ORIGINAL PAPER

The evaluation of interstitial Cajal cells distribution in non-tumoral colon disorders

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Abstract

Interstitial cells of Cajal (ICC) are pacemakers that generate electric waves recorded from the gut and are important for intestinal motility. The aim of the study was to evaluate the distribution of interstitial cells of Cajal in colon specimens from patients with idiopathic chronic pseudo-obstruction and other non-tumoral colon disorders as compared with samples from normal colon. The distribution pattern of ICC in the normal and pathological human colon was evaluated by immunohistochemistry using antibodies for CD117, CD34, and S-100. In two cases with intestinal chronic idiopathic pseudo-obstruction we found a diffuse or focal reducing number of Cajal cells, the loss of immunoreactivity for CD117 being correlated with loss of immunoreactivity for CD34 marker. Our study revealed that the number of interstitial cells of Cajal also decrease in colonic diverticular disease and Crohn disease ($p<0.05$), whereas the number of enteric neurones appears to be normal. These findings might explain some of the large bowel motor abnormalities known to occur in these disorders. Interstitial Cajal cells may play an important role in pathogenesis and staining for CD117 on transmural intestinal surgical biopsies could allow a more extensive diagnosis in evaluation of chronic intestinal pseudo-obstruction.

***Keywords*:** Cajal interstitial cells, bowel motility, CD117.

Introduction

Ramon y Cajal described Cajal interstitial cell a century ago, like a primitive neuron of the bowel. In the present, it is characterized like a cell with immunophenotype and ultrastructural features of a smooth muscle cell and neural cell. It is a part of networks distributed in intramuscular and intermuscular submucosal layers of digestive tract, from the esophagus to internal anal sphincter, and has a role of pacemaker in motility of gastrointestinal tract.

Two big plexus – myenteric plexus Auerbach and submucous plexus Meissner, which contain different types of distinct functional neurons, compose enteric nervous system. These include ganglion cells and glial cells connected with Cajal interstitial cells.

In the last years, there were multilevel researches (histology, immunohistochemistry, electron microscopy and molecular biology) in order to obtain a complex picture of Cajal cell physiopathology, to create an algorithm of diagnosis, evolution and prognostic of non-tumoral obstructive intestinal diseases.

Interstitial Cajal cells were described along the digestive tract, using many basic ultrastructural criteria for their identification, proposed by Huizinga JD *et al.* [1] and added under the name of "gold standard". At this level, cells have the main role of pacemaker, which generate and propagate slow electrical waves. Moreover, Cajal cells action as intermediate in neuronal control of bowel muscular activity, as sensors for elongation and coordinators of spatial bowel motility [2].

Idiopathic chronic intestinal pseudo-obstruction is a rare disease characterized by episodes like mechanic obstruction in the absence of organic, metabolic or systemic disorders [3]. In patients with this kind of pathology were identified some motor dysfunctionalities of the bowel, but anomalies and neuropathies of Cajal cells were identified only in a part of the cases. Other studies showed a decrease of the Cajal cells number in the cases of chronic intestinal pseudo-obstruction or the absence of immunoreactivity cells for CD34 and CD117 markers [4, 5].

The c-kit/CD117 receptor is immunohistochemically identified both at Cajal cells level and in a series of other human normal cells, like hematopoietic stem cells, mastocytes, melanocytes, basal skin cells, germinal cells and in breast epithelium. The activation of this tyrosine-kinase receptor, which is followed by immunohisto-chemical expression of c-kit protein, is done by somatic mutations in different stromal tumors, acute myeloid leukemia and mastocytosis or by auto-/paracrine mechanism in pulmonary and ovarian carcinomas.

Recent studies suggest that cells from stromal tumors positive for CD34 marker with proved c-kit

immunoreactivity can be differentiated in Cajal-like cells [6].

The aim of the study was to analyze the distribution and density of Cajal interstitial cells in normal and in non-tumoral colon diseases.

