Hirschprung’s disease in different settings – a series of three cases from a tertiary referral center

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Abstract
Failure of neural crest cells to migrate from neural crests during intrauterine development result in partial or total aganglionosis of the colon in newborn. Hirschsprung’s disease (HD) represents the clinical manifestation of this pathogenic process, currently accounting for the majority of lower intestinal obstruction in the first period of life. Our aim was to present a series of three cases presenting to our tertiary care center with a range of symptoms, all benefiting from surgery and consequent pathology examination of biopsy or resection pieces. The first case was of a male newborn that presented several years ago with common symptoms for HD (abdominal distension, vomiting and the total lack of intestinal passage for feces). Coming from young healthy parents after normal labor, the newborn displayed signs of Down’s disease after physical examination. After abdominal radiography, the patient underwent surgery and consecutive pathology revealed notable signs of Crohn’s disease (CD): massive stasis in the serosa and submucosa, chronic inflammatory infiltrate and lack of nervous cells in both plexuses and mucosa. Immunohistochemistry revealed low intensity CD34 membrane staining for fibroblast-like ganglion cells while CD117 staining showed few nervous cells within the mucosa. The second case presented before one year of age with an infectious background, already being operated upon with colostoma. We performed corrective surgery of the colostoma and consecutive pathology showed low CD117 cytoplasmic staining and intensely positive NSE (neuron specific enolase) staining within myenteric plexuses. Finally, the third and most recent case was that of a 4-year-old boy with an early diagnosis of megacolon and no previous surgery, who we evaluated by laparoscopy with five biopsies and consecutive S100 staining revealed a small number of nervous cells within nervous plexuses. In conclusion, an early diagnosis of HD is essential for successful therapeutic measures. Histology and, more recently, immunohistochemistry, represent the gold-standard procedures needed to objectify the diagnosis.

Keywords: Hirschsprung’s disease, surgical approach, immunohistochemistry, pathology.

Introduction
Hirschsprung’s disease (HD), the congenital megacolon, is considered to be the most common cause of lower intestinal obstruction in newborn, caused by the failure of neural crest cells to migrate and form normal plexuses and bowel innervation [1–3]. This phenomenon may affect variable portions of the colon, from the internal anal sphincter and proximally extending. It is considered to be more prevalent in males, with an incidence of approximately one in 500 live births [1, 3]. The affected length is usually up to the rectosigmoid (75% of all cases) while it may be confused to shorter segments (rectum) or parts of the rectum. It can also affect longer segments or even the entire colon [1, 2]. During diagnostic imaging, the proximal portion of the intestine appears dilated, followed by either gradual or sudden transition to a normal caliber, as an effort to overcome the partial obstruction [4]. This is a process that accentuates in time, therefore older patients display hypertrophy of the colon musculature as well as increased dilation [4, 5]. Common symptoms are pathognomonic since childbirth and early signs point to the disease; complications are frequent and the condition should be diagnosed early on, benefiting from surgical treatment that may overcome direct implications of the disease.

Pathology examination of biopsy or resection pieces is extremely important in characterizing HD and it may be required for adequate treatment and ongoing management, as well as future measures [6–8].

HD is strongly associated with Down’s syndrome, as roughly 5 to 15% of all HD patients also have trisomy 21 [9–11]. The disease etiology is still unknown; however, several genetic factors have been identified, as well as a family history in approximately 10% of cases [1, 12]. Other anomalies include those of the cardiovascular, gastrointestinal or urogenital system [1].
Aim

Considering the impact of the disease on the survival and quality of life of the infant, we present here our experience in a series of three cases diagnosed with congenital megacolon who benefited from surgery in our service. We present a wide range of symptoms and presentation features, along with a comprehensive pathology description and immunohistochemistry assessment following both open surgery and laparoscopic approach.

Case reports

We present below a series of three cases with congenital megacolon who presented in our Pediatrics Clinic between 2011 and 2014.

