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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

Table 2
Post-pull-through Hirschsprung disease patients with subsequent biopsies of neorectum +/− redo pull-through procedure.

	Original Pull-Through								Biopsy of Neorectum						Redo pull-through						
			Resection Length (cm)		Putative Features of Transition Zone (Proximal Margin)				Putative Features of Transition Zone						Putative Features of Transition Zone						
PATIENT	Procedure	Age	Total (Site)	Agang. Segmt	Part. Agang.	Hypogang.	Any nerve > 40 μm (max)	400× fields with ≥ 2 nvs > > 40 μm	Age	Type	Agang	Hypogang.	Any nerve > 40 μm (max. in μm)	400× fields with ≥ 2 nvs > > 40 μm	Procedure	Age	Length (cm)	Part. Agang. (dist. in cm)	Hypogang. (dist. In cm)	Any nerve > 40 μm (dist, max)	400× fields with ≥ 2 nvs > 40 μm (dist)
1	So	3d	27 (TV)	26	+	+	−	−	6y	S	+	n/a	+ (<50)	1	Sw	6y	28	+ (3.5)	+ (19)	+ (4 cm; 115 μm)	+ (2 cm)
2	So	12d	14 (Sig)	14	+	−	−	−	2y	S	+	n/a	+ (<50)	−	Sw	3y	7.5	+ (1)	+ (3.5)	+ (1 cm; 76 μm)	+ (1 cm)
3	Sw	9d	11.5 (Sig)	10	+	+	+ (58)	2	13 m	FT	+	−	+ (94)	4	Redo pull-through has not been performed						
4	So[a]	6 m	23 (DC)	18	+	+	+ (81)	2	18 m	FT	−	−	+ (60)	1	Sw	2y	8	−	−	+ (1 cm; 85 μm)	+ (1 cm)
5	So (2 st)[b]	2 m	ns (DC)	>6	−	−	−	−	3y	FT	−	−	+ (<50)	0	Redo pull-through has not been performed						
6	Sw	5d	32 (TV)	20	−	−	−	−	21 m	S	−	n/a	+ (ns)	1	Redo pull-through has not been performed						
7	So (2 st)[b]	6 m	21 (D)	14	−	−	−	−	5y	FT	−	−	+ (91)	>5	Redo pull-through has not been performed						
8	Sw	2 m	14 (Sig)	6	−	−	−	−	4.5y	FT	−	−	+ (ns)	1	Redo pull-through has not been performed						
9	So	7d	23 (DC)	7	−	−	−	−	45d	FT	−	−	−	−	Sw	6.5 m	12	−	−	−	−

Abbreviations: Part. Agang., contiguous aganglionosis of 1/8 or more of circumference; Hypogang, hypoganglionosis; Agang, aganglionosis; max, maximal submucosal nerve diameter in tissue section; dist, maximal distance from aganglionic segment at which finding was observed; Pat, patient; So, Soave; Sw, Swenson; 2 st, two-stage procedure; ns; length not specified; TV, transverse colon; DC, descending colon; Sig, sigmoid colon; S, submucosal; FT, Full-thickness; y, years; m, months; d, days; nvs, nerves; +, present; −, absent; n/a, not applicable.

[a] Includes 3 cm "twisted" segment resected 6 months after initial pull-through at age 7 days.

[b] Segmental resection performed at time of ostomy placement; proximal margin pathology refers to the proximal end of ostomy takedown.