Material and methods

Colon specimens were obtained from 25 cases (study group) with different non-tumoral diseases presented in Table 1 and the study was carried out in accordance with local ethical guidelines. We used 11 specimens (control group) from subjects undergoing hemicolectomy for non-obstructing colorectal cancer taken at least 5 cm from the resection margin in tumor free areas.

Table 1 – ***Types of bowel disorders in study group***

No. of cases	Clinical and histological diagnosis
2	Idiopathic chronic intestinal pseudo-obstruction
5	Crohn disease
2	Ulcerative colitis
2	Colonic diverticulosis
2	Gluten enteropathy
11	Reactive repair lesions in intestinal bowel wall
1	Hamartomatous lesion

The resection samples were fixed in 10% neutral buffered formalin for 24 hours. For conventional histology, 4 μm thick paraffin wax embedded serial sections were stained with Hematoxylin–Eosin.

In order to evaluate the enteric nervous system and identify Cajal cells we investigated the immuno-reactivity for CD117 (1:400 dilution, DAKO), CD34 (1:25 dilution, DAKO), S-100 protein (1:800, DAKO). Paraffin wax embedded sections were dewaxed and rehydrated through a decreasing alcohol series up to distilled water. For immunohistochemistry we used Avidin–Biotin Complex method with diaminobenzidine tetrahydrochloride as the chromogen and counterstained with Mayer's Hematoxylin. Each protocol included positive and negative controls of immunoreactivity and the evaluation of immunoreactivity was done on serial sections in order to appreciate the relative distribution of antigens in the tissue. For each patient, the number of immunoreactive cells was calculated and expressed as the mean number of cells in 10 microscopic high-power fields, for each region of interest.

The percent of positive cells on a specimen was semi-quantitatively estimated: negative – none of the positive cells at 40× magnification; weak positive (+) – visible cells only at 40× magnification or occasional weakly positive; moderate positive (++) – easy distinguished cells at low magnification 10×; intense positive (+++) – cells intense positive at 10× magnification.

An image processing and analysis program (Image J) was used for counting of positive cells at immunohistochemistry. Statistical analysis was performed with SPSS software program for chi-square test, in order to compare the number of interstitial cells of Cajal in pathological and normal colon samples and a statistical significant difference was considered for $p<0.05$.

Results

In both groups (control and study), the nerve plexus architecture appeared normal on Hematoxylin–Eosin staining. In the control group, CD117 was expressed in large bowel in few Cajal cells around the myenteric plexus (Figures 1 and 2), but a major part of the cells were present around muscular layers. CD34 was positive in the cells around myenteric plexus and more numerous as compared with CD117 immunoreactivity ($p<0.05$).

Study group includes two male patients with repeated episodes of intestinal occlusion and diffuse intestinal dilatation in the absence of an associated systemic disorder or administration of drugs known to result in bowel dysmotility. In the colon specimens from these patients with idiopathic colonic pseudo-obstruction, we observed a marked diffuse (Figure 3) or focal decreasing of Cajal cells CD117 positive and fibroblasts CD34 positive.

In one case of acute diverticulitis and four cases of chronic inflammatory bowel disease, an inflammatory infiltrate cytological composed of neutrophils, lymphocytes, macrophages was localized around muscular or nervous structures. One patient with diverticulosis showed a feature of diffuse and nodular chronic inflammation in mucosa. Diverticulosis is a disease with a multifactor etiology and an important role has the motor abnormalities of the large bowel.

Immunohistochemical evaluation revealed a normal number of myenteric and submucous plexuses, but the number of Cajal cells (Figure 4) was significantly decreased ($p<0.05$).

In cases with inflammatory pathology we observed that tissues from two patients with Crohn disease showed a complete loss of interstitial Cajal cells in longitudinal and circular muscular layer and a significantly decrease of their number at the myenteric plexus (Figures 5 and 6).

Cajal cells are abundant in small bowel in some patients with inflammatory lesions and that could conduce to desynchronize of pacemaker electrical activity.

In one case of Crohn disease, Cajal interstitial cells from small bowel showed atrophy and a vacuolar degeneration together with decrease of Cajal cells number. In ulcerative colitis Cajal cells have no degeneration signs.

