aganglionosis and nerve trunk hypertrophy. On conventional staining with HE, confirming the presence of a single ganglion cell (Fig. 3) excludes the diagnosis of HD, and a minimum of 50 HE stained levels are examined at our institution before aganglionosis can be concluded.[37] Adequate biopsies with greater than 50% submucosa are important in making the correct diagnosis. In the developing world, clinical presentation and biopsy stained with HE with or without radiologic studies may be sufficient for diagnosis, as presentation can be markedly delayed and access to advanced diagnostic techniques is limited.[5] Nerve trunk hypertrophy is perhaps the most striking difference between normal controls and HD biopsies, and fibers can be highlighted with adjunct staining for AChE to aid in diagnosis (Table 3).[38]

AChE staining is carried out on fresh frozen specimens snap frozen in liquid nitrogen and sectioned by a cryostat for histochemical processing. Cholinergic nerve fibers that are AChE positive in the submucosa are hypertrophied in HD, making AChE staining more prominent.[39] Modifications to this method such as freezing at -20°C enable the broader application of these techniques to centers not equipped with the most advanced laboratory capabilities.[40] The absence of characteristic AChE staining does not exclude HD in the neonatal period when most biopsies and diagnoses take place. Additionally, very short segment HD may be missed if biopsies are taken too high and demonstrate normal AChE staining. AChE staining has been associated with relatively high rates of inter-observer variability in results interpretation, leading to a push toward other ancillary staining methods that may be more straightforward such as calretinin (Table 3).[41-43]

Calretinin immunohistochemical staining can be carried out on formalin fixed paraffin embedded tissue, making it more widely applicable than AChE staining.[44] Calretinin is a calcium binding protein that is normally found in enteric neurons within the submucosal plexus. Calretinin immunoreactivity (CRir) extends into the aganglionic segment 1-2 cm within the transition zone in HD, and may demonstrate false negatives (CRir positive or weak staining) in HD patients if biopsy is within the transition zone or if the aganglionic segment is very short. Like AChE staining, CRir interpretation can be challenging, especially if biopsies are taken within the transition zone; however results may be more consistent, and it may be a more favorable stain than AChE.[42,43] CRir is a useful adjunct, and may be better than AChE staining, but not a stand-alone substitution for HE examination for ganglion cells and nerve trunk hypertrophy (Table 3).[38,45]

Other markers such as lactate dehydrogenase, nicotinamide adenine dinucleotide phosphate-diaphorase, and succinate dehydrogenase have been used less commonly to identify ganglion cells, and though additional markers are available to stain hypertrophied nerve trunks such as rearranged during transfection oncoprotein, these require more advanced histologic staining techniques that limit their widespread use, and are beyond the scope of this manuscript.[1,2,43,46]

## Conclusions

Rectal biopsy remains the gold standard for the diagnosis of HD. It is a safe procedure with a low complication rate when performed by an experienced pediatric surgeon or gastroenterologist. Though constipation accounts for many visits to the pediatrician, a careful history and focused physical examination should reveal those patients with a history of delayed passage of meconium, abdominal distention, or vomiting who require rectal biopsy to exclude HD. Despite a relatively high incidence of inadequate specimens requiring follow up and repeat biopsy, rectal biopsy with the adjunct of AChE staining for nerve trunk hypertrophy or CRir in addition to the classic HE staining demonstrating an absence ganglion cells in the eyes of an experienced pediatric pathologist is diagnostic of HD. Future directions for study include noninvasive imaging, which has shown good results in a rat model by optical coherence tomography that correctly identified segments of aganglionosis, and serum protein markers with 100% sensitivity and specificity fingerprint chromatograms for early detection of HD, and this research is promising.[47,48]

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**Competing interest:** No conflicts of interest.

**Contributors:** Muise ED and Cowles RA conceived of the manuscript. Muise ED wrote the first draft and both Muise ED and Cowles RA revised the manuscript into final form. Cowles RA is the guarantor.

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