Diagnosis of Hirschsprung's disease with particular emphasis on histopathology. A systematic review of current literature

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Abstract

Hirschsprung's disease (HD) is a disorder that involves several medical specialties such as paediatric gastroenterology, paediatric surgery, and pathology. Hirschsprung's disease is a congenital bowel innervation disorder characterised by the absence of ganglion cells in myenteric (Auerbach) and submucosal (Meissner) plexus in the distal colon in its classical form. Rapid and accurate diagnosis of HD is a key element in further treatment patterns. The efficiency of different diagnostic methods used in HD patients may vary. Using one limited diagnostic procedure can lead to as much as a few per cent of overlooked cases. In recent years, rectal biopsy was recognised as an important diagnostic tool that allows for a definitive HD diagnosis with an accuracy of 95% of cases. A correct diagnosis depends on the localisation of the biopsied sample, its representativeness, the number of specimens, and proper interpretation of microscopic studies supported by histochemical and immunohistochemical methods. When several methods are used and all diagnostic criteria are used, the diagnostic sensitivity can almost eliminate cases of undiagnosed patients.

Introduction

Hirschsprung's disease (HD) is one of the diseases that, in the majority of cases, are treated by paediatric surgery. It a congenital condition of intestine innervations leading to a lack of ganglion cells in the area of the Auerbach plexus and Meissner plexus in the distal section of the large intestine. The disease prevalence is estimated at the level of 1 : 5000 live births. The disease more often afflicts male than female patients at a ratio of around 4 : 1 [1, 2]. Its pathogenesis is not fully understood, and various hypotheses have been examined. The most popular of these assumes that the cause of aganglionic intestine is the disorder of target cell migration from the primary neutral tube's neural crest in the intrathecal direction during embryonic development between the 4th and 12th week of pregnancy [3]. As a consequence there are no ganglion cells in the part or along the whole large intestine. Other hypotheses suggest degeneration processes of ganglion cells, abnormal differentiation in changed microenvironment, as well as premature cell necrosis [4]. However, HD in most cases is not a genetic disease. Nowadays, there are eight identified genes that are probably related to the disease. The recent studies are focused on the role of REarranged during Transfection (RET) proto-oncogene on the 10q11.2.2. chromosome, which present in 50% of family cases and in 20% of sporadic cases of the disease occurrence, as well as in some patients with the so-called "long segment" type of HD. The RET proto-oncogene mutations were found in numerous cases of IIA type multiple endocrine neoplasia (MEN) (e.g. medullary thyroid cancer and adrenal tumours). In 30% of cases, HD occurs as one phenomenon among the many others found in congenital defect syndromes, and in 70% of them as an isolated defect. Down's syndrome (trisomy 21 chromosome) is the most common chromosome de-

fect accompanied by HD; it afflicts 10% of patients with Down's syndrome [3, 5, 6]. Other disease that predispose to HD include congenital deafness, hydrocephaly, bladder diverticula, Meckel's diverticula, renal agenesis, cryptorchidism, Waardenburg syndrome (skin pigmentation disorder and deafness), and neuroblastoma [7, 8].

Hirschsprung's disease is classified according to the length of aganglionic section (Figure 1). The most common form, covering 75–80% of cases, is the conventional form with short aganglionic segment (S-HSCR). The aganglionic segment is present in the distal part of the sigmoid colon and rectum. In 10% of cases long aganglionic segment (L-HSCR) can be observed extending from the rectum, sigmoid colon, and colon up to the splenic flexure. The rarest form of the disease with the most severe clinical course is total colonic aganglionosis (TCA) observed in 5% patients. The last described form of HD is ultra short segment (HSCR) in which the aganglionic section is very short in the anal canal above the pectinate line [9, 10].

Intestine with abnormal innervations does not function with normal motility. It means that the peristaltic wave is not properly conducted and the aganglionic segment is in a state of permanent contraction causing acute or chronic occlusion of the bowel. The part of the intestine above the section affected by the lesion undergoes significant secondary dilatation. In 70–90% of cases, clinical symptoms appear in the first days after birth. If a newborn infant does not pass meconium within 24–48 h after birth, HD should be suspected [1, 2, 11]. Around 80% of patients in the first months of their lives demonstrate defecation problems and, additionally, dietary problems, delayed physical development, significant flatulence, and emesis. Other patients do not show any symptoms until late childhood, when the clinical symptoms include chronic constipation, malnutrition, and physical development delay. Some patients may suffer from diarrhoeas that may also raise suspicion of severe complications of HD, i.e. acute enteritis with a 30% fatality rate [12, 13].