Case No. 1

The first case is of a male newborn who first presented on November 2011 with abdominal distension, vomiting and complete absence of fecal passage. Both parents were less than 30-year-old, employed and with no notable illnesses. He was the first child, born naturally with an Apgar score of 9 and weighing 2.8 kg. Upon birth, the patient presented Mongoloid face, Down syndrome stigmata – cutis laxa, full-moon face, low implanted ears and patient presented Mongoloid face, Down syndrome stigmata. The abdomen and one alimentary vomiting with fecaloid aspect. Venous perfusions and antibiotherapy. During the course and superficial breathing, necessitating incubation, endo-racial cyanosis and hypotonia, protrusion of the tongue deformed nasal pyramid. After birth, he presented gene– cutis laxa, full-moon face, low implanted ears and patient presented Mongoloid face, Down syndrome stigmata. The abdomen and one alimentary vomiting with fecaloid aspect. A nasogastric probe was placed and gastric washing was performed (fecaloid residue) with rectal probing.

The abdominal radiography did not show pneumoperitoneum, with hydroaeric levels on the small bowel at the lower and middle abdominal level. No laboratory alterations were recorded at that time.

Surgery revealed the total absence of intestinal ganglia between the anus and the sigmoid; thus, colostoma was performed on the superior third of the sigmoid, in a dilated area, evacuating normal meconium. No sigmoid resection was performed at that time as the newborn presented in an altered state, with cyanosis and pulmonary symptoms.

The newborn returned in February 2012 for a stenosis of the colostoma. Physical examination revealed an enlarged abdomen with erythema adjacent of the colostoma. Surgical retouch was performed, with positive outcome. The patient received antibiotherapy and was dismissed.

Two years later, in March 2014, the baby returned for further surgery; barium transit showed a normal aspect from the colostoma, situated at the level of the splenic flexure and up to the distal portion of the descending colon where a smaller caliber appears. We did not encounter any laboratory abnormalities thus a third surgical intervention was performed with Duhamel type abdominal pelvic descent. The post-operative follow-up was positive, with an improvement in both number and consistency of stools.

The pathology exam revealed massive stasis in the serosa and submucosa of the colic wall and an extensive edema, with no ganglia cells at the level of the myenteric plexus. Chronic inflammatory infiltrate with follicular placement was encountered at this level, with mucosal ulcerations and atrophy of the external muscularis. Lymph follicles were enlarged, with adjacent stasis adipose tissue and total absence of nervous cells. We could observe on one of the biopsy pieces small groups of 3–4 nervous cells where a submucosal plexus should have been situated and generalized atrophy of the muscle fibers of the external muscularis.

Immunohistochemistry revealed low intensity CD34 marking of the membranes of fibroblast-like cells within the small, degenerate ganglions that were present, intensely diffuse and showing upon quantitative analysis the paucity of these cells (Figures 1–3). Ganglions appeared disorganized, with a less than normal population of nervous cells. Further CD117 immunostaining (Figures 4 and 5) revealed positive cytoplasmic marking of nervous cells within nervous plexuses with various types of aplasia and structural abnormalities.

Case No. 2

The second case was of a 10 months baby girl that presented in April 2014, being previously diagnosed with congenital megacolon for which cecostomia was performed eight and a half months prior to the presentation in our service. The baby presented for abdominal discomfort and transit alterations, febrile (38°C) and an altered state, with rhinorrhea and congestive pharynx. Upon clinical examination, the girl presented with discrete sensibility at the lower point of the post-operative scar where painful tumefactions, erythema and the aspect of a suture granuloma were observed. Intestinal transit was present on both colostoma and anus. Laboratory identified leukocytosis (11 600/mm³). Medical supportive treatment and antibiotherapy were instituted.

Surgery was performed, suturing the colostoma, cecostomy, peritoneal drainage and appendicectomy. The post-operative evolution was favorable.

Microscopic evaluation of surgical pieces revealed stasis through the intestinal wall, lymphoplasmocytary inflammatory infiltrate with follicular disposition in some parts and submucosal sclerosis. Few degenerated ganglia cells were identified within the myenteric plexus.

Following immunohistochemical evaluation, we found atrophic ganglia with low CD117 cytoplasmic staining (Figure 6), as well as intensely positive NSE (neuron specific enolase) staining of cells within submucosal and myenteric plexuses (Figures 7 and 8).

