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MODERN CLINICAL, INSTRUMENTAL, AND MORPHOLOGICAL APPROACHES TO THE EARLY DIAGNOSIS OF HIRSCHSPRUNG DISEASE IN CHILDREN

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Abstract

Background. Hirschsprung disease is a congenital developmental disorder characterized by the absence of enteric ganglion cells in the distal intestine, resulting in functional intestinal obstruction. The clinical presentation of the disease depends on the length of the aganglionic segment and the age of the child and may range from acute intestinal obstruction in the neonatal period to chronic constipation in older children. In some cases, the nonspecific nature of the clinical manifestations contributes to delayed diagnosis.

Objective. To analyze, based on contemporary scientific literature, the diagnostic capabilities and limitations of clinical signs, contrast enema, anorectal manometry, rectal biopsy, histochemical, and immunohistochemical examinations in the early diagnosis of Hirschsprung disease in children.

Materials and Methods. A targeted narrative literature review was conducted. Priority was given to scientific studies indexed in PubMed/MEDLINE, international clinical guidelines, systematic reviews, meta-analyses, and multicenter studies. Major diagnostically relevant sources published between 2006 and 2025, as well as fundamental studies addressing the pathogenesis of the disease, were analyzed.

Results. Delayed passage of meconium, abdominal distension, bilious vomiting, and persistent constipation beginning early in life are the principal clinical features that raise suspicion of Hirschsprung disease. The mean sensitivity and specificity of contrast enema are 70% and 83%, respectively, while those of anorectal manometry are 91% and 94%, respectively. In a large systematic review, rectal suction biopsy demonstrated a mean sensitivity of 96.84% and a specificity of 99.42%. Nevertheless, the reliability of biopsy findings depends on the presence of an adequate amount of submucosa in the specimen and on the quality of morphological assessment. In a recent meta-analysis of calretinin immunohistochemistry, sensitivity was 98% and specificity was 99%.

Conclusion. Early diagnosis of Hirschsprung disease should not rely on a single diagnostic test but should be based on timely clinical suspicion, targeted use of instrumental diagnostic methods, and confirmation of the diagnosis through histopathological examination of a representative rectal biopsy specimen. Calretinin immunohistochemistry is an important ancillary method that improves diagnostic accuracy, particularly when conventional histological examination yields equivocal findings.

Keywords: Hirschsprung disease, children, infants, constipation, intestinal obstruction, contrast enema, anorectal manometry, rectal biopsy, aganglionosis, calretinin.

Introduction. Hirschsprung disease is a congenital developmental disorder of the enteric nervous system characterized by the absence of ganglion cells in the submucosal and myenteric nerve plexuses of the distal intestine. During embryogenesis, disruption of the migration, proliferation, differentiation, or survival of neural crest-derived cells along the intestine results in the formation of an aganglionic segment of variable length [1]. The absence of ganglion cells

impairs normal relaxation and propulsive peristalsis of the distal intestinal segment. Consequently, functional distal obstruction develops, while progressive intestinal dilatation and megacolon may subsequently occur in the proximal segment.

Hirschsprung disease occurs, on average, in approximately 1 in 5,000 live births and is relatively more common in boys [1]. The clinical presentation of the disease depends on the length of the aganglionic segment, the age of the patient, and the presence of complications such as enterocolitis. The classic manifestations of Hirschsprung disease during the neonatal period include delayed passage of meconium, abdominal distension, and bilious vomiting. The 2024 Chinese expert consensus identified failure to pass meconium within the first 24 hours after birth as an important clinical sign warranting suspicion of Hirschsprung disease [2].

At the same time, the disease may not be diagnosed during the neonatal period in all patients. Particularly in short-segment aganglionosis, symptoms may be less pronounced, and the child may present for several months or years with chronic constipation, abdominal distension, and a persistent need for enemas or laxatives. In such cases, misinterpretation of the condition as functional constipation may lead to delayed diagnosis. The clinical importance of timely diagnosis of Hirschsprung disease is not limited to determining the timing of surgical treatment. Prolonged distal obstruction, fecal stasis, and Hirschsprung-associated enterocolitis may aggravate the clinical course of the disease and worsen the child's general condition.