Diagnostics

Precise and quick diagnosis of HD is the key to accurate treatment. Diagnostic procedures should be carried out in neonates. Without proper treatment at an early age a significant group of children may suffer from severe complications later in life. These complications include acute enteritis or toxic megacolon [2]. The diagnostic effectiveness of the various methods applied for confirmation of clinical suspicion of HD can fluctuate, sometimes leading to failure of proper diagnosis. Imagining techniques are helpful in HD diagnosis. However, their sensitivity has been found to be close to 80% [14].
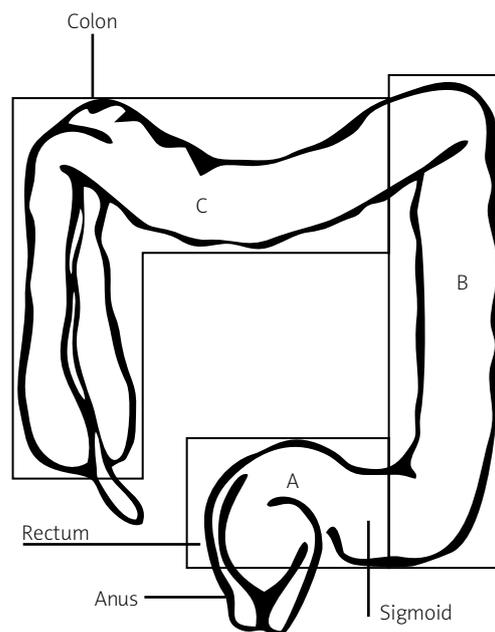

Figure 1. Classification of Hirschsprung's disease according to the aganglionic segment length. A – Conventional form with short aganglionic segment (S-HSCR); A and B – a form with long aganglionic segment (L-HSCR); A, B, and C – total colonic aganglionosis (TCA)

An abdominal radiograph can show intestinal loop distention with fluid levels, whereas contrastive coloclyster with a series of radiographs taken on different days can demonstrate conus-shaped part of intestine, the so-called "transition zone", the section where properly innervated intestine (widened) descends into the aganglionic segment (narrowed). If the "transition zone" is not visible, prolonged contrast evacuation can raise suspicion of HD, confirmed later by an image taken after 24 h. The major disadvantage of imaging techniques is their inadequacy in children under 3 months of age, whereas for TCA and HSCR the radiological images could be normal. Another weakness of such techniques is the risk of perforation during contrast administration in patients with acute enteritis [15, 16].

One of the characteristics of HD patients' is their inability to relax the anal internal sphincter in the response to extension, which was used as a diagnostic finding in anorectal manometry [8]. The test demonstrates 90% sensitivity. Unfortunately, it can only be carried out in patients at least 12 months old because the relax reflex of the anal internal sphincter may not be developed in infants [17]. Manometry is useful for screening in case of constipation in older children.

The most common non-invasive diagnostic methods, namely radiologic imaging and anorectal manom-
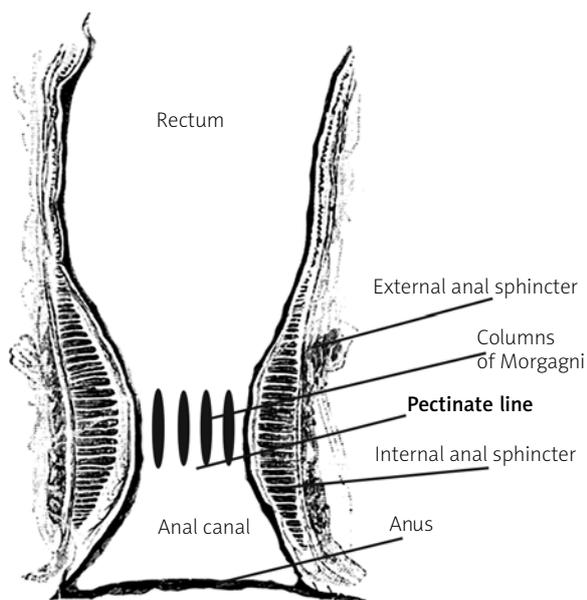
Figure 2. Rectal anatomical structure. The place of obtaining materials – at least 2 cm above the pectinate line

