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Should the search for ganglia in the distal rectal fistula in patients with anorectal malformation be abandoned?☆,☆☆

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ABSTRACT

Purpose: Occurrence of Hirschsprung's disease in anorectal malformation (ARM) patients is rare, but many surgeons still ask to pathologists to search for ganglia in the terminal rectum/fistula; the histological procedure is time and money consuming and the results confounding. A consecutive series of ARM patients, in which the presence of ganglia in terminal rectum was revised, is herein presented.
Materials and methods: Rectal specimens of ARM patients who underwent corrective surgery in the last 6 years were retrieved. The histological protocol included H&E staining and calretinin immunohistochemistry. Each specimen is processed until all material is examined if no ganglia are retrieved after the first twelve sections.
Results: Forty cases were examined. Eight patients were younger than 1 month of age at operation. The mean length of the specimen was 1.5cm (range: 1–3 cm). Upon clinical request, ganglia were searched in 15/40 cases (37.5%) and resulted absent in 10/15 (66.5%). All patients have been followed and none developed signs or symptoms suggestive for Hirschsprung.
Conclusions: The practice to search for ganglia in the terminal rectum/fistula in ARM patients should be abandoned, as incidence of associated colorectal diseases is rare. Moreover, the procedure is expensive both in terms of laboratory's reagents and working time of expert pathologists and technicians.
Level of Evidence: Level IV (Case Series with no Comparison Group)

Anorectal malformation (ARM) and Hirschsprung disease (HD) are the most common low gastrointestinal congenital anomalies that cause intestinal obstruction at birth. ARM is a spectrum of congenital malformations caused by the abnormal development of the anus and rectum occurring during embryonic life [1]. HD is a clinical condition characterized by bowel aganglionosis from the anus to variable proximal extent [2]. The estimated incidence of both conditions is 1 in 2500-5000 live births, without significant gender differences for ARM and male preponderance for HD [3,4]. Correlation between ARM and HD is very rare and reported in few studies, mainly single center series [5–9]. Nevertheless, many pediatric surgeons consider screening for HD in ARM patience of great importance and routinely suggest rectal biopsy at the time of pull-trough to search for ganglia, despite the absence of clinical HD signs [6,9].

Aim of this study is to support the evidence the practice to search for ganglia in the terminal rectum/fistula of ARM patients should be avoided because of the potential for misinterpretation and waste of human and technical resources.

1. Materials and methods

Patients with ARM who underwent corrective surgery during the last 6 years at the Pediatric Surgery of Ca'Foncello Hospital of Treviso were included in the present analysis. For each case, specimen of fistula or terminal rectum trimmed before performing the anoplasty was obtained during the pull-through procedure and sent to pathologists. Pathology reports were retrieved to find out how many samples underwent systemic search for ganglia upon surgeon request.

The protocol adopted by pathologists consisted in an extensive examination of the material by a two steps procedure, routinely used for HD diagnosis for both small biopsies and larger samples [10,11]. After the evaluation of sample adequacy, a first set of twelve 3-5μm sections stained with hematoxylin and eosin (H&E) and a final section for Calretinin (DAK-Calret1, prediluted, Agilent Dako) immunohistochemistry is obtained. If ganglia are retrieved, no further analysis is required and HD is ruled out. If no ganglia are retrieved, all the material (or up to 100 slides) is examined with H&E and calretinin, together with evaluation of neural hypertrophy (nerve fibers diameter >40 μm).

When ganglia research was not specifically requested by surgeons, exclusively an H&E morphologic revision was performed.

Abbreviations: ARM, anorectal malformation; HD, Hirschsprung disease; H&E, hematoxylin and eosin; ATZ, anal transition zone.

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2. Results

A total of 40 patients, 21 females and 19 males, mean age at surgery 4.9 months (range: 3 days–3.25 years) were retrieved. According to the Krickenbeck classification [12], patients showed the following types of ARM: 47.5% perineal fistula (n = 19 patients), 15% recto-vestibular fistula (n = 6), 15% recto-urethral fistula (n = 3 prostatic and 3 bulbar), 7.5% recto-vesical (n = 3), 7.5% without fistula (n = 3), the remaining 7.5% (n = 3) were one each of H-type recto-vaginal fistula, anal stenosis and covered cloacal exstrophy. Eight infants were younger than 1 month. All patients were treated with a more or less extended PSARP, accordingly to the severity of the malformation. The patient with a covered cloacal exstrophy required a laparotomic approach to correct the defects.