Objective of the study. To analyze, on the basis of contemporary scientific evidence, the capabilities, diagnostic accuracy, and limitations of modern clinical, radiological, functional, and morphological diagnostic methods used for the early detection of Hirschsprung disease in children.

Materials and methods. The present study was conducted as a targeted narrative literature review. The literature search covered publications available up to December 2025.

To identify the principal scientific evidence, articles indexed in PubMed/MEDLINE, international expert consensus statements, ERNICA clinical guidelines, systematic reviews, meta-analyses, and multicenter clinical studies were analyzed.

Source selection was based on the principle of prioritizing the highest available level of evidence relevant to diagnostic decision-making, including clinical guidelines, systematic reviews, meta-analyses, and multicenter studies.

Results.

Establishing Clinical Suspicion of Hirschsprung Disease

The analysis of the literature indicates that the most important stage in the early diagnosis of Hirschsprung disease is timely clinical suspicion of the condition.

During the neonatal period, the following signs are diagnostically important:

- failure or delay in the passage of meconium within the first 24–48 hours after birth;
- progressive abdominal distension;
- bilious vomiting;
- signs of functional distal intestinal obstruction;
- absence of spontaneous defecation;
- forceful passage of large amounts of gas and stool after rectal examination or stimulation.

The 2024 expert consensus emphasized that delayed passage of meconium beyond 24 hours is observed in a substantial proportion of patients with Hirschsprung disease and that the disease should be actively excluded in such cases [2]. Digital rectal examination helps exclude anorectal malformations and assess the condition of the distal bowel. In Hirschsprung disease, the rectal ampulla is often empty, and forceful expulsion of gas and stool may occur after withdrawal of the examining finger. However, this sign is not pathognomonic for the disease.

In older children, the age at onset of constipation is diagnostically important. Persistent constipation beginning during the first weeks or months of life, abdominal distension, difficulty with spontaneous defecation, and a regular need for enemas require exclusion of organic pathology, including Hirschsprung disease.

Contrast Enema. Contrast enema allows evaluation of colonic anatomy and the probable transition zone in children with suspected Hirschsprung disease. The classic radiological feature of the disease is the transition zone between the distally narrowed aganglionic bowel and the proximally dilated ganglionic segment.

Additional findings include:

- an abnormal rectosigmoid index;
- dilatation of proximal intestinal segments;
- changes in the bowel wall and contours;
- delayed evacuation of the contrast medium.

In the systematic review by de Lorijn et al., contrast enema demonstrated a mean sensitivity of 70% (95% confidence interval, 64-76%) and a specificity of 83% (74-90%) [3]. These findings indicate that contrast enema is an important anatomical diagnostic examination; however, it is insufficient as a standalone method for either confirming or excluding Hirschsprung disease.

Particularly in neonates and in patients with long-segment or total colonic aganglionosis, the classic transition zone may not be clearly defined. Therefore, a normal contrast enema does not definitively exclude Hirschsprung disease.

Anorectal Manometry. The principal role of anorectal manometry in the diagnosis of Hirschsprung disease is to evaluate the rectoanal inhibitory reflex (RAIR). In a healthy child, distension of the rectum with a balloon causes reflex relaxation of the internal anal sphincter. In Hirschsprung disease, this reflex is usually absent because of aganglionosis. In a systematic review, anorectal manometry demonstrated a mean sensitivity of 91% (85-95%) and a specificity of 94% (89-97%) [3]. However, the diagnostic accuracy of the test depends on the child's age, examination technique, catheter type, patient movement, sedation status, and the experience of the specialist interpreting the findings. Absence of the RAIR raises strong suspicion of Hirschsprung disease, but anorectal manometry cannot replace morphological confirmation of the diagnosis.

Rectal Biopsy and Histopathological Confirmation. Definitive confirmation of Hirschsprung disease is based on histopathological examination of a representative rectal biopsy specimen [2,4].

In clinical practice, the following biopsy techniques are primarily used:

1. rectal suction biopsy;
2. punch biopsy;
3. open or full-thickness rectal biopsy.