etry, are not options for neonates. Recently, anal biopsy has been considered as the important diagnostic tool, with 95% accuracy in HD diagnosis. Moreover, when additional immunohistochemical studies were carried out, the correct diagnosis was shown to have very high sensitivity, up to 99.7% [18, 19]. Accurate diagnosis depends on the site of biopsy, the representativeness of the samples taken, the number of specimens, and finally the pathologist's skill. If all the criteria are met, diagnostic sensitivity can even reach 100%. As far as histology is concerned, the basic criterion of HD diagnosis is the lack of ganglion cells in the submucosal or intramuscular nerve plexus of the intestinal wall and the presence of hypertrophic nerve fibres and trunks. There are numerous ways of carrying out various forms of biopsy to obtain materials for tests, e.g. transmural, submucosal, and serosal-muscular. Suction biopsy is recommended in most centres, which is believed to be a simple, safe, fast, and inexpensive method [19, 20]. This technique does not require general anaesthesia or surgical suturing. It allows for avoidance of numerous other complications as well. Due to its high adequacy, simplicity, and lack of side effects, suction biopsy has become the method of choice in HD diagnosis. Standard transmural rectal biopsy is recommended in children after more than one non-diagnostic suction biopsy [14]. The key point of the biopsy is the place from which the material was obtained. In children such material must be obtained at least 2 cm above the pectinate line (Figure 2) [16]. Aldridge and Campbell in 1968 con-

firmed the presence of the area of reduced number or even lack of ganglion cells and significant hypertrophy of nerve fibres up to 1–2 cm from the edge of the pectinate line [21].

If the material is not obtained accurately, in the histopathological examination a pathologist can observe the presence of the so-called "anal transition zone" with epithelium different than that from the large intestine, showing characteristics of squamous epithelial cells (morphology resembling uroepithelium) in the proximity of the pectinate line. The presence of this area should be described in detail in the histopathological report to avoid false positive HD diagnosis. Suction biopsies are more difficult to interpret than conventional biopsies because they show only the surface submucosal nerve plexus. Transmural biopsies with intramuscular nerve plexus present in the specimen pose fewer difficulties in