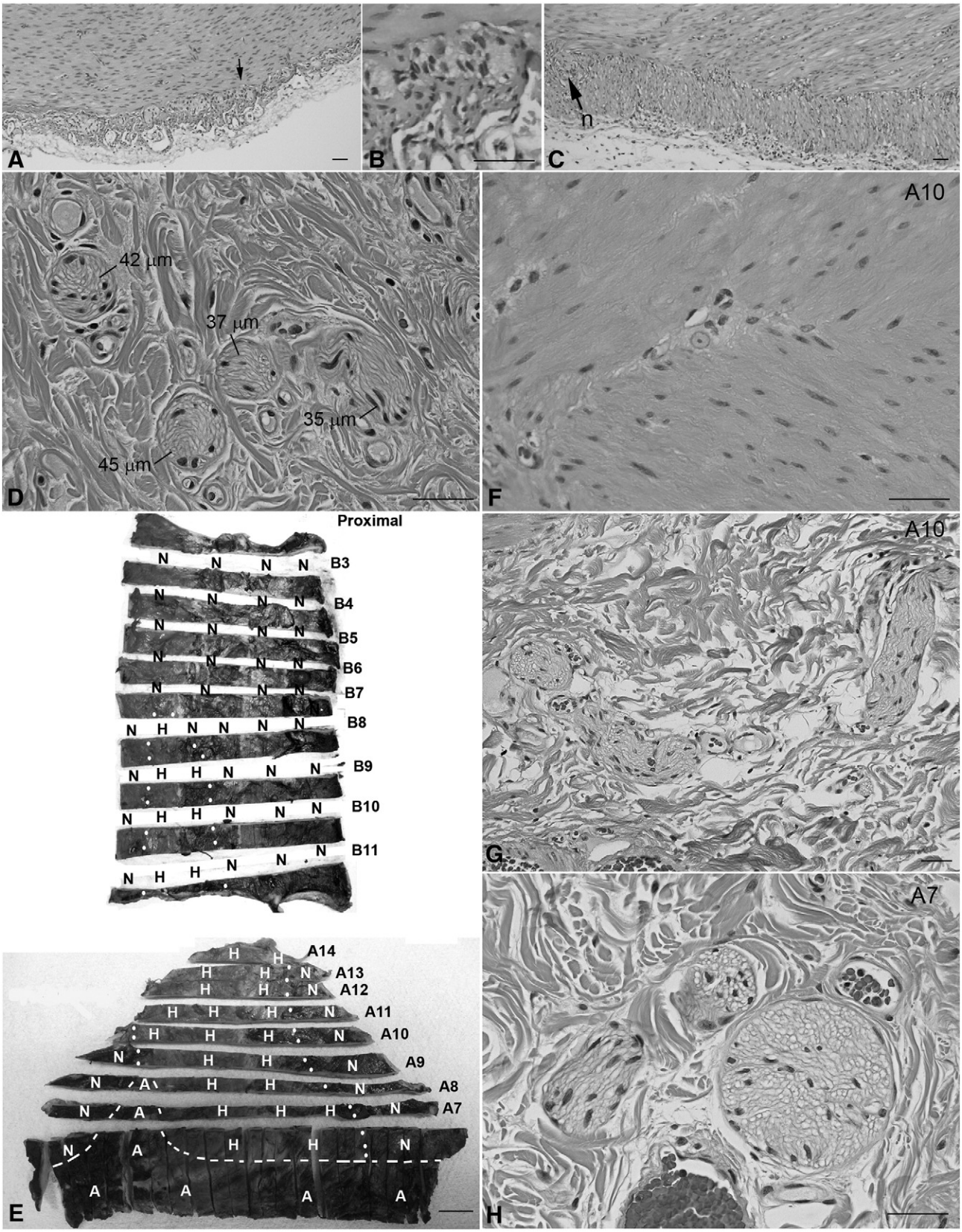

Fig. 1. Transitional zone pull-through (patient 1). (A–C) Microscopic fields from the proximal surgical margin of the patient's original pull-through resection show small, but otherwise well-formed ganglia along part of the circumference (A, arrow is area enlarged in B). Other portions of the circumference (C) are devoid of ganglion cells, but contain myenteric nerves (n). Hypertrophic submucosal nerves were not present (not shown). (D) The submucosa of a rectal biopsy performed 6 years later contains 400× fields with 2 nerves more than 40 μm in diameter. (E) The redo pull-through resection was received as two segments (serosal surface inked with longitudinal stripes to facilitate histological mapping), which were thoroughly sampled for histological analysis (alphanumeric designations to right identify corresponding transverse full-circumference sections). The dashed line at the distal end shows the anastomotic scar from the original pull-through. Regions of histopathological aganglionosis (A), hypoganglionosis (H), and normoganglionic bowel (N) are mapped. (F) A representative portion of severely hypoganglionic myenteric plexus contains a lone ganglion cell surrounded by scant neuropil. (G, H) Microscopic fields of submucosa from transverse sections A10 and A7 each demonstrate multiple nerves with diameters >40 μm. Scale bars: E, 1 cm; all others, 40 μm.