Mean specimen's length was 1.5 cm (range: 1-3 cm) and in 37.5% of cases (15/40) the surgeon asked to search for ganglia. A normal pattern of ganglia was present in 33.5% of these selected cases (5/15) while in 66.5% (10/15) a pattern of aganglionosis was detected (Fig. 1). These latter cases belonged to 1 patient with Down Syndrome and ARM without fistula, 2 with recto-vestibular fistula (1 of them with vaginal agenesis), 5 with perineal fistula, and 2 with recto-urethral fistula (prostatic and bulbar, respectively). In the other 62.5% of cases (25/40), when ganglia research was not specifically requested by surgeons, pathologists found absence of ganglia in 11 patients, after exclusively H&E examination. None of the aganglionic specimen showed other histological findings, such as thickness of submucosal nerve trunks, nor clinical signs typical of Hirschsprung's disease.

In all the cases, in spite of the histopathological absence of ganglia, no further diagnostic investigations were performed, nor treatments suggested. It was considered that the specimen belonged to the anal transition zone (ATZ), a short tract of terminal rectum where ganglia can be physiologically absent. The patients were regularly followed in the outpatient colorectal clinic, as well as every other ARM patient who undergoes surgery in our center. No one developed signs and symptoms of HD during a mean follow-up of 3.5 ± 2.1 years, such as abdominal distension and severe constipation not responding to bowel management, failure to thrive, and enterocolitis, nor required surgical treatment for constipation; no significant differences concerning the bowel function were found between the two groups of patients.

3. Discussion

ARM and HD are commonly associated with other congenital anomalies and malformations [13], but the occurrence of both conditions in the same patient is quite rare (from 2.3% to 3.4%) and few cases have been described in literature. In a recent systematic review, the Authors included 38 studies published from 1952 to 2013 – 28 single case reports and 10 case series of 2,465 ARM patients - with a result of 90 cases without gender predominance [14]. Twenty-three cases were syndromic patients, mostly affected from Currarino and Down syndromes. The type of ARM was reported in 59 patients, with a higher incidence in males without fistula (n = 15) and females with recto-vestibular fistula (n = 11). The diagnosis of HD was delayed by a median of 8 months from the diagnosis of ARM. Indeed, the classical HD clinical signs typically seen in newborns, such as abdominal distension and delayed passage of meconium, were masked by ARM or misinterpreted as ARM post-operative complications. In very few patients, the clinical suspicion for HD was supposed before ARM corrective surgery because of a not functioning stoma created in an aganglionic colonic tract. In these patients the simultaneous correction of both defects was performed [8]. However, in the majority of cases this was not possible and diagnosis of HD was delayed after pull-through procedure. It was based on