interpretation. Nevertheless, they could be accompanied by some serious complications [22]. Aganglionosis is usually connected with hypertrophy of nerve fibres. H + E staining remains the method of choice for identification of ganglion cells (Figure 3 A). Regular biopsies for H + E slides studies require "only" fixation of material in buffered formalin and then standard processing, whereas application of additional histochemical staining for acetylcholinesterase (AChE) is connected with additional biopsy and freezing of the obtained material as soon as possible and then processing through a complicated procedure. Histochemical staining of frozen tissues for AChE demonstrates a larger mesh of thick, dense, and irregular nerve fibres within muscularis mucosa of segments with lesions (Figure 3 B). Hyperactivity of AChE becomes pathognomonic for Hirschsprung's disease. Therefore, histochemical staining together with H + E staining is the gold standard in HD diagnosis. Recent studies have demonstrated the high specificity of AChE staining but with inadequate sensitivity (up to 85%) [23–25]. False negative results are mostly connected with superficial biopsies (without muscularis mucosa), immaturity of the enzyme system (found in patients under the age of 2), technical variations in staining [26], young age of patients (AChE activity characteristic for HD is observed in 83% of children under 3 months but then grows with the patient's age) [24, 27], HSCR, TCA [28, 29], and Down's syndrome [23]. Typical morphological AChE staining pictures characteristic for HD are detected only in the distal part of the large intestine (beneath the splenic flexure) because innervations of this intestinal section are different by parasympathetic fibres of the spinal cord at the S2–S4 segments. Thus, diagnosis applying AChE staining of specimens taken from the ascending colon and transverse colon does not provide reliable information [27, 28, 30]. On
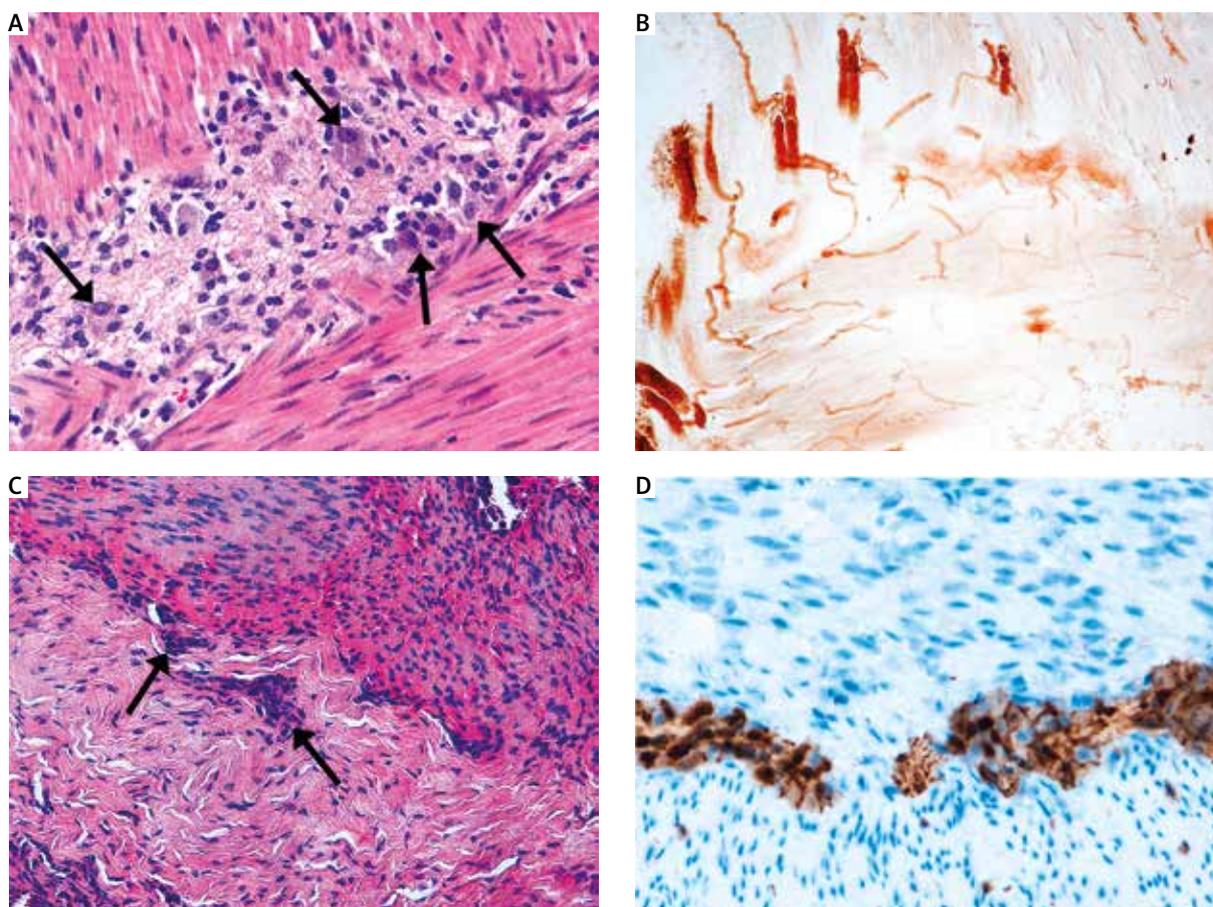
Figure 3. A – Intramuscular nerve plexus in H + E staining, arrows indicate numerous ganglion cells. **B** – Histochemical staining for the presence of acetylcholinesterase (AChE), larger mesh of thick, dense, and irregular nerve fibres. **C** – Specimen taken from the neonate intestinal wall in H + E staining, arrows indicate irregular, small, and immature (dysplastic) ganglion cells. **D** – S100 staining highlighting the presence of ganglion cells by expression of Schwann's cells and nerve cells. Primary magnification 20×

