Constipation in (early) infancy and childhood : pathogenesis and diagnostic procedures

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Chapter 7

Diagnosis of Hirschsprung's disease; a prospective, comparative accuracy study of common tests

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Abstract

Background

Full thickness biopsy is considered the gold standard for Hirschsprung's disease (HD), however, it is invasive and requires general anesthesia. Whether other tests can replace full thickness biopsy is unclear.

Aim

To compare the diagnostic accuracy of contrast enema (CE), anorectal manometry (ARM) and rectal suction biopsy (RSB) for the detection of HD.

Methods

Following a prospective protocol, infants suspected of HD underwent all three index tests. Children with two or more positive index test results or children, who continued to have severe bowel problems, underwent a full thickness biopsy as reference standard. Clinical follow-up was the reference standard in all other children.

Results

Between 2000 and 2003, 111 consecutive patients (67 boys, median age: 5.3 months) suspected of HD were enrolled. HD was found in 28 out of 111 patients. RSB had the highest sensitivity (93%) and specificity (100%), but values were not significantly different from CE (sensitivity=76%; specificity=97%) and from ARM (sensitivity=83%; specificity=93%). Inconclusive test results occurred in 8 infants with CE, in 15 infants with ARM due to agitation and in 2 infants with RSB .

Conclusion

Rectal suction biopsy is the most accurate test to diagnose Hirschsprung's disease, and has the lowest rate of inconclusive test results.

Introduction

In the majority of infants and children with constipation, no obvious cause can be identified. A rare cause of constipation is Hirschsprung's disease (HD). HD is characterized by the absence of ganglion cells from the anal rectum to a variable length up to the duodenum. The extent of the aganglionic segment varies, but in most patients the lesion does not extend beyond the rectum and sigmoid colon. In a few cases however, the aganglionosis may involve the colon and in extremely rare cases up to the duodenum[1]. The clinical symptoms of HD may become manifest in the neonatal period, which include lack of passage or delayed passage of meconium beyond 24 hours, and signs and symptoms of large bowel obstruction such as biliary vomiting, a distended abdomen, severe defecation problems and/ or enterocolitis.

Several tests, based on different features of the condition, are employed in the work-up of patients suspected for HD. The presence of a caliber change, with a dilated normal colon to a narrowed aganglionic bowel may be demonstrated by contrast enema (CE). Anorectal manometry (ARM) assesses the recto-anal inhibitory reflex (RAIR), which is absent in children with HD. The third option consists of a rectal suction biopsy (RSB), which shows an elevated cholinesterase activity and may even show aganglionosis in case of HD. Since RSB is a superficial biopsy, it is not always possible to detect with certainty the presence or absence of ganglion cells. Therefore, all positive RSB test results were confirmed with a full-thickness biopsy (FTB) which contains rectal mucosa and underlying muscle. This test provides the most definitive answer, but is invasive and requires general anesthesia.

There has been considerable debate about the most appropriate diagnostic approach for HD, since CE, ARM and RSB all produce false-negative and false-positive test results[2,3]. Each of these tests has both advantages and disadvantages in terms of availability, technical difficulty, radiation exposure, and invasiveness. The diagnostic accuracy of each investigation has never been compared directly in a prospective study. Therefore the aim of our study was to compare the diagnostic accuracy of contrast enema, anorectal manometry and rectal suction biopsy in infants suspected of HD.

Methods

Between 2000 and 2003, we enrolled 122 consecutive patients suspected of HD in our prospective study. All patients had severe defecation problems from birth and abdominal distension and / or could not be weaned off from laxative treatment. Data regarding gestational age and first passage of meconium after birth were collected. All patients were referred to our outpatient paediatric motility clinic in a tertiary medical hospital by paediatricians or paediatric surgeons to exclude or confirm HD. Anorectal manometry was performed in our outpatient paediatric motility unit. The CEs were performed at the paediatric radiology suite and a paediatric surgeon, mostly on an outpatient basis, performed all RSB's. CE, ARM and RSB were performed in arbitrary order, based on the availability and planning situation in the hospital. Eleven out of the 122 infants did not undergo all investigations and were therefore excluded from the study. Reasons not to perform all three tests were: no permission given by the parents to perform all three tests in 4 infants; parents who did not show up for one or more investigations in 4 infants. RSB was considered too invasive due to prematurity in 2 children and due to autoimmune hepatitis in 1 child. In all other 111 children the three tests were performed within 3 weeks. The investigators were informed about the clinical status of the patients but did not know the outcome of the other tests. All data were prospectively collected on pre-designed case record forms.