In non-specific, non-inflammatory non-tumoral lesions and gluten enteropathy we could not find a significant change of the number or distribution of Cajal cells in our cases (Figure 7).

Discussion

The role of Cajal interstitial cells as intestinal pacemakers has been presented in different experimental studies, which have shown that a decrease of Cajal cell number lead to the absence of slow waves and is accompanied by delay of intestinal motility.

In the upper gastrointestinal tract a lack or paucity of

Cajal cells has been found in diseases associated with gastric and small bowel motility [7, 8] but data on human colonic ICC are still scarce, especially in pathological conditions.

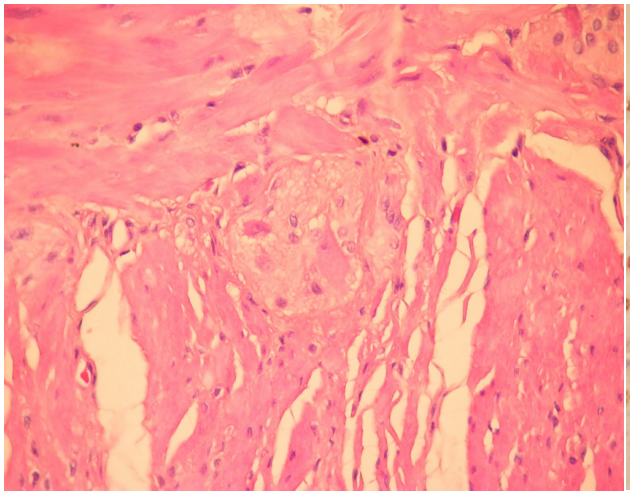

Figure 1 – ***Myenteric plexus in colon wall without significant inflammatory infiltrate (HE stain, 400×)***

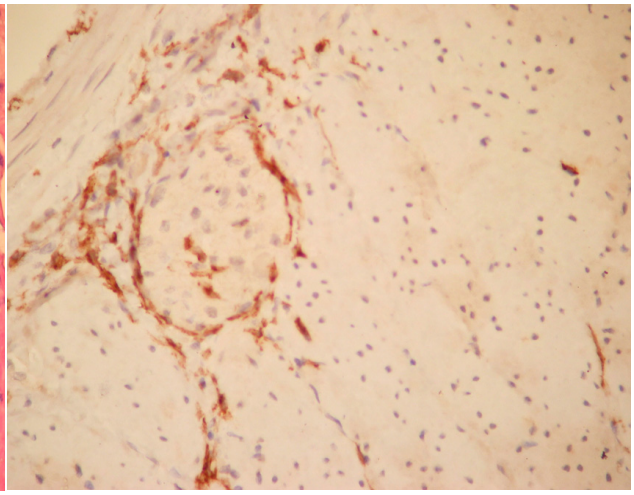

Figure 2 – ***CD117 positive Cajal interstitial cells in normal intestinal wall, 400×***

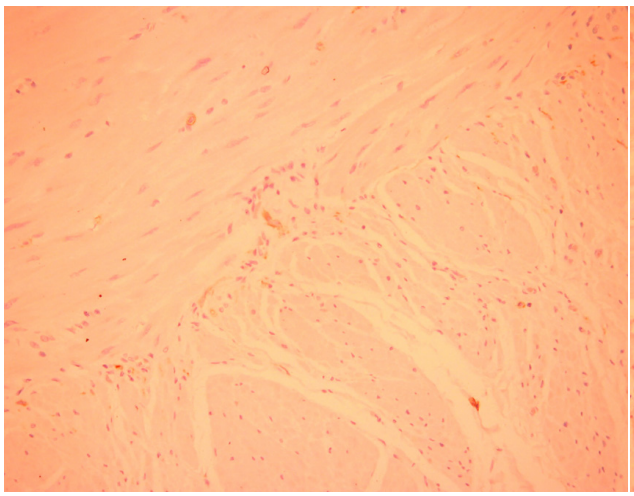

Figure 3 – ***Diffuse reduction of Cajal cells in a case with idiopathic colon pseudo-obstruction (CD117 immunoreactivity, 400×)***