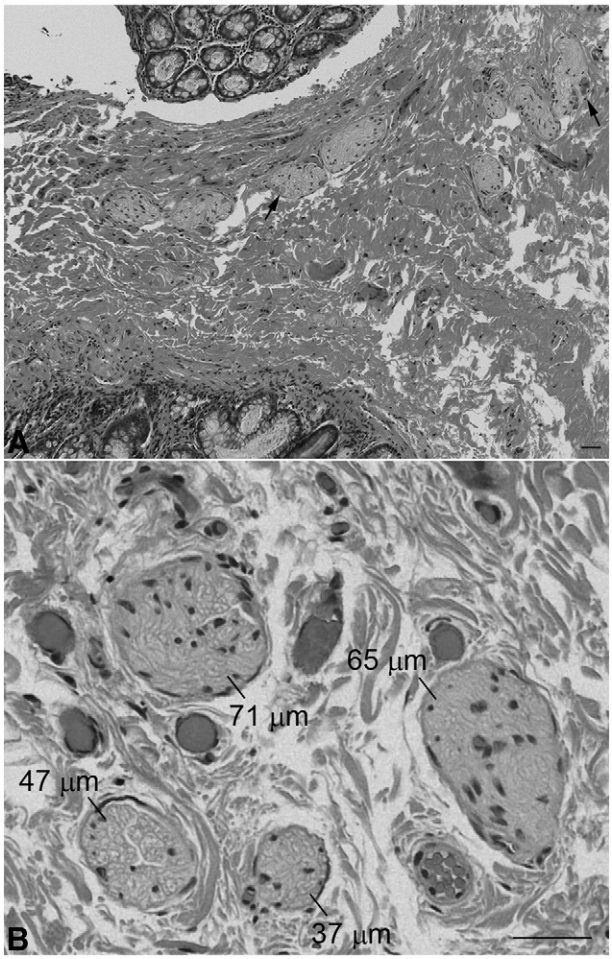

Fig. 2. Submucosal nerves in post-pull-through rectal biopsy (patient 7). (A, B) Two microscopic fields from the submucosa of a rectal biopsy performed 4.5 years after two-stage pull-through procedure. Ganglion cells (arrows) and multiple nerves >40 μm thick are present, including three such nerves in a single 400× field (B). Scale bars: 40 μm.

neorectal biopsy or redo pull-through resection. Both indices of submucosal nerve hypertrophy were present at the proximal margin of his original pull-through and both types of post-pull-through specimens.

For the other 5 patients, no histopathological feature of transition zone was present at the proximal margins of their initial resections. However, neorectal biopsies from some of these patients contained one or more submucosal nerve >40 μm (4 patients) with or without a 400× field with ≥2 such nerves (3 patients); (Fig. 2) the exception being the youngest (patient 9), whose rectal biopsy was obtained at 45 days of age (38 days post-pull-through). Patient 9 is problematic in that nerve hypertrophy was noted subjectively in multiple biopsies by the outside pathologist and used as a rationale for redo pull-through, but could not be confirmed retrospectively by objective nerve measurements.

2.2. Age-related changes in submucosal nerve caliber in control populations

As exemplified by our case series, most patients with Hirschsprung disease with persistent postoperative obstructive symptoms are not rebiopsied until a year or more after their initial pull-through procedure. The neorectums of these patients cannot be consider "normal" in that they derive from more proximal bowel of individuals with an underlying enteric neurodevelopmental disorder, and they have been surgically traumatized. In this context, no ideal control population exists, but data regarding the caliber of submucosal nerves at different points along the length of the colons of children at different ages may be relevant (Supplemental Table 1).

Measurements of submucosal nerve thicknesses obtained from an autopsy/surgical series show that nerves are generally larger in the rectum than other parts of the colon and that nerve caliber increases with age. Under the age of 1 year, only 3/17 controls contained hypertrophic submucosal nerves, defined as any nerve >40 μm, and these were confined to the distal rectum (Table 3). In older children, the prevalence of nerve hypertrophy increased and large nerves were also found in more proximal large intestine. One or more nerves >40 μm thick were observed in a representative section of the distal rectum of all patients older than1 year. In the proximal rectum and sigmoid colon, such large nerves were observed in 13/45 and 7/29 controls respectively, and only in individuals older than 1 year. More proximal colonic involvement was found only in controls >5 years of age.