Contrast enema

Paediatric radiologists or residents performed the radiological examination of the colon in a routine manner using standard CE techniques. Children did not have bowel preparation prior to the CE. A diluted (1:3) hyperosmolar water-soluble contrast medium (amidotrizoic acid) was administered rectally. A small rectal catheter was used and placed in the rectum as distally as possible. No balloon catheters were used. All CE images were read by the same paediatric radiologist (AS). The classical finding in patients with HD is that of a caliber change between a small or normal-sized distal aganglionic segment and a dilated proximal ganglionic bowel[4]. The presence or absence of this caliber change was considered to be a positive or negative test result, respectively.

Anorectal manometry

In all children anorectal perfusion manometry, using a purpose-built silicone rubber micromanometric anorectal catheter (od 2.0 mm), was performed after bowel preparation with an enema. The catheter incorporated a 1.5-cm-long sleeve sensor and an array of 3 side-holes spaced 0.5 cm apart for measurement of anal sphincter pressures and 1 side-hole located 0.5 cm proximal of the sleeve for measurement of basal pressure within the rectum. All side-holes were perfused with sterile degassed water at a rate of 0.2 mL/min. An air channel was present on the tip of the catheter. Rectal distension was produced with a highly compliant 4-cm-long distending rectal balloon, tied at the end of the catheter.

The catheter was positioned with the sleeve straddling the anal sphincter high-pressure zone and the balloon in the rectum. To elicit a RAIR, 2 to 60 mL of air was insufflated in the balloon for rectal distension. The reflex was defined to be normal when rectal distension produced a relaxation of the anal sphincter pressure of at least 5 mmHg over 2 to 5 seconds. When the RAIR was observed 3 times, it was concluded that ARM could not support the diagnosis of HD. Recording sessions lasted 30 minutes on average. Measurements were performed and assessed by FdL and MB.

Rectal suction biopsy

In all patients 4 RSBs were taken at 4 different sites: 2 and 4 cm from the dentate line anteriorly, and 2 and 4 cm from the dentate line posteriorly. The suction biopsy specimens were examined for routine histology and for acetylcholinesterase (AchE) histochemistry. Bowel specimens were fixed in 4% formaldehyde buffered with phosphate-buffered saline (PBS), dehydrated, and embedded in paraffin. Tissue sections were made at 5µm, stretched, and dried at 57°C overnight. The sections were rehydrated in an alcohol series, endogenous peroxidase activity was blocked by 1% H_2O_2 in methanol for 1 hour.

Acetylcholinesterase activity was determined as previously described by Karnovsky and Roots[5]. Nonspecific acetylcholinesterase was inhibited by 2.5 x 10^6 mol/L tetra-isopropylpyrophosphoramide (ISO-OMPA)[6]. Ganglion cells were determined with a hematoxylin-eosin staining. A RSB was considered positive if the acetylcholinesterase activity was elevated in combination with an absence of ganglion cells. If ganglion cells were present, HD was excluded. The biopsies were

evaluated by one experienced histopathologist with specific interest and expertise for HD (FtC).

Final diagnosis

The final diagnosis of HD was made by the absence of ganglion cells in a full thickness biopsy or from the operative specimen, or was rejected by thoroughly clinical follow-up including a hospital visit to confirm the disappearance of complaints. Children with two or more positive index test results or children who continued to have severe complaints despite intensive laxative treatment during follow-up were verified through a full thickness biopsy (n=32). In all other children clinical follow-up was used to demonstrate the disappearance of complaints. The minimum duration of follow-up was six months.

Full thickness biopsy specimens were obtained under general anesthesia by a paediatric surgeon at 2 cm above the dental line, posteriorly. These specimens were handled in a routine manner in the pathology laboratory of our tertiary center. Full thickness specimens were examined for the presence or absence of ganglion cells in Auerbach's plexus located between the longitudinal and circular muscle layers of the bowel wall. The same histopathologist examined all sections.