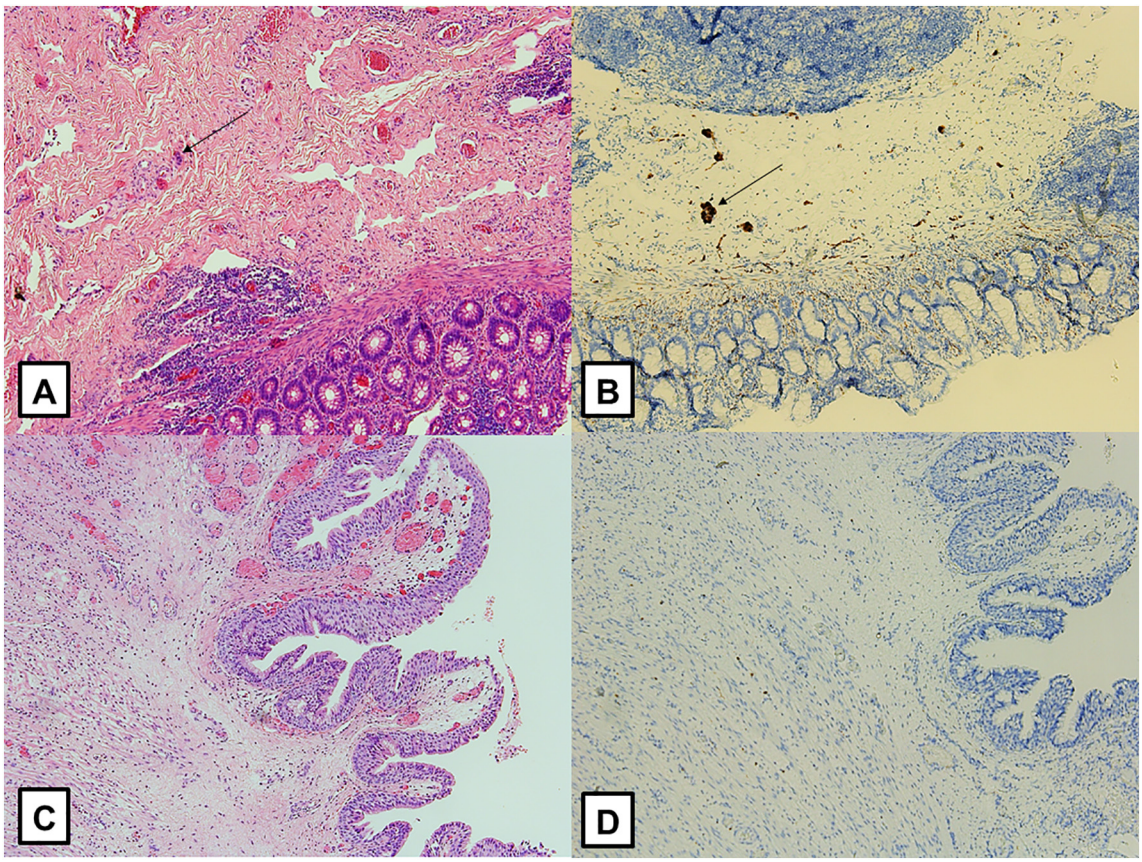

Fig. 1. Two cases of ARM (A-B and C-D) in which ganglia were searched in the resected distal rectum/fistula. Case 1: A (H&E) and B (Calretinin stain): presence of ganglia in submucosa (arrow). Case 2: C (H&E) and D (Calretinin stain): no ganglia in submucosa. 100× magnification. Mast cells are shown as internal controls for calretinin.

rectal biopsies taken as signs and symptoms developed [6,9]. The follow-up of only 20% patients was detailed and 88% of them were continent.

For all these reasons, several surgeons still perform rectal biopsies during colostomy or pull-through procedure and ask the pathologists to process them for ganglia in ARM patients. According to our database, in the last 6 years 40 ARM patients were operated, but in only 37.5% of cases the surgeon asked to search for ganglia. The decision appeared to be based upon surgeon preference and not on clinical conditions. In the present analysis, for the 10 patients without ganglia after specific histopathological protocol examination, a wait and see approach was adopted, avoiding harmful over treatments. The same approach was assumed for all the remaining cases, where the retrospective histopathological analysis highlighted the morphological absence of ganglia in further 11 cases, supporting our position about the usefulness of ganglia search in all ARM patients.

Abnormalities of the innervation pattern in the terminal rectum and fistula are commonly seen in samples taken during pull-through procedure. Holschneider et al. examined the terminal rectum/fistula of 52 patients and in 96% different abnormalities were detected, such as aganglionosis, hypoganglionosis, disganglionosis, and neuronal intestinal dysplasia type B [15]. In particular, all fistulas resulted aganglionic without identifiable nerve structures. Eighty-four percent of patients were regularly followed-up and the majority did not manifest any pathological clinical condition. The minority (25%) developed severe constipation, but no information about further surgery is reported and the Authors concluded the bowel function does not necessarily correlate with histology.

In general, the ganglion cells are normally absent 1-2 cm above the dentate line, that is within the anal transition zone [16]. ATZ is the middle portion of the surgical anal canal, where the anal squamous epithelium gradually changes into the colorectal type mucosa. Normally, the ATZ starts from the dentate line and extends for almost 0.6-2 cm upward [17]. Considering the fistula/terminal rectum of ARM patients usually contains the typical structures of the ATZ, ganglion cells may be physiologically absent if specimen are sampled from these areas, independently of the severity of malformation (Fig. 2).

Different views are expressed by Raboei [6], Poeanru et al. [7], Nmadu et al. [9], who always recommend rectal biopsy at the time of pull-through since they consider the association of ARM and HD well described in literature and not as rare as commonly thought. However, only the first author specified the prevalence of ARM and HD association found in his center, that is 3/53 cases (5.66%), almost twice what is reported in the literature [6]. He justifies this high percentage with the consanguinity of all his patients, that may had increased the incidence of malformations, and the presence of 2 patients with Down syndrome.