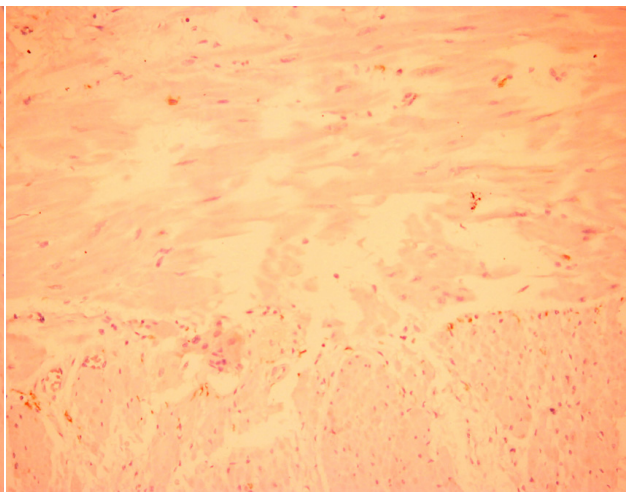

Figure 4 – ***Paucity of Cajal interstitial cells in diverticulosis (CD117 immunoreactivity, 200×)***

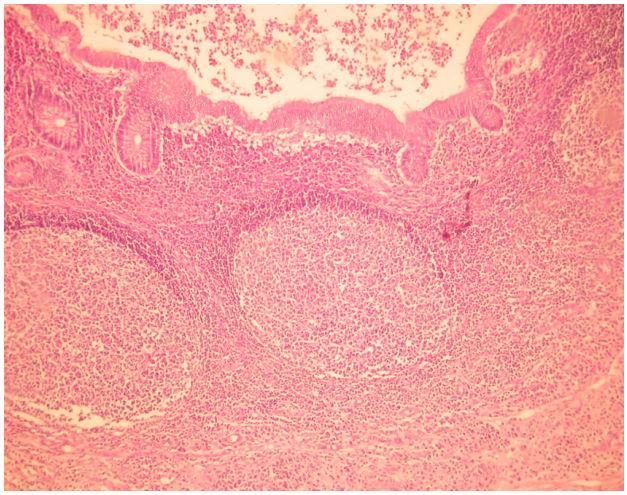

Figure 5 – ***Crohn disease with active chronic inflammation and lymphoid nodules hyperplasia (HE stain, 40×)***

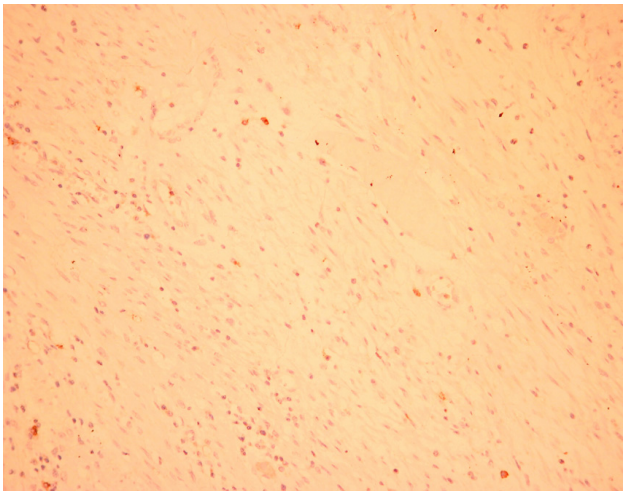

Figure 6 – ***Reducing number of Cajal cells in Crohn disease, diffuse lymphocytes infiltrate (CD117 immunoreactivity, 200×)***