Rectal suction biopsy is widely used in infants and young children because of its minimally invasive nature. However, the diagnostic specimen must contain an adequate amount of submucosal tissue. In the systematic review by Friedmacher and Puri, which included 58 studies and 14,053 rectal suction biopsies, the mean sensitivity of this method was 96.84%, while its specificity was 99.42% [5]. At the same time, specimens were inadequate for diagnosis in 10.07% of cases, and repeat biopsy was required in 8.46% [5]. Thus, the mere fact that a biopsy has been performed is insufficient; specimen quality is one of the principal determinants of diagnostic reliability.

In a 2023 study involving five centers, 69 of 82 biopsies, or 84.2%, were suitable for a conclusive diagnostic interpretation, whereas 13 biopsies, or 15.8%, were inconclusive [7]. The main cause of inconclusive results in suction biopsies was an insufficient amount of submucosal

tissue. Therefore, when an inconclusive result is obtained, specimen quality should be reassessed, and repeat biopsy or full-thickness biopsy should be considered when necessary. The principal morphological criterion for Hirschsprung disease is the absence of ganglion cells in the corresponding nerve plexuses. Hypertrophied nerve fibers may serve as an additional diagnostic feature [4].

Histochemical and Immunohistochemical Diagnosis. Standard hematoxylin and eosin staining allows the diagnosis to be established in many cases. However, particularly in neonates, ganglion cells may be small and morphologically immature. This increases the risk of misinterpretation [4]. Acetylcholinesterase histochemistry has been used for many years to assess nerve fiber activity in the aganglionic segment.

Calretinin immunohistochemistry helps identify ganglion cells and certain nerve fibers in the normal intestine. The absence of calretinin-positive nerve fibers in the aganglionic segment is considered a finding supportive of Hirschsprung disease. In the systematic review and meta-analysis by Claxton et al., which included 107 studies and 10,891 children, the pooled sensitivity of calretinin immunohistochemistry was 98%, and the pooled specificity was 99% [8]. In the same meta-analysis, acetylcholinesterase demonstrated a sensitivity of 94% and a specificity of 99% [8]. Although these results demonstrate the high diagnostic value of calretinin, the authors noted heterogeneity in the quality of the included studies. Therefore, immunohistochemical examination does not replace an adequate biopsy specimen and evaluation by an experienced pathologist.

Comparative Characteristics of Diagnostic Methods

Diagnostic method	Main diagnostic criterion	Sensitivity	Specificity	Main advantage	Important limitation
Contrast enema	Transition zone and colonic anatomy	70%	83%	Minimally invasive and provides anatomical information	A normal examination does not exclude the disease
Anorectal manometry	Absence of the RAIR	91%	94%	Functional assessment	Depends on age and methodology
Rectal suction biopsy	Morphological identification of aganglionosis	96.84%	99.42%	Enables definitive diagnosis	An adequate amount of submucosa may not be obtained
Calretinin IHC	Absence of calretinin-positive nerve fibers	98%	99%	High diagnostic accuracy in complex histological cases	Does not replace biopsy as a standalone test

Note: Because these values were obtained from different systematic reviews and meta-analyses, they should not be interpreted as a direct head-to-head comparison within a single study.

Diagnostic Algorithm Based on Synthesis of the Literature.

Based on the synthesis of the analyzed international guidelines, systematic reviews, and diagnostic studies, the following practical sequence is considered appropriate:

Stage 1. Clinical suspicion. Delayed passage of meconium, abdominal distension, bilious vomiting, and constipation present from birth or the first months of life are identified.

Stage 2. Physical and anorectal examination. The abdomen, perineum, and anal opening are assessed. Anorectal malformations are excluded.

Stage 3. Instrumental assessment. Depending on the clinical circumstances, contrast enema is used to assess colonic anatomy and the transition zone, while anorectal manometry is used to evaluate the RAIR.

Stage 4. Morphological confirmation. If a justified clinical or instrumental suspicion of Hirschsprung disease persists, a representative rectal biopsy specimen is obtained and subjected to histopathological examination.

Stage 5. Clarification of inconclusive findings. If the specimen is inadequate or the morphological conclusion is inconclusive, the tissue preparation should be reassessed by an experienced pathologist, additional methods such as calretinin and/or AChE should be considered, and repeat or full-thickness biopsy should be performed when necessary.