Regarding our cohort of patients, all of them - including those without ganglia - had been regularly followed up in the dedicated outpatient clinic and no one developed HD symptoms. As described, the most common clinical presentation of HD after the ARM pull-through is persistent constipation and abdominal distension refractive to bowel management [9], along with episodes of sub-acute obstruction relieved only by colonic decompression [6], development of necrotizing enterocolitis [6], or clinical pattern resembling HD without radiological signs [8]. None of our patients developed such signs and symptoms, therefore the interpretation of “aganglionosis” in the rectal specimen/fistula can be explained as part of the physiological absence of ganglia in the terminal rectum or as temporary immaturity of ganglia. Hence, the presence of constipation in ARM patients, if not associated to the typical clinical features of HD previously mentioned, should be interpreted as a dysmotility pattern that characterizes the anorectal malformations, rather than the possibility of HD. Therefore, a wait-and-see approach together with an appropriate bowel management, results in a wise decision that avoids to over treat these patients with colon resection. This practice, indeed, could lead to disastrous consequences in terms of fecal continence and bowel management, considering that in ARM patients the more colon is preserved the better the prognosis is [18]. Before the PSARP era, some surgeons performed Hirschsprung's type pull-through to correct the anorectal malformation [19] and this practice had led to incontinence in some patients not only for the loss of the rectal reservoir, in addition to the lack of a true anal canal and deficient sphincters, but also for the critical role that the large bowel plays in the physiologic continence, such as to slow the stool transit and absorb the excess of water [20]

Furthermore, the histopathological process to search for ganglia is very time and cost consuming, requiring also the aid of dedicated specialists with specific expertise in the field in order to avoid potential false positive HD-diagnosis related to anatomic variations, tissue handling, and tissue specific characteristics.

4. Conclusion

In our opinion, the systematic search for ganglia in all specimens of distal rectum/fistula of ARM patients should be avoided, because of

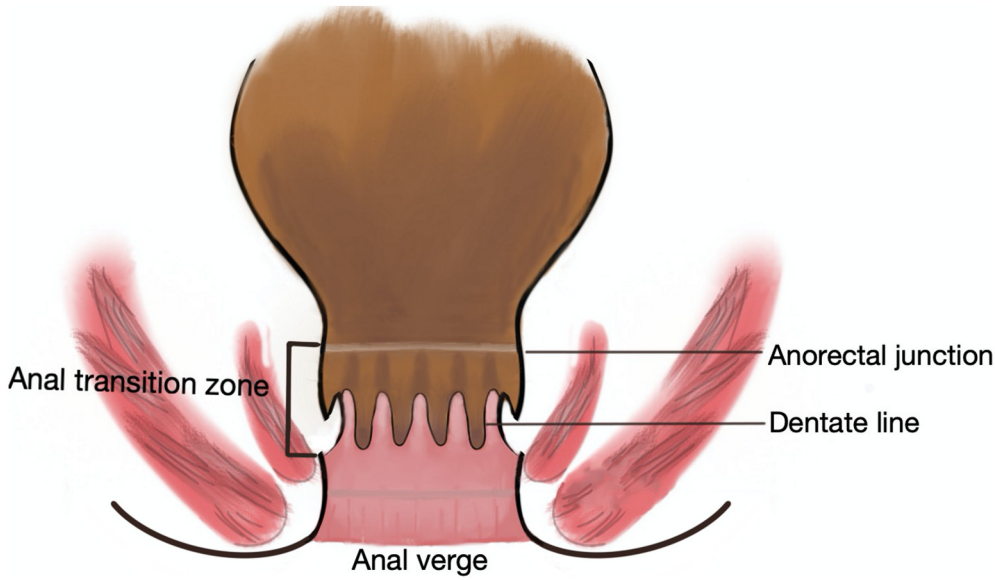

Fig. 2. Schematic graphic representation of the anal canal and the anal transition zone.

the extreme rarity of coexistent HD in ARM, the potential of misinterpretation with serious consequences, and, last but not least, the costs in terms of laboratory resources. The procedure should be considered only in selected ARM patients with a malfunctioning stoma, familial history of HD, Down syndrome, and consanguinity.

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