

TITLE PAGE

Title:

Rectal mucosal/submucosal biopsy under general anesthesia ensures optimum diagnosis of bowel motility disorders

Running Title:

Hirschsprung's disease and rectal mucosal biopsy

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Abstract

Purpose: We reviewed all rectal mucosal/submucosal biopsies (RMSBx) performed between 1986 and 2015 focusing on specimen quality, incidence of complications during and after biopsy, and parental satisfaction.

Methods: From 1986 to 2004, RMSBx were performed without general anesthesia (GA) (N-GA; $n = 98$) and from 1995 to 2015 were performed under GA (GA; $n = 525$). In GA cases, some sections were stained intraoperatively and examined by a pathologist and pediatric surgeon immediately to identify ganglion cells.

Results: Mean ages at RMSBx were similar (2.7 vs. 2.5 years; $p = \text{NS}$). There were significantly more inadequate specimens in N-GA [18/98 (18.4 %) vs. 0/525 (0 %); $p < 0.0001$]. Incidence of rectal bleeding requiring transfusion was significantly lower in GA [0/525 (0 %) versus 2/98 (2.0 %); $p = 0.024$]. Parents of GA subjects willingly consented to RMSBx when told GA would facilitate diagnosis. Incidentally, RMSBx was more expensive in GA (US\$1320 versus US\$294; using ¥120 = US\$1).

Conclusion: RMSBx performed under GA are safe and all specimens obtained included submucosa appropriate for optimum diagnosis in all the cases.

INTRODUCTION

Hirschsprung's disease (HD) is diagnosed by confirming the absence of intramural ganglion cells and demonstrating the presence of thickened nerve trunks [1]. In 1965, Dobbins and Bill first described a technique for suction rectal biopsy (SRBx) performed without general anesthesia (GA) for the diagnosis of bowel motility disorders, such as HD and allied HD, by demonstrating the presence of submucosal ganglion cells [2]. Thereafter, SRBx became the diagnostic gold standard for HD. SRBx is intended to take both mucosal and submucosal layers. However, Friedmacher and Puri [3] reported in their systematic review that inadequate specimens (the specimen contained only lamina propria or a small area of submucosa) were obtained in 10.1 % (9.3-10.9 %) after SRBx and there is a small but definite risk for rectal bleeding that if severe may require blood transfusion [3] or cause rectal perforation [1].

To improve biopsy specimen quality and eliminate complications, we began performing rectal mucosal/submucosal biopsy (RMSBx) under GA in 1995 after we experienced SRBx case who required blood transfusion for rectal bleeding. In the GA cases, a pathologist and a pediatric surgeon with specialized knowledge of HD innervation used hematoxylin and eosin (HE) staining to examine some specimens immediately in the operating room to ensure that adequate submucosa were present. Here, we reviewed all RMSBx performed between 1986 and 2015 focusing on specimen quality, incidence of

complications during and after biopsy, and parental satisfaction.

MATERIALS AND METHODS

Subjects

The subjects for this study were 623 patients with severe intestinal dilatation or chronic constipation, who were indicated for RMSBx to exclude disorders of bowel motility, such as HD and allied disorders, at our institution between 1986 and 2015.

Of the 623 patients, 98 patients had RMSBx as SRBx without GA between 1986 and 2004 (N-GA group). The remaining 525 patients had RMSBx under GA between 1995 and 2015 (GA group). The parents of all the GA group subjects were advised of the risks and cost of GA and all consented willingly to RMSBx once it was explained that diagnosis would be facilitated.

Rectal mucosal/submucosal biopsy techniques

In the N-GA group, RMSBx involved a conventional blind suction technique (Fig.1) without GA. Details are described elsewhere [4]. Specimens were obtained from two sites on the posterior wall of the rectum, 1 and 2 cm proximal to the dentate line; however, the exact place where the biopsies were taken from is uncertain, since the biopsies were not taken under direct vision. Half of each specimen was preserved in formalin for staining with HE and the other half was snap frozen and serial sections stained for acetylcholinesterase (AChE) histochemistry.