This algorithm is not a verbatim reproduction of any individual international guideline but represents a practical diagnostic sequence developed through synthesis of the analyzed evidence.

Discussion. The principal problem in the early diagnosis of Hirschsprung disease is not the absence of diagnostic tests but rather delayed clinical suspicion and misinterpretation of the diagnostic capabilities of different investigations. Although delayed passage of meconium is an important “red flag” during the neonatal period, its absence does not completely exclude Hirschsprung disease. Likewise, chronic constipation alone is not a specific sign in older children; diagnostically, the timing of symptom onset is particularly important.

Contrast enema remains a useful method for obtaining anatomical information in Hirschsprung disease. However, its sensitivity and specificity reported in systematic reviews are lower than those of morphological methods. Therefore, failure to identify a transition zone cannot scientifically be equated with the conclusion that “Hirschsprung disease is absent” [3]. Anorectal manometry has higher pooled diagnostic performance than contrast enema, and the presence of the RAIR may provide important evidence against Hirschsprung disease, whereas absence of the RAIR strengthens the need for morphological confirmation. However, particularly in young children, manometric findings are sensitive to methodological and technical conditions.

Rectal biopsy is a decisive step in the diagnostic algorithm. However, it is incorrect to interpret biopsy automatically as a “100% accurate test.” Systematic reviews have also reported inadequate specimens and the need for repeat biopsy [5]. Multicenter clinical data likewise demonstrate that one of the principal causes of diagnostic uncertainty is an insufficient amount of submucosal tissue in the biopsy specimen [7]. Therefore, diagnostic quality depends on three factors: obtaining the biopsy from the correct level, ensuring an adequate volume of tissue, and having the specimen evaluated by a specialist experienced in the pathology of Hirschsprung disease.

Calretinin immunohistochemistry is a promising, highly accurate ancillary component of modern morphological diagnosis. In a 2025 meta-analysis, its pooled sensitivity and specificity were 98% and 99%, respectively [8]. At the same time, these figures do not provide grounds for interpreting calretinin as a standalone screening method that can replace biopsy.

Updated ERNICA recommendations also maintain the central role of histopathological diagnosis in the management of Hirschsprung disease [9]. The consequences of delayed diagnosis are complex. In the study by Ullrich et al., which included 679 patients from 14 centers, age at diagnosis was not associated with 30-day postoperative complications or the need for revision of the pull-through procedure [10]. However, children diagnosed at an older age were more likely to subsequently require fecal diversion and additional interventions related to constipation or fecal incontinence.

Therefore, the general conclusion that “delayed diagnosis worsens surgical outcomes in all patients” is not fully supported by the available evidence. A more accurate conclusion is that early diagnosis is important for timely recognition of prolonged obstructive symptoms, recurrent

hospitalizations, and disease-associated enterocolitis. Hirschsprung-associated enterocolitis is a serious complication that may develop both before and after surgery. Its clinical manifestations include abdominal distension, diarrhea, fever, vomiting, lethargy, and deterioration of the general condition [11]. Delaying diagnosis in such patients may be dangerous.

Thus, the available scientific evidence supports an approach based on the principle of clinical suspicion → targeted instrumental assessment → representative biopsy → histopathological confirmation, rather than the concept of a single "best diagnostic test" for Hirschsprung disease.

Conclusion.

1. The first and most important step in the early diagnosis of Hirschsprung disease is the timely establishment of clinical suspicion. Delayed passage of meconium, abdominal distension, bilious vomiting, and constipation beginning early in life require active exclusion of the disease.
2. Although contrast enema and anorectal manometry are important instrumental investigations, neither can independently provide definitive confirmation of Hirschsprung disease or completely exclude it.
3. Definitive confirmation of Hirschsprung disease is based on histopathological examination of a representative rectal biopsy specimen. The presence of an adequate amount of submucosa in the biopsy specimen is critically important for diagnostic reliability.
4. Calretinin immunohistochemistry is an ancillary morphological method with high diagnostic accuracy and is particularly useful when the results of conventional histological examination are inconclusive.
5. The most evidence-based diagnostic approach consists of the stepwise integration of clinical, radiological, functional, and morphological data, thereby reducing the risk of diagnostic errors and unjustified exclusion of the disease.

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