Statistical analysis

Sensitivity and specificity were calculated for all three index tests together with 95% confidence interval using the method of Wilson[7]. We tested for differences in sensitivity, specificity and inconclusive results between tests by using the McNemar test for paired observations[8]. The diagnostic odds ratio was used to test whether the overall accuracy of an index test differed between subgroups of patients (young versus old). Patients with inconclusive test results were excluded in the calculation and comparison of sensitivity, specificity, and diagnostic odds ratio. In all analyses, two-sided P-values less than 0.05 were considered statistically significant.

Results

Table 1 outlines the patient characteristics. Of the 111 children included in the study, 67 (60%) were boys. The median age of the total group was 5.3 months (range 4 days-12 years). A final diagnosis of HD was made in 28 (25.2 %) children. The other 83 children were classified as having functional constipation. The median age at intake was significantly lower in children with HD compared to children with functional constipation. As expected, significantly more children with HD had a delay in meconium passage compared to children with functional constipation.

A total of 28 patients showed two or more positive index test results. In all 28 children, HD was confirmed by FTB. In four patients, however, less than 2 index tests were positive for HD. Since these patients presented with such severe and acute defecation problems laparotomy was necessary to relieve colonic distension. During the surgical procedure a FTB was performed, which definitely excluded HD in these 4 infants. In 21 out of the total of 28 infants with HD (75%) the length of the aganglionic segment was limited to the rectosigmoid. In 5 patients (18%) the aganglionic segment extended to the splenic flexure and in 1 patient to the ascending colon. Only 1 patient had a total aganglionosis coli. The children who did not undergo a FTB were followed up for at least 6 months. After these 6 months 68% of these children developed a normal defecation pattern without the use of laxatives, 9% had a normal defecation pattern with the use of laxatives while mild defecation problems needing oral laxatives persisted in 23% of the children. In these latter 32% of children, RSB had been normal.

Contrast enema

Table 1. Patient characteristics.

	Total group (N = 111)	HD (N = 28)	FC (N = 83)	P-value HD vs FC
Percentage of boys	60%	68%	58%	p=0.35
Median age at intake in months	5.3	4.0	6.0	p=0.01
< 1 month (%)	19%	39%	12%	
1-6 months (%)	33%	18%	37%	
6-12 months (%)	16%	29%	12%	
≥ 12 months (%)	32%	14%	39%	
Prematurity	17%	4%	22%	p=0.03
Delayed meconium passage (>24 hours)	54%	81%	44%	p=0.001
Median defecation frequency/week (range)	2.0 (0.0-49.0)	0.5 (0.0-24.0)	2.0 (0.0-49.0)	p=0.30

HD=Hirschsprung's disease, FC=functional constipation

Table 2 shows the sensitivity and specificity for each index test. A caliber change was seen in 19 out of 28 patients (68%) with HD. No caliber change was observed in 6 patients with histological proven HD. Five of them were younger than 1 year. In the other 3 patients, CE was inconclusive for technical reasons: the catheter was not removed from the rectum during the recording period, and / or too fast injection of contrast which sometimes leads to overdistension of the bowel. Furthermore, a caliber change was present in 2 patients, both younger than 1 year, with functional constipation. In eight infants, CE was non-conclusive, mostly because of technical failures as mentioned above. No side effects occurred during or after the CE procedures. The sensitivity and specificity of a CE was 76% and 97%, respectively. The overall accuracy of a CE is both not significantly different in infants <1 months compared to infants ≥1 month (100% versus 90%, p=0.27) or in children >1 year compared to younger children (94% versus 89% respectively, p=0.21).

Anorectal manometry

A positive test (absence of the RAIR) was found in 19 children out of the 28 children with HD. Surprisingly, 4 children with histological proven HD showed a relaxation of the IAS upon balloon distension. Three of them were younger than 1 month. This resulted in a sensitivity of 83%. In 15 infants the results of ARM could not reliably be analyzed due to agitation during the procedure, five of these patients turned out to have HD. Even after inflation of up to 60 ml of air in the rectal balloon, 5 children with functional constipation showed no reflex relaxation of the sphincter complex leading to a specificity of 93% (95% CI 0.85-0.97). No side effects occurred during or after the ARM procedures. The accuracy of ARM was not significantly different in infants younger than 1 month compared to infants ≥1 month (<1 month = 84%, ≥1 month = 93%, p=0.70) or in children older than 1 year compared to children ≤1 year (94% versus 90% respectively, p= 0.47).

