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Suction rectal biopsy in the diagnosis of Hirschsprung's disease and chronic constipation

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Abstract. Suction rectal biopsy has gained increased acceptance as the means of definitively diagnosing Hirschsprung's disease as well as excluding this diagnosis when evaluating the child with chronic constipation. During the 11-year period from 1 July 1974 through 30 June 1985 at the University of Michigan, Mott Children's Hospital, 309 suction rectal biopsy specimens were evaluated. Of these, 293 were done for the evaluation of chronic constipation and/or Hirschsprung's disease. The remaining 16 were performed as a part of the work-up in patients with neuromuscular, glycogen storage, inflammatory bowel, or other diseases. Forty-two (14%) of the 293 patients were diagnosed as having Hirschsprung's disease at an average age of 14.4 months. This diagnosis was ruled out in the remaining 251 patients, whose age at biopsy averaged 2.7 years. There was one false-negative reading representing an incidence of 0.3%, with no false-positives. There were no complications. The suction rectal biopsy is a bedside or clinic procedure that reliably provides pathologic material adequate for the accurate diagnosis or exclusion of Hirschsprung's disease and offers a number of advantages over manometric, radiographic, histochemical, and open, full-thickness biopsy techniques.

Key words: Hirschsprung's disease – Diagnosis – Suction rectal biopsy

Introduction

Although Hirschsprung's disease was first described in 1888 [8], more than 60 years passed before the concept of routine pathologic examination of the rectosigmoid submucosal and myenteric plexuses became more widely applied [6, 14]. This procedure was advanced through the classic work of Swenson and Bill in 1948, when they and others advocated the use of general or spinal anesthesia and open, full-thickness rectal biopsy to make a definitive diagnosis [16]. Open, full-thickness biopsy remains the standard procedure at a number of institutions and although several approaches, including radiologic, manometric, and histological techniques have been investigated in recent years, suction biopsy has emerged as an important diagnostic tool in patients with a history or radiologic evidence suggestive of Hirschsprung's disease. At our institution, suction biopsy is the initial and definitive modality used to evaluate the infant or child with constipation. The results with the use of this technique over the past 11 years are the subject of this clinical review.

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Materials and methods

The clinical records, pathologic reports, and selected specimens from all patients undergoing suction rectal biopsy between 1 July 1974 and 30 June 1985 were reviewed. This patient population included those being studied to rule out Hirschsprung's disease, as well as the few patients with suspected neuromuscular or metabolic disease. A total of 309 specimens were reviewed. All pathologic material had been initially read or has been reviewed by one of the authors (KPH). During this same 11-year period, 99 patients with Hirschsprung's disease were cared for at Mott Children's Hospital, 75 of whom had been primarily diagnosed and treated at our institution. Forty-two of these 75 patients underwent suction rectal biopsy.

The technique of suction rectal biopsy at our institution has been described previously and is briefly recounted here [23]. The multipurpose pediatric biopsy tube and capsule

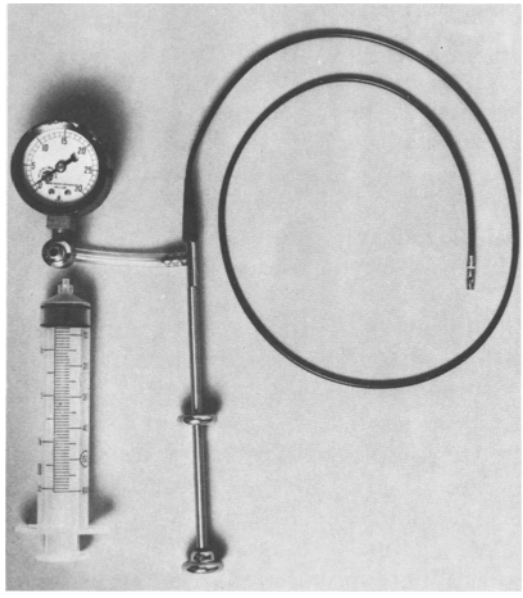
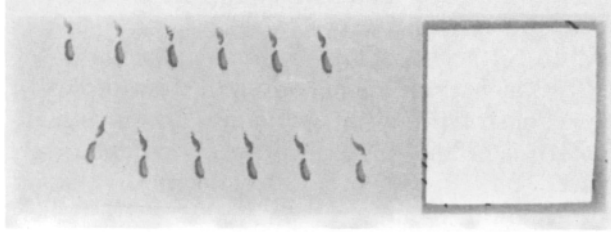


