IMPROVING DIAGNOSTIC METHODS FOR HIRSCHSPRUNG'S DISEASE IN CHILDREN.

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Annotation: *Hirschsprung disease (HD) is a disorder that affects several medical specialties such as pediatric gastroenterology, pediatric surgery, and pathology. Hirschsprung disease is a congenital disorder of intestinal innervation characterized by the absence of ganglion cells in the muscular (Auerbach) and submucosal (Meissner) plexuses in the distal colon in its classic form. Rapid and accurate diagnosis of HD is a key element in further treatment regimens. The effectiveness of various diagnostic methods used in patients with HD may vary. The use of a single limited diagnostic procedure may result in several percent of missed cases. In recent years, rectal biopsy has been recognized as an important diagnostic tool, allowing a definitive diagnosis of HD with an accuracy of 95% of cases. The correct diagnosis depends on the localization of the biopsy sample, its representativeness, the number of samples and the correct interpretation of microscopic examinations supported by histochemical and immunohistochemical methods. When using several methods and all diagnostic criteria, the sensitivity of the diagnosis allows to practically exclude cases of undiagnosed patients.*

Key words: *Hirschsprung's disease, diagnostics, histopathology*

INTRODUCTION

Hirschsprung disease (HD) is one of the diseases that are treated in most cases by pediatric surgery. It is a congenital disorder of intestinal innervation resulting in the absence of ganglion cells in the region of Auerbach's plexus and Meissner's plexus in the distal colon. The prevalence of the disease is estimated at 1:5000 live births. The disease affects males more often than females, with a ratio of about 4:1 [1, 2]. Its pathogenesis is not fully understood, and various hypotheses have been considered. The most popular of them suggests that aganglionic bowel is caused by impaired migration of target cells from the neural crest of the primary neutral tube in the intrathecal direction during embryonic development between the 4th and 12th weeks of pregnancy [3]. As a result, ganglion cells are absent in part or throughout the colon.

Hirschsprung disease is classified according to the length of the aganglionic segment. The most common form, accounting for 75–80% of cases, is the usual

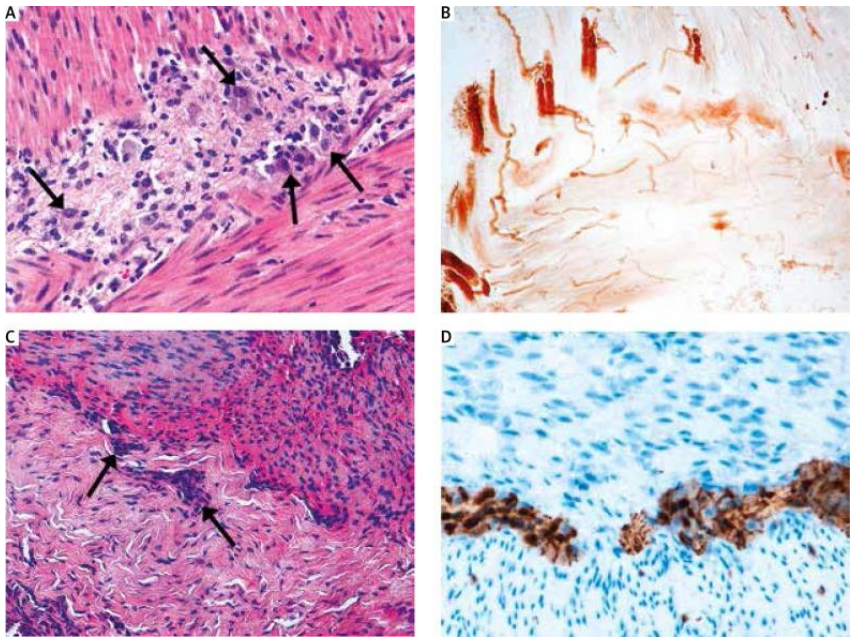
short aganglionic segment (S - HSCR). The aganglionic segment is present in the distal sigmoid and rectum. In 10% of cases, a long aganglionic segment (L - HSCR) can be observed, extending from the rectum, sigmoid and colon to the splenic flexure. The rarest form of the disease with the most severe clinical course is total colonic aganglionosis (TCA), observed in 5% of patients. The last described form of GD is the ultrashort segment (HSCR), in which the aganglionic segment is very short in the anal canal above the pectineal line [9, 10]. The bowel with abnormal innervation does not function with normal motility. This means that the peristaltic wave is not conducted properly and the aganglionic segment is in a state of constant contraction, causing acute or chronic intestinal occlusion. The portion of the intestine above the affected area undergoes significant secondary dilation. In 70–90% of cases, clinical symptoms appear in the first days after birth. If a newborn baby does not pass meconium within 24–48 hours after birth, GD should be suspected [3–6]. About 80% of patients show problems with defecation in the first months of life and, in addition, feeding problems, delayed physical development, significant flatulence and vomiting. Other patients do not show any symptoms until late childhood, when clinical symptoms include chronic constipation, malnutrition and delayed physical development. Some patients may suffer from diarrhea, which may also raise suspicion of severe complications of GD, i.e. Acute enteritis with a 30% mortality rate [8, 9, 12].