If the index of nerve hypertrophy was raised to consider only nerves >50 μm hypertrophic, then submucosal nerves of only 1 of 17 infants less than 1 year of age met this criterion and only in sections from the distal rectum (Table 3). The more stringent cut-off of 50 μm produced

Table 3
Putative indices of nerve hypertrophy in non-Hirschsprung controls.

	Number of Controls Fulfilling Specified Index of Nerve Hypertrophy (% of Total)							
	Distal Rectum (<3 cm)				Proximal Rectum			
Age Range	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf
<1 y	17	3 (18%)	1 (16%)	3 (18%)	26	0 (0%)	0 (0%)	0 (0%)
1–5 yrs	10	10 (100%)	9 (90%)	7 (70%)	12	3 (50%)	2 (17%)	0/13 (0%)
5–18 yrs	9	9 (100%)	9 (100%)	8/8 (100%)	7	7 (100%)	7 (100%)	6 (86%)
	Sigmoid				Descending			
	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf
<1 y	15	0 (0%)	0 (0%)	0 (0%)	15	0 (0%)	0 (0%)	0 (0%)
1–5 yrs	7	2 (29%)	1 (7%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
5–18 yrs	7	5 (71%)	2 (29%)	3 (43%)	7	5/6 (83%)	3 (43%)	2 (29%)
	Transverse				Ascending			
	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf	N	Any nerve > 40 μm	Any nerve > 50 μm	≥2 nvs, >40 μm per hpf
<1 y	15	0 (0%)	0 (0%)	0 (0%)	15	0 (0%)	0 (0%)	0 (0%)
1–5 yrs	4	0 (0%)	0 (0%)	0 (0%)	4	0 (0%)	0 (0%)	0 (0%)
5–18 yrs	8	5 (63%)	3 (38%)	2 (25%)	7	3 (43%)	1 (14%)	1 (14%)

N, total number of patients in each group, unless specified in denominator.

Table 4
Average diameter (μm) of largest nerve greater than 40 μm among controls with any nerves >40 μm (number of controls).

Age Range	Distal Rect (<3 cm)	Prox Rect	Sigm	Desc	Trans	Asc
<1 y	49 (5)	na	na	na	na	na
1–5 yrs	67 (10)	49 (6)	52 (2)	na	na	na
5–18 yrs	69 (9)	81 (7)	62 (5)	58 (6)	70 (5)	51 (3)

nearly identical age- and spatial-trends in the distribution of hypertrophic nerves observed as with 40 μm.

Similar trends were also obtained when the presence of a 400× field containing 2 or more nerves >40 μm was employed as an alternative index of nerve hypertrophy, in that the frequency of hypertrophy in the distal rectum increased with age (Table 3). However, involvement of bowel proximal to the rectum was only identified in a subset of controls from the oldest age group (5–18 years). Therefore, by any definition used, "hypertrophic nerves" were relatively common in the distal rectum of individuals without Hirschsprung disease after the age of 1 year, but nonexistent in colon proximal to the sigmoid, except after age 5.

An explicit effort was not made to map the locations of large nerves in the submucosa, but most of the large nerves were deep, often adjacent to the muscularis interna. Nonetheless, more superficial large nerves were encountered in the distal rectum of at least one control from each age group. In a given tissue section, the maximal submucosal nerve diameter and the number of high power fields containing ≥2 nerves >40 μm in diameter also tended to increase with age (Tables 4 and 5).

3. Discussion

3.1. Current diagnostic criteria for nerve hypertrophy based on diameter > 40 μm are not justified after 1 year of age

Histopathological delineation of the transition zone in Hirschsprung disease has considerable practical importance because incomplete resection of the transition zone is regarded as a reason for persistent obstructive symptoms after a pull-through procedure and a potential indication for redo surgery. Histological identification of submucosal nerve hypertrophy alone has been touted as diagnostic of transition zone [2,5,9,10], and objective criteria for hypertrophy based on nerve diameter greater than 40 μm have been extrapolated from data collected by Monforte-Munoz et al. [7], who evaluated suction rectal biopsies with ganglion cells as controls. Indeed, autopsy and surgical control samples in the current study confirm that submucosal nerves larger than 40 μm are normally rare under age 1 year, even in the distal rectum, and are not encountered proximal to the rectum before age 5 years.