Rectal suction biopsy

A positive biopsy was found in 25 out of 28 children with HD. Two children, both younger than 1 month, had a false-negative test result for RSB. Although the RSB in these infants showed a normal acetylcholinesterase activity, no ganglion cells could be identified. In 1 child the RSB was not conclusive and 1 more inconclusive RSB result occurred in a patient without HD. In both inconclusive cases the biopsy

was too superficial and did not contain muscularis mucosa. RSB produced no false-positive test results. One patient had rectal blood loss after the RSB procedure, but no surgical re-examination was needed to stop this. This meant that the sensitivity and specificity of RSB was respectively 93% (95% CI 0.77-0.98) and 100% (95% CI 0.96-1.00), excluding the 2 patients with inconclusive test results. The accuracy of RSB was 90% in infants <1 month and 100% in infants ≥1 month (p=0.11) whereas the accuracy was 100% in children >1 year and 97% in children ≤1 year (p=0.32).

Direct comparison of accuracy

Table 2 shows a raw overview of test results per indextest. The pairwise comparison of sensitivity and specificity between different tests showed that RSB had the highest sensitivity (93%), but it was not significantly different from CE (76%, p=0.29) and from ARM (83%, p=0.69). The specificity of the RSB was higher (100%) compared to CE (97%, p=0.50) and ARM (93%, p=0.06).

Table 2. Raw data of test results per index test and comparison of sensitivity, specificity and inconclusive tests between contrast enema, anorectal manometry and rectal suction biopsy in patients suspected of Hirschsprung's disease.

	Contrast enema	Anorectal manometry	Rectal suction biopsy	CE vs ARM p-value	CE vs RSB p-value	ARM vs RSB p-value
Sensitivity (95% CI)	76% (57-89%)	83% (63-93%)	93% (77-98%)	1.00	0.29	0.69
TP / (TP+FN)	19 / (19+6)	19 / (19+4)	25 / (25+2)			
Specificity (95% CI)	97% (91-99%)	93% (85-97%)	100% (96-100%)	0.22	0.50	0.06
TN / (TN+FP)	73 / (73+2)	68 / (68+5)	82 / (82+0)			
Inconclusive - no. (%)	11 (9.9%)	15 (13.5%)	2 (1.8%)	0.17	0.11	0.002

TP=true positive test result; FN=false negative test result; TN=true negative test result; FP=false positive test result; CI=confidence interval, CE=contrast enema, ARM=anorectal manometry, RSB=rectal suction biopsy

Discussion

In this prospective study we compared the accuracy of contrast enema, anorectal manometry and rectal suction biopsy in patients suspected of Hirschsprung's disease (HD). Our results demonstrate that rectal suction biopsy (RSB) is the most accurate test in diagnosing Hirschsprung's disease with a sensitivity and specificity of 93% and 100%, respectively. This was however, not significantly different from contrast enema (CE) (76% and 97% respectively) and anorectal manometry (ARM) (83% and 93% respectively). RSB also had the lowest rate of inconclusive test results and provides a histological diagnosis.

In accordance with the literature, approximately 75% of the infants with HD were boys[1]. Furthermore, as also shown in our study, up to 80-90% of the infants with HD fail to pass meconium in the first 24 hours of life[1]. Delayed passage of meconium by itself, seems not a good discriminative clinical symptom to differentiate between HD and functional constipation, as 30-40% of children with functional constipation and approximately 30% of healthy preterm infants have delayed meconium production[9,10]. In patients presenting with delayed passage of meconium and other important clinical signs of HD such as vomiting and abdominal distension, however, rapid diagnostic tests are necessary. Severe intestinal obstruction leading to enterocolitis, remains the commonest cause of morbidity and mortality in HD[1].

The hallmark to diagnose HD using a CE is the presence of a caliber change[10]. In accordance with earlier studies[10-12], no caliber change was found in 6 patients with HD (30%), of which 5 were younger than 1 year and in 1 patient with total aganglionosis coli. Taxman et al suggested that in young infants the caliber change is more difficult to demonstrate[10]. False-negative test results are also reported in 75% of children with total aganglionosis[13,14]. Furthermore, rectal wash-outs and even digital rectal examinations may decompress the distended proximal bowel with distortion of the caliber change leading to false-negative test results[4]. We have no explanation why false-positive test results were found in 2 patients with functional constipation, both younger that 1 year of age. Long-term follow-up of these patients revealed no defecation problems thereby excluding HD. Although the CE is a simple test to perform, the radiation exposure is high and an experienced paediatric radiologist is essential to evaluate and score the x-ray results.