Figure 1

In the GA group, a wedge biopsy under direct vision after anal dilatation with

the patient in the lithotomy position was performed under GA to obtain a mucosal/submucosal biopsy specimen from one or two sites on the posterior wall of the rectum 1-2 cm proximal to the dentate line. The place where the specimens were taken from is certain, since the specimens were taken under direct vision. Each specimen was snap frozen intraoperatively and some frozen sections were stained with HE and examined by a pathologist and a pediatric surgeon with specialized knowledge of HD innervation to determine: (1) if adequate submucosa was present; (2) if mature ganglion cells were present; and (3) if hypertrophied nerve fibers coexisted in the submucosa. If the pathologist and pediatric surgeon decided that a specimen was inadequate for diagnosing HD, because it contained only lamina propria or a small area of submucosa, another specimen was taken. Half of each specimen considered adequate by both the pathologist and the pediatric surgeon was preserved in formalin for staining with HE, and serial sections of the other half were stained for AChE histochemistry.

Diagnostic criteria for bowel dysmotility disorders

We diagnosed HD based on HE and AChE staining of RMSBx specimens if there was absence of mature ganglion cells in the submucosa and the presence of thick nerve trunks and dramatically increased numbers of nerve fibers in the submucosa. We also defined chronic constipation as adequate numbers of mature ganglion cells in the submucosa, no thick nerve trunks, and no

dramatically increased numbers of nerve fibers in the submucosa; intestinal neuronal dysplasia (IND) as hyperplasia of the submucous plexus with increased AChE activity around submucosal vessels [5]; and isolated hypoganglionosis (IH) as decreased plexus size and number of ganglion cells [6].

Statistical analysis

Data were reported as mean \pm standard deviation (SD). Fisher's exact test was used for categorical data (Prism software; Graphpad Software, Inc., San Diego, CA).

Ethics

This study was approved by the Ethics Committee of Juntendo University School of Medicine and complies with the Helsinki Declaration of 1975 (revised 1983).

RESULTS

Mean ages at RMSBx were similar (2.7 ± 3.2 vs. 2.5 ± 3.3 years; $p = \text{NS}$).

There were significantly more inadequate specimens in the N-GA group compared with the GA group [18/98 (18.4 %) vs. 0/525 (0 %), respectively; $p < 0.0001$]. In the GA group, all specimens were adequate to establish a definitive diagnosis.

Of the 525 patients in the GA group, 107 patients were diagnosed with HD, 415 patients were diagnosed with chronic constipation, 1 patient was diagnosed with IND, and 2 patients were diagnosed with IH. In the N-GA group, 50 patients were diagnosed with HD, 27 patients were diagnosed with chronic constipation, 2 patients were diagnosed with IND, and 1 patient was diagnosed with IH. All the 18 inadequate cases in the N-GA group required repeat wedge biopsy under GA.

The incidence of rectal bleeding requiring transfusion was significantly lower in the GA group compared with the N-GA group [0/525 (0 %) vs. 2/98 (2.0 %), respectively; $p = 0.024$].

Incidentally, RMSBx under GA was more expensive than SRBx without GA (US\$1320 vs. US\$294; using ¥120 = US\$1).

DISCUSSION

HD is characterized by the absence of mature ganglion cells in the terminal large intestine, and diagnosed if there is histologic proof of such absence [7]. Thus, RMSBx is mandatory. Dobbin's and Bill's SRBx technique [2] was modified by Noblett in 1969 with the introduction of a dedicated SRBx tube that was particularly recommended for use in neonates and small children [8]. He took 116 biopsy specimens from 45 children of all ages, including newborn infants with this instrument, and all the specimens were considered adequate for the diagnosis of HD [8]. SRBx without GA was a considerable advance for the diagnosis of HD, because it can be performed readily in a hospital ward or at an outpatient clinic [4]. However, there are some disadvantages of SRBx performed without GA.

Biopsy specimens may be inadequate and include only mucosa and a small portion of submucosa; the exact site from which the tissue was taken is often uncertain, and natural variability in the distribution of ganglion cells in the distal rectum could result in a false-negative diagnosis of HD [7]. Our approach is that if there is sufficient submucosa present, then a biopsy is adequate for diagnosis.

The complication rate of SRBx without GA reported by Friedmacher and Puri in their systematic review was 0.7 % (0.5-0.9 %), with persistent rectal bleeding requiring transfusion occurring in 0.5 % (0.4-0.8 %) and bowel perforation in 0.1 % (0.02-0.2 %) [3].

We actually use SRBx under GA in neonates and cases with a very high suspicion of HD, and wedge biopsies in older children and cases with an indistinct transition zone to obtain as obtain a good specimen of submucosa (Fig. 2). From experience, SRBx specimens taken from older children tend to be inadequate, because the rectal submucosa of older children is more difficult to obtain because the mucosa is much thicker than in neonates. Biopsy may need to be repeated.