Accurate and rapid diagnosis of GD is the key to proper treatment. Diagnostic procedures should be performed in neonates. Without proper treatment at an early age, a significant group of children may suffer from serious complications later in life. These complications include acute enteritis or toxic megacolon [7,9]. The diagnostic performance of different methods used to confirm the clinical suspicion of GD may vary, sometimes resulting in failure to make a correct diagnosis. Imaging methods are useful in the diagnosis of GD. However, their sensitivity has been found to be close to 80% [8, 10]. Abdominal radiograph may show distension of intestinal loops with fluid levels, while contrast colonoscopy with serial radiographs taken on different days may show a cone-shaped portion of intestine, the so-called “transition zone”, an area where the properly innervated intestine (dilated) descends into the aganglionic segment (narrowed). If the "transition zone" is not visible, prolonged evacuation of contrast may raise suspicion of HD, later confirmed by an image taken 24 hours later. The main disadvantage of imaging techniques is their inadequacy in children under 3 months, whereas in TCA and HSCR Radiological images may be normal. Another disadvantage of such methods is the

risk of perforation during contrast administration in patients with acute enteritis [10, 12].

One of the characteristics of patients with GD is their inability to relax the internal anal sphincter in response to stretch, which has been used as a diagnostic feature in anorectal manometry [8, 12]. The test demonstrates 90% sensitivity. Unfortunately, it can only be performed in patients at least 12 months of age, since the internal anal sphincter relaxation reflex may not be developed in infants [7, 11]. Manometry is useful for screening constipation in older children.

The most common non-invasive diagnostic methods, namely radiographic examination and anorectal manometry, are not suitable for neonates. Recently, anal biopsy has been considered an important diagnostic tool with an accuracy of 95% in the diagnosis of GD. Moreover, when additional immunohistochemical studies were performed, it was shown that the correct diagnosis has a very high sensitivity, up to 99.7% [5, 8]. Accurate diagnosis depends on the biopsy site, the representativeness of the samples taken, the number of samples and, finally, the skill of the pathologist. If all criteria are met, the diagnostic sensitivity can even reach 100%. As for histology, the main criterion for the diagnosis of GD is the absence of ganglion cells in the submucosal or intramuscular nerve plexus of the intestinal wall and the presence of hypertrophied nerve fibers and trunks. There are many ways to perform different forms of biopsy to obtain materials for research, for example, transmural, submucosal and seromuscular. Most centers recommend aspiration biopsy, which is considered a simple, safe, rapid and inexpensive method [6, 7]. This technique does not require general anesthesia or surgical suturing. It also avoids many other complications. Due to its high adequacy, simplicity and absence of side effects, aspiration biopsy has become the method of choice in the diagnosis of GD. Standard transmural rectal biopsy is recommended in children after more than one non-diagnostic aspiration biopsy [4]. The key point of the biopsy is the site from which the material was obtained. In children, such material should be obtained at least 2 cm above the pectineal line (Figure 2) [6, 7]. Aldridge and Campbell in 1968 confirmed the presence of an area of a reduced number or even absence of ganglion cells and significant hypertrophy of nerve fibers at a distance of up to 1–2 cm from the edge of the pectineal line [11, 12].