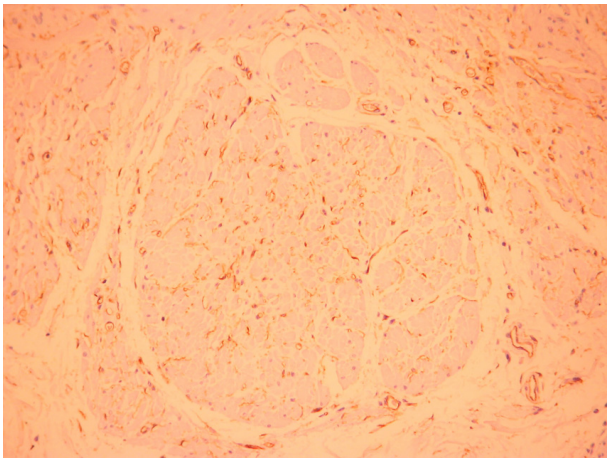

Figure 7 – ***Non-specific inflammatory lesions with normal distribution of Cajal cells at immunohistochemistry for CD117, 200×***

Chronic intestinal pseudo-obstruction represents a particular and rare syndrome characterized by impaired gastrointestinal propulsion together with symptoms of bowel obstruction in the absence of any lesions occluding the intestinal lumen. Studies on Cajal cells showed a marked reducing number or total absence of these interstitial cells in colons with this disorder [9, 10]. We studied the colon of two young male patients with idiopathic chronic pseudo-obstruction and found a diffuse reduction of Cajal cell, number of cells being significantly decreased as compared with normal colon.

In our study, in tissues from Crohn's disease patients, the density of interstitial Cajal cells was reduced throughout the tunica muscularis compared to control intestinal tissues ($p<0.05$). This feature is concordant with other studies [11] in which tissues from Crohn's disease patients showed an almost complete abolition of interstitial cells of Cajal within the longitudinal and circular muscle layers and a significant reduction in numbers at the level of the myenteric and deep muscular plexuses. In Crohn's disease, there are focal Cajal cells with vacuolar degeneration but in ulcerative colitis, the colonic Cajal cells have not signs of degeneration. These features of Cajal cells were described in a recent study [12] on small bowel affected by inflammatory diseases with atrophy and vacuolar degeneration of Cajal cells in Crohn's small bowel disease and a hyperplastic feature of Cajal cells in ulcerative colitis.

In cases with colonic diverticulosis we observed a significantly reduced numbers of colonic Cajal cells compared with tissues controls ($p<0.05$). In a recent study [13] on diverticular disease has been showed that there is a significant reduction of all subpopulations of interstitial Cajal cells and of enteric glial cells, whereas the enteric neuronal population appeared to be normal. This kind of alterations of Cajal number could explain, at least in part, the motor disturbances in patients with diverticulosis (high motility, abnormal response to food, etc.).

Experimental studies [14] revealed that intra-muscular ICC have receptors for and respond to some neuromediators and are required in mice for responses to the exogenous and endogenous neuromediators nitric oxide and acetylcholine. However, the mechanisms underlying this requirement remain unclear.

The pseudo-obstruction and disturbance of intestinal motility, which occurs in patients with Crohn's disease and diverticulosis, may be a consequence of the loss populations of interstitial cells of Cajal within the tunica muscularis or loss of their function. It is not possible to indicate the cause of Cajal cell loss; it could be possible that reducing or losing of the Cajal cell function to determine decrease or elimination of electric waves with decrease of contractile response and as result a delay in intestinal transit.

Conclusions

In intestinal chronic idiopathic pseudo-obstruction appear a diffuse or focal reducing number of Cajal cells, the loss of immunoreactivity for CD117 being correlated with loss of immunoreactivity for CD34 marker. Our study revealed that the number of interstitial cells of Cajal also decrease in colonic diverticular disease and Crohn's disease, whereas the number of enteric neurons appears to be normal. These findings might explain some of the large bowel motor abnormalities known to occur in diverticulosis and Crohn's disease.

Transmural intestinal surgical biopsies are helpful in evaluation of chronic intestinal pseudo-obstruction and allow a more extensive diagnosis. Further studies on motor abnormalities are needed to explore the gastrointestinal tract motility.

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