Figure 2

In conclusion, because RMSBx under GA were performed under direct vision, the places where the specimens were taken from is certain (exactly, 1 and 2 cm from the dentate line), and hemostasis is also performed under direct vision. On the contrary, during SRBx without GA, the biopsy site is not exactly 1 and 2 cm from the dentate line, because the biopsy was taken in a blind manner. While RMSBx under GA was more expensive, the extra cost is justified, because parents generally prefer an invasive procedure to be performed accurately once without complications and surgeons can focus better.

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FIGURE LEGENDS

Fig. 1

Suction rectal biopsy instrumentation.

a Reusable type

b Disposable type

Fig. 2

Example: A specimen from an 11-year-old girl who had severe constipation.

a SRBx specimen obtained under GA and a wedge biopsy specimen under GA (x20 HE in both). *SRBx* suction rectal mucosal biopsy, *GA* general anesthesia.

b SRBx specimen under GA (x100 HE). The submucosa is inadequate, showing only lamina propria and muscularis mucosa.

c Wedge mucosal/submucosal biopsy specimen obtained under GA (x40 HE).

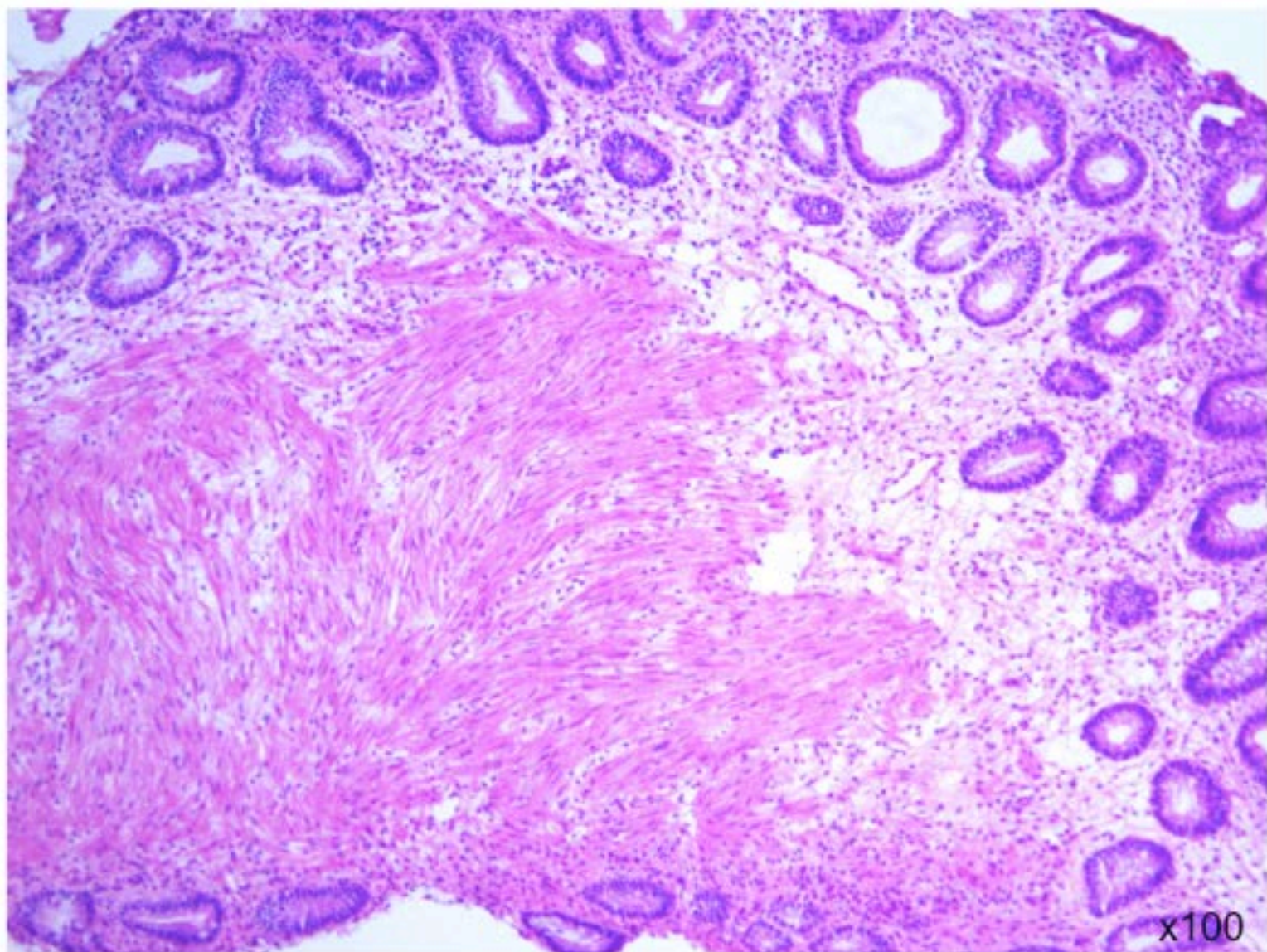
Adequate submucosa was obtained. Both nerve fiber trunks and ganglion cells were confirmed in the submucosa.

