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Submucosal nerve diameter of greater than 40 μm is not a valid diagnostic index of transition zone pull-through

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ABSTRACT

Background: Submucosal nerve hypertrophy is a feature of the transition zone in Hirschsprung disease and has been used as a primary diagnostic feature of transition zone pull-through for patients with persistent obstructive symptoms after their initial surgery. Most published criteria for identification of hypertrophy rely on a nerve diameter of greater than 40 μm, based primarily on data from a relatively small number of infants with Hirschsprung disease and controls. The validity of these objective measures has not been validated in appropriate controls for post-pull-through patients.

Scientific approach: The primary pull-through specimens and post pull-through biopsies +/− redo pull-through resections from a series of 9 patients with Hirschsprung disease were reviewed to assess the prevalence of submucosal nerves >40 μm in diameter and 400× microscopic fields containing two or more such nerves. Similar data from multiple colonic locations were collected from a series of 40 non-Hirschsprung autopsy and surgical controls.

Results: The overwhelming majority of Hirschsprung patients harbored submucosal nerves >40 μm in their post-pull-through specimens independent of other features of transition zone pathology, and despite normal innervation at the proximal margins of their initial resections. Measurement of submucosal nerve diameters in autopsy and surgical non-Hirschsprung control samples indicated that nerves >40 μm are normal in the distal rectum after 1 year of age and are found in more proximal colon at older ages.

Conclusions: These results suggest that diagnostic criteria currently used to recognize submucosal nerve hypertrophy in the neorectum after a pull-through for Hirschsprung disease are not justified and should not be regarded as definitive evidence for transition zone pull-through.

Submucosal nerve hypertrophy is a well described feature in the aganglionic distal rectums of most Hirschsprung disease patients [1]. Both the density and caliber of submucosal nerves are increased. These nerves arise predominantly from pelvic autonomic ganglia, which are a normal source of extrinsic innervation to the rectum and left colon [2]. In Hirschsprung disease, particularly short segment disease, submucosal nerve hypertrophy extends beyond the aganglionic segment into ganglionic bowel, and is considered a diagnostic feature of the transition zone — neuroanatomically abnormal bowel between the aganglionic segment and more proximal normally innervated intestine [3–5]. Other features of transition zone are absence of ganglion cells from a significant portion of the bowel circumference or moderate-to-severe hypoganglionosis of the myenteric plexus [6].

Recognition of submucosal nerve hypertrophy in rectal biopsies is a helpful diagnostic feature of Hirschsprung disease, and is usually assessed subjectively by experienced pathologists. However, a quantitative index of nerve hypertrophy was introduced by Monforte-Munoz and colleagues, who identified one or more nerve with a diameter > 40 μm in 90% of aganglionic suction biopsies from Hirschsprung disease patients, as opposed to 0% of ganglionic biopsies from non-Hirschsprung patients [7]. A recent study of rectal biopsies from 92 Hirschsprung disease patients indicated that biopsies from a third contained no nerve >40 μm thick, and showed that rectal nerve hypertrophy was more often absent in patients with long segment disease [8]. The overall prevalence of nerve hypertrophy was less than reported by Monforte-Munoz et al., possibly because of fewer long segment cases in the latter series. As neither study addressed the sensitivity of rectal biopsy in predicting nerve hypertrophy in the subsequent resections, it is unclear how well biopsy data reflect the actual incidence of nerve hypertrophy in aganglionosis.

A significant problem is the lack of sufficient control data to established diagnostic criteria for submucosal nerve hypertrophy in older patients. Most patients with obstructive symptoms after pull-through surgery are managed conservatively for many months to years before redo surgery is considered. In an autopsy series of non-Hirschsprung controls up to 6 months of age, Kapur and Kennedy reported 400× fields with ≥2 nerves >40 μm nerves in the distal 1–4 cm of the rectum in 3 of 9 patients [4]. Coe et al. examined the colectomy specimens from 5 patients with familial adenomatous polyposis

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Table 1
Clinical findings in Hirschsprung disease patients with subsequent biopsies of neorectum +/− redo pull-through procedure.

Patient	Sex	Associated Anomalies	Postoperative Findings	Contrast Enema	Manometry
1	F	No	C, OI, EC, DC, RI	Very dilated rectum and distal sigmoid with more normal caliber proximal bowel.	No
2	M	No	C, AD, EC, RI	Short segment narrowing at the location of the rectal vault with marked dilation of the proximal colonic bowel loops.	No
3	M	No	C, EC, RI	Elongated anal canal with no evacuation of contrast after 30 min.	Intraanal pressures between 60 and 80 mmHg, no rectoanal inhibitory reflex
4	M	PFO, PDA	C, AD, EC, RI, BTX	Dilated 15–20 cm segment proximal to anastomosis (CE).	Absent rectoanal inhibitory reflex)
5	M	No	C, AD, pEC	Not available	Not available
6	M	No	C, OI, AD, EC, RI, BTX	Mild caliber narrowing of the descending colon and rectum relative to the ascending and transverse colon.	Intraanal pressures between 50 and 60 mmHg and no rectoanal inhibitory reflex, and abnormal colonic manometry with no HAPCs.
7	M	No	NRI	Normal caliber rectum and colon.	No
8	M	No	C, OI,RI	Diffuse retention of stool throughout the colon.	No
9	M	No	C, AD, EC	Not available	Not available

Abbreviations: C, constipation; OI, overflow incontinence; EC, enterocolitis; DC, surgical decompaction of stool; RI, rectal irrigations; AD, abdominal distension; pEC, probable enterocolitis; BTX, botox injection into anal sphincter; NRI, nonretentive incontinence; PFO, patent foramen ovale; PDA, patent ductus arteriosus; HAPCs, high amplitude peristaltic contractions; M, male; F, female.