An unintended consequence of the pioneering paper by Monforte-Munoz et al. was the notion that a submucosal nerve >40 μm thick is abnormal, independent of age, location, or prior surgery. In fact, Monforte-Munoz et al. only studied suction rectal biopsies from 20 Hirschsprung disease patients (ages 2 days–3 years) and 50 controls (ages not specified). Therefore, in the context of the initial pull-through in a young infant, these morphometric criteria are probably valid. Nonetheless, some surgeons believe that "a nerve trunk that is larger than 40 μm is positively predictive of a transition zone" and consider a rectal biopsy that contains a single 400× field with ≥2 nerves >40 μm as adequate justification for redo pull-through surgery [5,9]. As a result, assessment of submucosal nerve hypertrophy has been advised as part of the histopathological evaluation of rectal biopsies from post-pull-through patients and intraoperative frozen sections during initial and redo pull-through surgery [3,9,10].

Table 5
Average number of high power fields containing 2 or more nerves >40 μm thick (number of controls).

Age Range	Distal Rectum (<3 cm)	Prox Rectum	Sigmoid	Descending	Transverse	Ascending
<1 y	0.2 (17)	0 (26)	0 (15)	0 (15)	0 (15)	0
1–5 yrs	1.3 (10)	0 (13)	0 (7)	0 (4)	0 (4)	0 (4)
5–18 yrs	3.4 (8)	3.4 (7)	1.9 (7)	1 (7)	0.9 (7)	0.7 (7)

The results of this investigation show that reliance on nerve diameters >40 μm alone to establish a diagnosis of transitional pull-through cannot be justified and should be abandoned. Autopsy and surgical control samples clearly demonstrate that neither of these putative indices is specific for transition zone independent of age, and that one or more 400× fields with ≥2 nerves >40 μm are present in the majority of control rectums after 1 year of age, and in more rostral colon at older ages. It might be argued that nerves >40 μm in diameter are rare in colon proximal to the rectum in children under age 5 years, and therefore finding such nerves in post-pull-through rectum is particularly meaningful since sigmoid or more proximal colon is used to fashion the neorectum in most Hirschsprung disease pull-through surgery. However, too little is known about extrinsic innervation of the neorectum to know whether submucosal nerve thickness should resemble age-matched site of origin versus age-matched rectum. Certainly, the native hypertrophic extrinsic nerves to the rectum are transected, either prior to their entry into the gut wall (Swenson procedure) or in the plane of the submucosa (Soave procedure). As the surgical anastomosis heals, it is conceivable that some of these damaged nerves innervate the neorectum. The potential for abnormal postsurgical neorectal innervation may be exaggerated in Hirschsprung patients, given that hypertrophic extrinsic innervation of their native aganglionic rectum is a common phenotypic feature [1], and not necessarily secondary to aganglionosis per se.

3.2. Postsurgical innervation of the "pulled-down" bowel by pelvic extrinsic nerves may produce larger than expected nerve diameters

Unfortunately, ideal comparators for post-pull-through rectum samples are virtually impossible to obtain because Hirschsprung patients who do not have postoperative bowel management issues generally are not biopsied. However, some potentially useful inferences seem reasonable based on our current understanding of the transition zone and the spectrum of pathology observed in some redo pull-through specimens. The collective data from histological, immunohistochemical, and whole-mount studies of the transition zone in Hirschsprung disease [1,2,4,6,11–17] indicate the following generalizations:

1. Overlap in the distribution of myenteric and submucosal ganglion cells is usually close, but not perfect. Isolated submucosal or myenteric aganglionosis may be present in part or all of the bowel circumference in a 1–2 cm long region at the distal end of the transition zone [4,14,15].
2. The interface between aganglionic and ganglionic bowel has an irregular circumferential boundary such that the circumference is partially aganglionic. Partial circumferential aganglionosis typically extends for less than 3 cm from the completely aganglionic segment [4,14,15].
3. Moderate or severe hypoganglionosis of the myenteric plexus often flanks areas of partial circumferential aganglionosis and typically extends for less than 5 cm from the aganglionic segment [4,18].
4. In the aganglionic bowel, submucosal nerve hypertrophy is most prominent (larger and more crowded nerves) in the distal rectum with decreasingly graded severity more proximally [2,12]. In young infants with long segment disease, large or crowded submucosal nerves are seldom encountered proximal to the descending colon [8]. The graded distribution of submucosal nerve hypertrophy often extends into transition zone, but seldom for more than 5 cm from the aganglionic segment [4].