Rice. 2

A – Intramuscular plexus stained with H + E , arrows point to numerous ganglion cells. **B** – Histochemical staining for acetylcholinesterase (ache), larger network of thick, dense and irregular nerve fibers. **C** – Sample taken from the intestinal wall of a newborn with H + E staining , arrows point to irregular, small and immature (dysplastic) ganglion cells. **D** – S 100 staining highlighting the presence of ganglion cells due to the expression of Schwann cells and nerve cells. Primary magnification 20×

If the material is not accurately obtained, on histopathological examination the pathologist may observe the presence of the so-called “anal transition zone” with epithelium different from the colonic epithelium, showing characteristics of squamous epithelial cells (morphology resembling uroepithelium) near the pectineal line. The presence of this area should be described in detail in the histopathological report to avoid a false-positive diagnosis of GD. Aspiration biopsies are more difficult to interpret than conventional biopsies, as they show only the superficial submucosal nerve plexus. Transmural biopsies with the intramuscular nerve plexus present in the specimen present fewer difficulties in interpretation. However, they may be accompanied by some serious complications [8, 10]. Aganglionosis is usually associated with hypertrophy of nerve fibers. H + E staining remains the method of choice for the identification of ganglion cells (Fig. 3A). Conventional biopsy for H + E slide examination requires “only” fixation of the material in buffered formalin and then standard processing, whereas the use of additional histochemical staining for acetylcholinesterase (ache) involves an additional biopsy and freezing

of the obtained material as soon as possible and subsequent processing with a complex procedure. Histochemical staining of frozen tissues for ache demonstrates a larger network of thick, dense and irregular nerve fibers in the muscularis of the segments with lesions (Fig. 3B). Hyperactivity of ache becomes pathognomonic for Hirschsprung disease. Therefore, histochemical staining together with H + E staining is the gold standard in the diagnosis of GD. Recent studies have demonstrated high specificity of ache staining , but with insufficient sensitivity (up to 85%) [2, 4, 5]. False negative results are most often associated with superficial biopsies (without muscularis mucosa), immaturity of the enzyme system (found in patients under 2 years of age), technical variations in staining [2-6], young age of patients (ache activity characteristic of GD is observed in 83% of children under 3 months of age, but then increases with patient age) [1-5], HSCR , TCA [4-6] and Down syndrome [7]. Typical morphological patterns of ache staining characteristic of GD are detected only in the distal colon (below the splenic flexure), since the innervation of this part of the intestine is distinguished by parasympathetic fibers of the spinal cord at the level of segments S2 – S4 .

Thus, diagnostics using ache staining of samples taken from the ascending and transverse colon does not provide reliable information [6-8]. On the one hand, this explains false-negative results in children with TCA - if the results of the initial biopsy are negative and the symptoms do not subside, a repeat biopsy at the age of 3 months is recommended [4]. On the other hand, false-positive results often occur in cases of hemorrhagic lesions in the obtained material. This phenomenon occurs due to the high concentration of ache in erythrocytes [1]. Centanes *et al* . [3, 5, 9] proposed a classification of three ache reactions depending on the age of the patients. In children under 6 months, the presence of thick nerve trunks and fibers is observed only in the muscular mucosa and submucosa. In children over 6 months, an abundant number of nerve fibers is observed in all three layers of the mucosa. The latter form does not dominate in any age group and is characterized by uneven thickening of nerve fibers in all three layers.

Conclusion. The diagnosis of Hirschsprung disease requires close collaboration between clinicians and pathologists. On the one hand, it requires carefully performed biopsies and properly prepared specimens sent to a pathology institute. On the other hand, a pathologist who has doubts about the diagnosis must be able to communicate with the clinician to discuss borderline or difficult cases. We hope that this article will improve the collaboration between these two groups of physicians.

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