(mean age 135 +/− 49 months) and found "rare instances of submucosal nerve hypertrophy and hyperplasia (two or more nerves ≥40 μm in diameter in a field at 400×)" in the two oldest patients. These published data raise concern that existing indices for nerve hypertrophy based on nerves >40 μm may not be justified.

1. Methods

Hematoxylin-and-eosin (H&E)-stained sections from the following groups of specimens and corresponding pathology reports were retrieved from the files at Seattle Children's Hospital between June, 2010 and December, 2015:

a. Patients with persistent obstructive symptoms after their initial pull-through resections (n = 9) (Table 1). Patients were included in this group only if they underwent rebiopsy of their neorectum +/− redo pull-through and only if available pathology slides included an en face section of the proximal margin from their original pull-through and all subsequent bowel biopsies or resections (Table 2). For patients in this group, sections from their original pull-through, including the proximal margin of the original resection were reviewed along with any subsequent rectal biopsy (n = 9) and/or redo pull-through resection (n = 3). Patients with total colonic aganglionosis (small intestinal pull-through) were excluded.
b. Autopsy and surgical control group composed of non-Hirschsprung disease patients with no history of Hirschsprung disease-like symptoms (Supplemental Table 1). The autopsy cohort (n = 27) excluded samples with significant autolysis and included the rectums from 9 patients used as controls in a prior study [4]. The surgical controls included colectomies from 13 patients with intractable colitis or polyposis. Only well-mapped colons with section locations specifically noted in relation to a surgical margin or other landmark were considered.

Unless otherwise specified, all histological sections from resected or autopsy colon were full-circumference, complete thickness, transverse samples of bowel wall taken at regular intervals along the length of the specimen. For Hirschsprung disease resections, intervals of approximately 1.5 cm were used and always included the proximal margin. For surgical and autopsy specimens, intervals were generally 10 cm, with closer intervals in the distal rectum of autopsy cases. Rectal biopsies were almost all designated by the surgeon as "full-thickness", but frequently lacked most or all of the muscularis propria.

H&E-stained sections were examined by conventional light microscopy using a Nikon Eclipse compound microscope equipped with a Nikon NIS Elements BR 3-2 digital imaging system. The latter was used to transmit images to an adjacent monitor, where digital calipers could be applied to measure nerve thicknesses (diameters). Diameters of submucosal nerves were measured at the widest point along their long axes. Nerves were considered "separate" if fibrovascular stroma intervened between their contours in the plane of section. All submucosal nerves in the sections were examined. The largest nerve diameter greater than 40 μm and the number of 400× fields with ≥2 nerves >40 μm were recorded.

The study was approved by the Institutional Review Board at Seattle Children's Hospital.

2. Results

2.1. Clinical and pathological findings in Hirschsprung patients with post-pull-through obstructive symptoms

Nine patients were identified who underwent primary Soave or Swenson pull-through procedures as neonates and went on to develop significant persistent obstructive symptoms after their original surgery (Table 1). All of these patients had complicated postoperative histories with persistent obstructive symptoms, which were addressed in most with some combination of laxatives, surgical decompaction, rectal irrigations, botulinum toxin injection of the anal sphincter, and/or surgery. The neorectum of each of these patients was rebiopsied 1.5 months to 5 years after their primary surgery and redo pull-through procedures were performed on 4 patients.

Collectively these cases exemplify how the presence/absence of submucosal nerve hypertrophy may be encountered when attempting to histologically identify transition zone in biopsy and resection specimens from Hirschsprung disease patients. I consider the first 3 patients in the entire series (Table 2) examples of unequivocal transition zone pull because partial circumferential aganglionosis and/or severe myenteric hypoganglionosis were present at the proximal margins of their primary resections and at the distal ends of their redo pull-through resections (Fig. 1). Interestingly, submucosal nerve hypertrophy, characterized by at least one nerve with a diameter > 40 μm or a 400× field with 2 or more such nerves, was only identified at the proximal margin of one of the three patients, who had the shortest resection of the group. In contrast, one or both of these putative indices of nerve hypertrophy were observed in each postoperative biopsy and the distal end of each redo pull-through specimen. For the two patients who underwent redo pull-through, submucosal nerves >40 μm and 400× fields with ≥2 such nerves were restricted to the distal 4 cm of their resections and did not extend as far rostral as myenteric hypoganglionosis.

Patient 4 is might be regarded as "probable" or "equivocal" transition zone pull-through in that partial aganglionosis was present at the proximal margin of his primary resection, but was not observed in either his