Fig. 1. Suction rectal biopsy instrument



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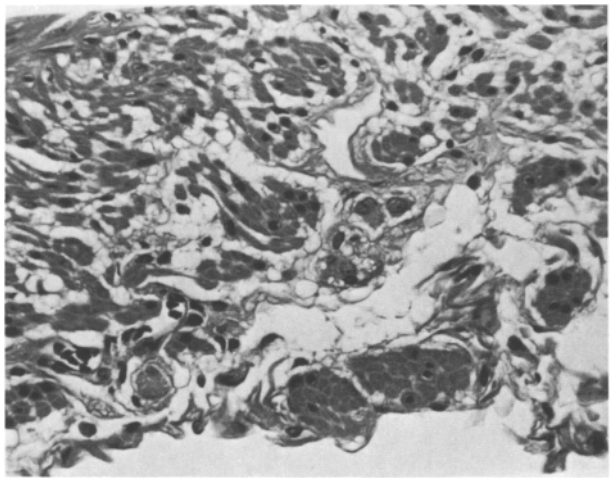

Fig. 3. Section of submucosa including muscularis mucosae at top with ganglion cells apparent (H&E×330)

(4.7 mm – Quinton Instruments, Seattle, Wash.) was utilized in all patients (Fig. 1). The instrument is kept clean and available for use in the clinic, patient ward, or the operating room, but need not be sterile. Following rectal examination and introduction of a small amount of lubricant, the instrument is inserted to a level approximately 2–2.5 cm proximal to the anal verge. The capsule is inserted in the closed position and directed posteriorly either with or without the examining finger present in the rectum. The biopsy capsule is then opened and 15–20 cm of water suction rapidly created by withdrawal through a 50 ml syringe. To ensure adequate sampling, the capsule must be closed immediately upon reaching peak pressure. If peak pressure is not sustained, some mucosa and submucosa will withdraw from the capsule port and the resulting specimen may be inadequate. The biopsy instrument is then withdrawn, occasionally with slight resistance and with minimal or no bleeding. The resultant specimen is 3–4 mm in diameter and 1–2 mm in depth and includes mucosa and submucosa. Several samples are routinely obtained, placed on filter paper to ensure proper orientation, and immediately immersed in formalin except in the few cases where frozen section evaluation is requested. Immediate proper cleaning and storage of the biopsy instrument is essential to preserve patency of the suction channel and sharpness of the biopsy capsule cutting element.

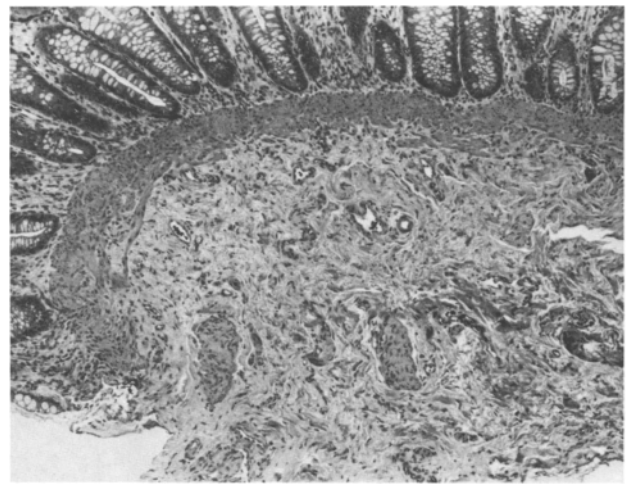

Fig. 4. Suction rectal biopsy with mucosa, muscularis mucosae and submucosa containing large nerves and no ganglion cells (H&E×83)

The biopsy specimens are allowed to fix and are routinely processed overnight in an Autotechnicon. The tissues are embedded in a plane such that the cut sections will shown mucosa overlying muscularis with submucosa on the bottom. The embedding is done by technicians familiar with mucosal biopsies, and multiple serial sections cut at 4–5 μm intervals are mounted until all tissue is processed. Multiple serial cuts are mounted on one slide (Fig. 2), allowing sequential tracing of clusters of ganglion cells if necessary. Generally, 20 sections per slide are mounted and 20 slides are made. If the biopsy is performed for the evaluation of neurologic disease, alternate slides are stained with hematoxylin and eosin (H&E), leaving the intervening slides for special stains. For the evaluation of Hirschsprung's disease, all slides are stained with H&E and then examined by a pathologist. The sections must contain submucosa in order to be adequate for evaluation. Depending on the mucosal thickness, the submucosa may not come into the plane of the section until the third or fourth slide. The submucosa is then examined for ganglion cells either singly or in clusters (Fig. 3). If ganglion cells are not seen, large nerve twigs (Fig. 4) are sought when examining all 20 sections on all slides. The mucosal surface is also carefully scrutinized for

squamous epithelium, which would indicate that the biopsy included some anal canal, an area normally without ganglion cells and thus inappropriate for diagnosis.

The diagnosis of Hirschsprung's disease is not made unless three criteria are met: (1) absence of ganglion cells, (2) the presence of hypertrophied nerve fibers, and (3) adequate areas of submucosa available for evaluation. Using the above techniques, the diagnosis is generally reported within 24 h of receipt of the sample.