the one hand, it explains the false negative results in children with TCA – if the primary biopsy results are negative and symptoms do not recede, one recommends a second biopsy at the age of 3 months [24]. On the other hand, false positive results are often in cases of haemorrhagic lesions present in the obtained material. Such a phenomenon occurs due to the high concentration of AChE in erythrocytes [30]. Chentanez *et al.* [25] proposed the classification of three AChE reactions according to the patients' age. Children below 6 months demonstrate the presence thick nerve trunks and fibres only in muscular mucosa and submucosa. In children over 6 months there are abundant nerve fibres in all three layers of the mucosa. The last form does not dominate in any age group and it is characterised by irregular thickening of nerve fibres in all three layers.

Pathologists are familiar with the problem of identification of ganglion cells from very small, overly su-

perficial, and mostly technically damaged specimens. Regular H + E staining can demonstrate the presence of various artefacts from the technical processing, which leads to additional interpretation problems. Moreover, the young age of patients is another difficulty. Neonates develop a small number of irregularly placed ganglion cells of very small size, often immature (dysplastic), which leads to problems in differentiation from endothelial cells (newly shaped vessels) and fibroblasts (Figure 3 C) [14]. The aforementioned difficulties contribute to the development of methods that could potentially simplify the HD diagnostic protocol. For this purpose one could perform numerous immunohistochemical studies (based on antibodies against S100, neuron-specific enolase [NSE], microtubule-associated protein-5 [MAP5], glucose transported-1 [GLUT-1], glial fibrillar acidic protein [GFAP], peripherin antibodies, and many others) to simplify and increase the accuracy
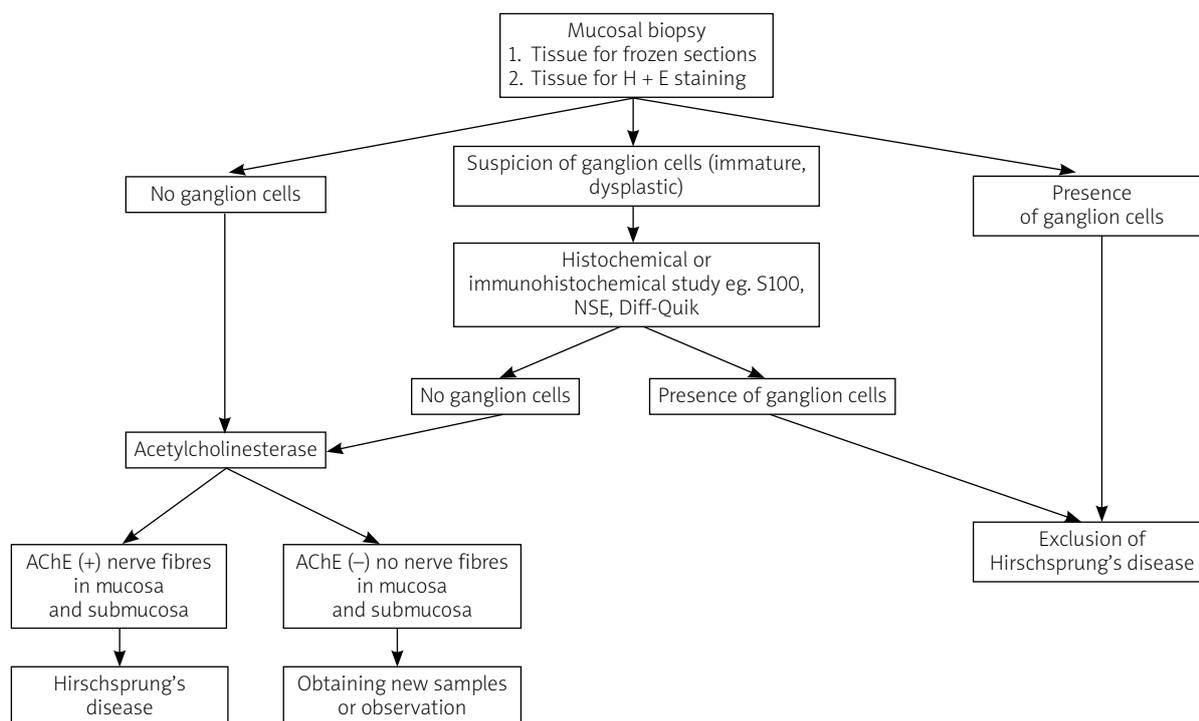
Figure 4. Diagnostic scheme

of ganglion cell identification. Neuron-specific enolase antibody demonstrates intensive expression in ganglions, which makes detection of small and immature cells easier, whereas S100 highlights the presence of ganglion cells by Schwann cell expression (Figure 3 D) [31]. Another recommended diagnostic method is histochemical staining with the Diff-Quik method. Ganglion cells show cytoplasm light blue colour. The method is easily accessible, inexpensive, and can be performed on frozen tissues directly.

Diagnostic scheme

Regular procedures should cover biopsies of two specimens from each suspicious intestinal section. One is for standard H + E staining and the other should be frozen for further AChE determination (Figure 4). If microscope examination confirms the presence of ganglion cells, one can reject HD. In other cases one should evaluate AChE reaction. The presence of thickened mucosal and submucosal nerve fibres confirms definitely HD. If the characteristic findings of AChE staining are not met, clinicians should consider obtaining additional specimens and should observe the patient. If problems with identification of ganglion cells occur in H + E slides, one has to perform immunohistochemical or histochemical tests (S100, NSE, Diff-Quik). Depending on the results, clinicians could recommend further treatment according to the aforementioned rules.

Summary

Hirschsprung's disease diagnostics requires close cooperation between clinicians and pathologists. On the one hand, it requires an accurately performed biopsy and properly prepared samples being sent to a pathology institute. On the other hand, a pathologist having diagnostics doubts should be able to communicate with a clinician to discuss borderline or complicated cases. We hope that the present paper enhances cooperation between those two groups of doctors.

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