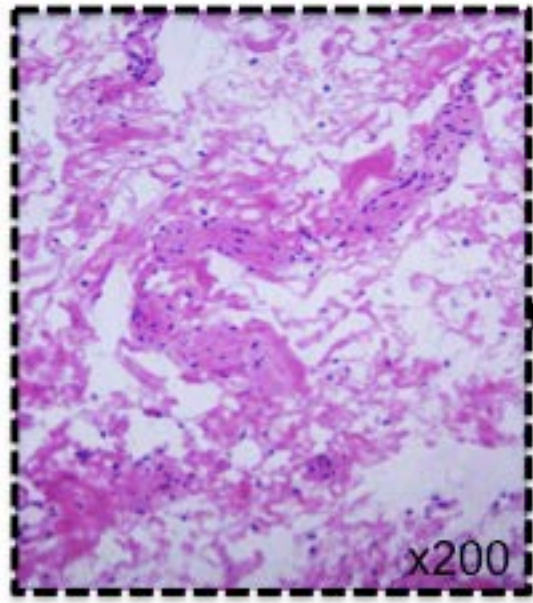


A

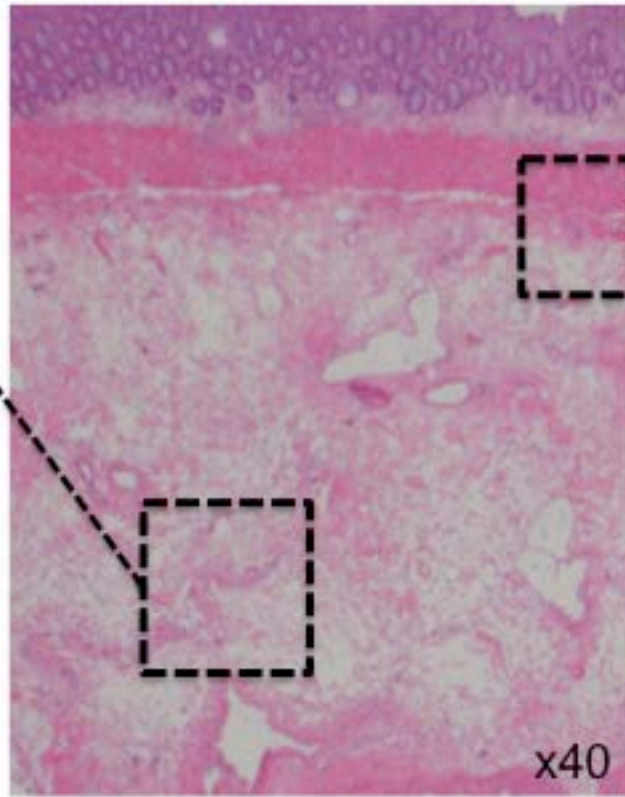


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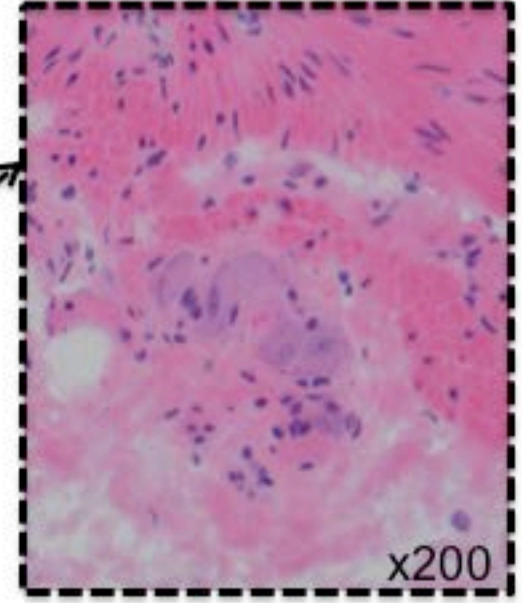




**Nerve fiber trunks
in the submucosa**



x40



Ganglion cell (+)