Results

Over the last 11 years, 309 suction rectal biopsy specimens have been examined, 293 of which were performed specifically for the evaluation of Hirschsprung's disease and/or chronic constipation and will constitute the focus of the remainder of this report. There has been no morbidity or mortality associated with this procedure, specifically, no instances of perforation or bleeding. There was only one incorrect reading, the very first specimen, representing a false-negative rate of 0.3%. Once a diagnosis was made by suction biopsy, open, full-thickness rectal biopsy was not done on any patient.

The subgroups of patients with and without Hirschsprung's disease are displayed separately in Tables 1 and 2, respectively. The average age at biopsy of patients with Hirschsprung's disease was 14.4 months; however, a more representative figure may be obtained by excluding two patients aged 15 and 16 years, which brings the average age to 5.7 months. The 42 patients with Hirschsprung's disease ranged in age from newborn to 16 years, representing 14% of the total series. Forty-eight percent of these patients were diagnosed within the 1st month of life, and 86% were diagnosed prior to 2 years of age. Only 6 patients were older than 2 years at the time of diagnosis. The patients all subsequently underwent a leveling colostomy, followed by the endorectal pull-through procedure.

The findings in the 251 patients (86% of the total series) proven not to have Hirschsprung's disease are displayed in Table 2. Fifty-five patients (22%) were biopsied before 1 month of age, while 117 (47%) were biopsied between 1 month and 2 years of age, 61 (24%) between 2 and 10 years, and 18 (7%) at more than 10 years of age. The average age at the time of diagnosis was 2.7 years, reflecting a patient population referred for the evaluation of encopresis and/or chronic constipation. This is in contrast with the first group of patients, who were generally biopsied to evaluate failure to pass meconium within the first 48 h of life, to further work-up barium retained for 24 h following barium enema to clarify symptoms of bowel obstruction or to evaluate children with abnormal stooling patterns. These patients were placed on a protocol for treatment of chronic constipation, including the use of stool softeners and enemas.

Sixteen patients were evaluated with suction

Table 1. Suction rectal biopsy in 42 patients with proven Hirschsprung's disease

	Patient age			
	Less than 1 month	1 month – 2 years	2 – 10 years	Over 10 years
Number of patients	20	16	4	2
Cumulative number	20	36	40	42
Percentage of subgroup	48%	38%	9%	5%
Cumulative percentage of subgroup	48%	86%	95%	100%
Percentage of total series (293)	7%	5%	1.3%	14%
Cumulative percentage of total series (293)	7%	12%	13.3%	14%

Table 2. Suction rectal biopsy in patients with ganglion cells

	Patient age			
	Less than 1 month	1 month – 2 years	2 – 10 years	Over 10 years
Number of patients	55	117	61	18
Cumulative number	55	172	233	251
Percentage of subgroup	22%	47%	24%	7%
Cumulative percentage of subgroup	22%	69%	93%	100%
Percentage of total series (293)	19%	40%	21%	6%
Cumulative percentage of total series (293)	19%	59%	80%	86%

rectal biopsy for neurologic, metabolic, or inflammatory bowel disease, but are not included in the above figures. They ranged in age from 9 months to 17 years, with an average of 6 years. Diagnoses made on the basis of these biopsies included glycogen storage disease, neuromuscular disease, amyloidosis, lipofuscinosis, and inflammatory bowel disease.

Discussion

Both investigation of the diagnosis of Hirschsprung's disease and evaluation of the constipated child have undergone remarkable evolution since the earliest gross and microscopic evaluations. More than 60 years passed following Hirschsprung's original clinical observations [8], however, before Swenson et al [17] in 1949 began to correlate the details of history, physical findings, and radiographic abnormalities with the functional colonic disturbance and established proximal colostomy as the initial and often life-saving treatment. The observation of abnormalities in ganglion cell populations of the myenteric plexus was made by Robertson and Kernohan in 1938 [13] and Tiffin et al. in 1940 [20].

Rectal biopsy was established as the mainstay of diagnosis by Swenson in 1955 [18]. With this technique, Swenson [14] subsequently reported a 98% diagnostic accuracy rate in follow-up studies; however, several disadvantages became evident, including the need for general anesthesia and occasional rectal bleeding.