Case No. 3

The third case was of a 4-year-old boy who presented for investigations in our center in January 2014. He was diagnosed with congenital megacolon during a previous check-up exam in a different clinic and currently presented protein caloric malnutrition. Laboratory values were as follows: hemoglobin 12 g%, 10 400/mm³ leukocytes, 360 000/mm³ thrombocytes, Quick time 114%, APTT (activated partial thromboplastin time) 24 seconds, urea 36 mg/dL, creatinine 0.23 mg/dL, AST (aspartate transaminase) 27 U/L, ALT (alanine transaminase) 10 U/L, serum ionogram – Na⁺ 138 mEq/L, Cl⁻ 107 mEq/L.
We performed laparoscopy, observing a narrowed rectosigmoidian section followed by an enlargement of the descendent colon. We performed five retrograde biopsies from the rectum, which were sent for pathology. The evolution was positive and no events were reported by the family until present.

Figure 1 – Anti-CD34 positive immunostaining of membranes of ganglion fibroblast-like cells. Anti-CD34 antibody, ×200.

Figure 2 – Low intensity CD34 positive immunostaining of membranes of ganglion fibroblast-like cells. Anti-CD34 antibody, ×200.

Figure 3 – Low intensity, diffuse CD34 positive immunostaining of membranes of ganglion cells. Anti-CD34 antibody, ×100.

Figure 4 – CD117 positive immunostaining within the cytoplasm of nervous cells of nervous plexuses. Anti-CD117 antibody, ×100.

Figure 5 – Low intensity CD117 positive immunostaining within the cytoplasm of nervous cells of nervous plexuses. Anti-CD117 antibody, ×100.

Figure 6 – Disorganized structure of ganglions, shown by low intensity CD117 positive immunostaining within the cytoplasm of nervous cells. Anti-CD117 antibody, ×100.
Microscopy of all five biopsies revealed atrophic muscularis and the degenerescence of smooth muscle fibers with groups of 4–6 ganglia cells with atrophic, degenerative aspect. Further immunohistochemistry involving NSE staining revealed the degenerated myenteric plexuses with atrophied cells, much reduced in both number and volume when compared with normal portions (Figures 9 and 10). We have identified positive cytoplasmic staining for S100 within nervous cells of such a plexus, again showing degenerative aspects and cellular paucity (Figures 11–13).
common features include the absence (or pronounced recurrent symptomatology. in diet and feeding habits due to previous surgery and colon [14]. Constipation was a symptom we have encoun-
tered in our cases and were early alarm signs for disease since first admission in our service. We did not encounter severe complications such as spontaneous perforations, which is reported in 3% of cases and is correlated with the extent of the disease, or toxic megaco-
lon [14]. Constipation was a symptom we have encountered in the older case, which we attributed to changes in diet and feeding habits due to previous surgery and common recurrent symptomatology.

The definite diagnostic test is the pathological examination of biopsy pieces from the rectal and affected areas; common features include the absence (or pronounced atrophy) of ganglionic cells, limited number of adrenergic fibers and subsequent changes in the musculature development as well as the other layers of the colonic mucosa [1, 6–8]. In our patients, we have identified common elements of such alterations, as well as immunohisto-
chemical features common to nervous cells of ganglia and nerve fibers within the intestinal wall.

Several papers in recent literature underline the importance of immunohistochemistry in proper diagnosis and characterization of HD cases; lack of CD117 and CD34 expression is a primary alteration of nervous cells in these cases [6–8]. Our results confirm this findings; usual staining also reflect the severe alterations that the remaining nervous cells display upon pathology examination.

Another issue with the young patients who suffer from HD is the subsequent alteration in the quality of life; surgical intervention is highly invasive and alters the physiology of bowel functions [15, 16]. Indeed, our cases presented with several issues after surgery; the first two benefited from colostoma with subsequent complications, which are inherent in this invasive procedure. Our first case underwent several readmissions in the first four years of life, with a low quality of life and high risk of infections complications; the second case, of a younger patient, showed how surgical complications can appear as fast as the first months after it being performed. Finally, in the third case we present alternative surgical interventions that can be performed, as well as different management in relation to age.

**Conclusion**

Early diagnosis of HD is essential for successful therapeutic measures. Both clinical presentations as well as standard tests are useful in establishing the necessary criteria for disease. Histology and, more recently, immunohistochemistry, represent the gold-standard procedures needed to objectify the diagnosis. The outcome is often dependent on the success of the surgical procedures instituted at an early stage.

**Conflict of interests**

The authors declare that they have no conflict of interests.

**Author contribution**

All the authors contributed equally to preparing this paper and share first authorship.

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**Figure 13 – Weakly positive S100 immunostaining present in some neuronal cells. Anti-NSE antibody, ×100.**


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