The literature contains conflicting data about the accuracy of anorectal manometry in neonates. Some studies reported that the RAIR does not occur in

premature or term infants until the 12th day after birth because of physiologic immaturity of anorectal function[15,16]. We demonstrated that premature infants older than 26 weeks' postmenstrual age have a well developed RAIR upon rectal distension[17,18]. In this study, 5 infants, of which 3 were < 1 year old, without HD showed an absence of the RAIR upon anorectal manometry (false positive test results). False-positive test results might be caused by insufficient inflation of the balloon. We used a maximum volume of 60mL, which might not have been enough to distend the rectum in some children, because of a congenital megarectum or a megarectum due to severe constipation. Furthermore, technical factors such as an air leak in the circuit might cause false-positive test results. False-negative test results were found in 4 infants, of which 3 were <1 month. In a study by Aaronson and Nixon[19], 26% of the patients with a final diagnosis of HD showed a normal RAIR. The authors attributed these false-negative results to displacement of the transducer probe with side hole sensors. Since the use of a sleeve sensor which allows for sphincter movement and pressure measurement over the length of the sleeve, the displacement can be avoided[17]. Therefore, it seems unlikely that our false results are due to displacement of the probe. Furthermore, they proposed that some of the false-negative results might be a consequence of relaxation of the external anal sphincter rather than the internal anal sphincter. We have no other explanation why the RAIR was present in those 4 children with HD.

Due to agitation during the procedure we were not able to reliably analyze the manometry tracings in a substantial number of patients. In order to improve cooperation it might have been helpful to perform anorectal manometry under mild sedation or anesthesia in these young infants. A recent study showed that the RAIR could be elicited even when anorectal manometry was performed under general anaesthesia[20].

Anorectal manometry is a non-invasive diagnostic test and is easy to perform in children older than 1 year of age. It therefore has often been suggested as an ideal screening tool. However, the equipment is expensive and extensive experience is necessary to perform this procedure to evaluate the test-results in infants younger than 1 year of age.

In our study, rectal suction biopsy (RSB) produced no false-positive test results. Two false-negative test results were found both in patients younger than 1 month old. Hamoudi et al[21] described up to 29% of false-negative test results in children

with HD, whereas others[22,23] reported that nearly 100% diagnostic accuracy was achieved with acetylcholinesterase histochemistry. Possible causes for false-negative test results include: variability in the biopsy site, too superficial taken biopsy material that lacks muscularis mucosa, immaturity of the enzyme system, technical variations in performance of the stain and the experience of individual pathologists[4]. Both infants with false-negative test results in our study continued to have severe bowel problems which needed daily bowel irrigation. Because of the ongoing clinical symptoms, despite intensive medical treatment, a FTB was performed which indeed showed HD in both infants. A RSB is relatively simple, efficient and incident-free procedure, although rectal bleeding, perforation or sepsis caused by RSB has been described[10,24].

To the best of our knowledge, no study has been performed in which CE, ARM and RSB were compared prospectively. A drawback of this study is that FTB was not performed in all children to make the final diagnosis. We considered it unethical to perform a FTB in all children, since it is an invasive procedure that requires general anesthesia. Therefore, a thorough clinical follow-up, using a standardized questionnaire to monitor the symptoms of constipation was carried out for each patient for at least 6 months. Another drawback of this study is that despite the large series of patients in the study (n=111), the number of patients with HD is still low (n=28). This means that the power to detect differences in sensitivity between the tests is still small.

In conclusion, RSB is a reliable quick and simple test that provides a tissue diagnosis and was the most accurate test to diagnose Hirschsprung's disease. A negative test result virtually rules out the disease when the tissue is obtained from the correct site and when the specimen contains at least a small amount of muscularis mucosa. If symptoms still strongly suggest the diagnosis of HD, we prefer to perform ARM, before repeating the RSB. If there is no ARM experience in the hospital, RSB should be repeated. In our opinion the value of a CE in the work up of HD is limited because of its fairly low sensitivity and high radiation exposure. Only if the diagnosis HD has been established, CE might be helpful for the surgeon to assess the localization of the caliber change and thus the length of the aganglionic segment.

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