It is important to note that these generalizations are largely derived from resection specimens of young infants with short segment Hirschsprung disease. In an older patient, the transition zone is almost certain to be longer simply owing to proportionate growth. The transition zone in long segment disease may also have different properties, as yet not well characterized.

Independent of the length of the aganglionic segment, submucosal nerve hypertrophy when present is a graded contiguous abnormality. These nerves arise predominantly from pelvic autonomic ganglia and ascend along and within the bowel wall [1,2,12]. Submucosal nerve hypertrophy only involves the transition zone when it extends through the entire length of the aganglionic segment. Therefore, persistence of hypertrophic nerves in the transition zone after a pull-through implies either that they were present in the proximal margin of the original pull-through specimen (or ostomy resection for a two-stage procedure) and persisted despite nerve re/transection during the original surgery or they result from innervation of the neorectum after the original pull-through.

The current series of patients supports a secondary innervation model. Submucosal nerve hypertrophy was not present in the proximal surgical margins of 6 of 8 patients who went on to exhibit one or both criteria for nerve hypertrophy in their post-pull-through rectal biopsies/resections. The two exceptions were patients with probable or definite transition zone pull-through, whose proximal surgical margins also showed partial circumferential aganglionosis/severe hypoganglionosis. I hypothesized that nerves, likely of extrinsic origin, innervate the distal neorectum, either as they would normal rectum or in a more exuberant manner because of surgical trauma and/or the patient's underlying neurodevelopmental disorder. As autopsy and surgical control data show that nerves >40 μm develop in the normal rectum with age, it is possible that similar innervation develops in the submucosa of more proximal colon, when it is relocated to the deep pelvis. Trauma-induced nerve hypertrophy may also be a factor, particularly since large nerves were common in colostomies, independent of Hirschsprung disease.

3.3. Diagnosis of transition zone after a pull-through procedure should not rely on existing published diagnostic criteria for nerve hypertrophy

A practical conclusion from the results of this study is that pathological diagnosis of transition zone pull-through should not rely upon existing 40 μm diameter-based criteria for submucosal nerve hypertrophy. Instead, a combination of clinical and pathological factors should be considered. Most specialized clinics with interdisciplinary teams to manage Hirschsprung patients with chronic postoperative symptoms have developed diagnostic algorithms that incorporate radiographic, manometric, therapeutic (e.g., botulinum toxin injection), and pathology data [9,10,19,20]. Pathological studies are an important part of these algorithms, but only one component in a multidisciplinary approach. From the latter perspective, the surgical pathology report from the original pull-through should be read to determine the length of resected ganglionic bowel and whether the neuromuscular microanatomy of the proximal surgical margin of the resected bowel closest to the anastomosis was normal. A length of resected ganglionic bowel less than 3 cm increases the risk of transition zone pull-through. When possible, the surgical pathology slides from the original pull-through procedure, especially the proximal margin, should be reexamined to exclude transition zone neuropathology (e.g., aganglionosis, severe hypoganglionosis). In some patients, biopsy of the neorectum just superior to the anastomosis may be helpful, particularly if the patient is examined under anesthesia as part of their workup. Generally a single full-thickness biopsy suffices, but if insufficient pathology information is present to confirm full-circumference distribution of ganglion cells at the original proximal resection margin, then four quadrant biopsies should be considered. The pathologist should handle the biopsy as a primary Hirschsprung disease diagnostic specimen, including many histological levels to exclude aganglionosis and ancillary stains (e.g., acetylcholinesterase, calretinin) as needed to facilitate this process. If ganglion cells are identified in all biopsies, transition zone pull-through should not be diagnosed histopathologically, but may be suggested cautiously if submucosal nerve hypertrophy is truly "florid" (crowded collections of markedly enlarged nerves).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jpedsurg.2016.06.007.

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