In 1960 Gherhardi [7] had previously confirmed that the level of aganglionosis in Hirschsprung's disease was identical in the submucosal and myenteric plexuses. In 1965, Dobbins and Bill [5] reported that the diagnosis of Hirschsprung's disease could be excluded by suction rectal biopsy. Investigations were continued by Smith in 1968 [15] and included descriptions of pathologic material from autopsies of fetuses, premature infants, and term infants. The author demonstrated sequential maturation of the myenteric plexus in a cranial-caudal direction and cautioned that appreciation of the degrees of maturation, as well as the presence or absence of ganglion cells, is important in the diagnosis of Hirschsprung's disease. The continued development of the rectal punch biopsy or suction rectal biopsy came from the work of Aldridge and Campbell in 1968 [2]. These authors confirmed the presence of a "hypoganglionic zone" within 1–2 cm of the anal verge and stressed that biopsy material should be obtained proximal to this level. They demonstrated that the density of ganglion cells in the submucosal plexus was sufficient above this level, with the maximum distance of approximately 1 mm between cells allowing accurate sampling and avoidance of a false-positive diagnosis due to sampling artifact.

Noblett first described suction rectal biopsy with a modified biopsy instrument in 1969 [11]. It was designed to yield a specimen 3.5–5 mm in diameter, with at least 2 mm of submucosa, and was specifically suited to the diagnosis of Hirschsprung's disease. This was followed by a 1969 report coauthored by Campbell [4], which continued to underscore the accuracy of the procedure in 111 patients. Reports of the widespread applicability and accuracy of suction biopsy have continued with the series of Yunis et al. in 1976 [24], Weintraub et al. in 1977 [23], and Andrassy et al. in 1981 [3]. In contrast, at the same time (1976) Pease et al. [12] reported their experience with punch biopsy, with only 65 of the 234 biopsies being performed without anesthesia. One patient developed a large retroperitoneal hematoma and another required hospitalization for bleeding. There were, however, no false-negative or false-positive results in this series.

The present study reemphasizes our enthusiasm for suction rectal biopsy for the definitive diagnosis of Hirschsprung's disease based on its 99.7% accuracy rate, the simplicity of the technique, and the absence of complications. Several points need to be emphasized, however. A pathologist experienced in preparing and reading these specimens is absolutely essential. Furthermore, regardless of the expertise of the team, we strongly caution against the routine use of frozen section diagnosis. Although frozen section diagnosis of Hirschsprung's disease by suction biopsy was accomplished in a number of patients in our series, this cannot always be done dependably because the orientation of the specimen required for predictable and accurate tissue cuts is unreliable and often requires that inordinate numbers of sections be made. Furthermore, frozen sections generally cannot be cut as thin as paraffin-embedded ones, nor can "ribbons" of successive sections be cut and mounted. For these reasons, despite the accuracy in experienced hands, frozen section interpretation of suction biopsy specimens is to be discouraged.

A number of diagnostic tests have been developed through the years for the investigation of Hirschsprung's disease. Barium enema has been helpful both in newborns and children, by either demonstration of a transition zone contrasting the

spasmodic aganglionic segment and dilated proximal segment, or by showing failure to evacuate barium 24 h after the study. Rarely, however, is the barium enema relied on as the sole means of diagnosis. Manometric diagnosis has been used alone and in conjunction with contrast studies and is based on the absence of the rectosphincteric reflex in which the internal anal sphincter relaxes with stretch stimulation of the rectum in normally innervated bowel. This technique does have the advantage of being noninvasive and suitable for use in the outpatient setting and, combined with barium enema, was the most frequent means of diagnosis of Hirschsprung's disease in 1628 Japanese patients as reported by Ikeda and Goto in 1984 [10]. The restricted applicability and limited accuracy rate of 90.8% [22] in excluding Hirschsprung's disease and the 74.3% accuracy rate in diagnostic confirmation make this technique relatively unattractive, however.

Histochemical diagnosis has been used with increasing frequency [9], and is based on the fact that nerve fibers in the lamina propria and muscularis mucosae of involved colon in Hirschsprung's disease are very prominent and demonstrate an increased acetylcholinesterase content. Wakely and Adams [22] have demonstrated more reliable diagnostic accuracy with this technique versus standard H&E staining (95% vs 83%). The technique can be used on suction rectal biopsy specimens and may require only 1–2 sections. The technical requirements for processing and staining, however, place the method at a relative disadvantage, thus limiting its use, and false-negative results have also been reported.

Suction rectal biopsy is especially well suited to our diagnostic and treatment protocol for children with chronic constipation and Hirschsprung's disease. For the reasons enumerated above and, given the experience of our pathologist, suction biopsy has proven to be extremely reliable in both diagnosis and exclusion of Hirschsprung's disease. The procedure is quickly performed in the office and, in nonemergent situations, allows confirmation of the diagnosis within 24 h. We favor endorectal pull-through for the definitive treatment of Hirschsprung's disease. Suction rectal biopsy, which does not involve full-thickness sampling of the rectal wall, does not interfere at all with the subsequent performance of this procedure and its submucosal dissection. Finally, the technique can be taught with relative ease to resident surgeons. Given the high degree of accuracy and ease of performance combined with simplicity and versatility of instrumentation, we continue to strongly favor suction rectal biopsy as the procedure of choice for the diagnosis of Hirschsprung's disease.

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