Research and Science Today
WWW.RSTJOURNAL.COM

DOI:

Title:	NEUROENDOCRINE CELL HISTOLOGY CHANGES IN THE EVOLUTION OF ULCERATIVE COLITIS
Authors:	Anamaria CURTE Gabriel BECHEANU Maria SAJIN

Section: MEDICAL SCIENCES

Issue: 1(19)/2020

Received: 3 February 2020	**Revised:** No
Accepted: 02 March 2020	**Available Online:** 15 March 2020

Paper available online HERE

RST Research and Science Today
WWW.RSTJOURNAL.COM
SPRING 2020
No. 1(19)/2020
ISSN-P: 2247-4455 / ISSN-E: 2285-9632

Medical Sciences

NEUROENDOCRINE CELL HISTOLOGY CHANGES IN THE EVOLUTION OF ULCERATIVE COLITIS

Anamaria CURTE[1]
Gabriel BECHEANU[2]
Maria SAJIN[3]

ABSTRACT:
BACKGROUND AND AIM*: NEUROENDOCRINE CELLS (NECS) HAVE BEEN IN FOCUS RECENTLY IN SEVERAL STUDIES ON PATIENTS WITH INFLAMMATORY BOWEL DISEASE SHOWING INCREASED NUMBERS IN THE CRYPTS AND SOMETIMES IN THE MUCOSAL LAMINA PROPRIA. THE AIM OF THIS STUDY WAS TO ASSESS HOW NEC HISTOLOGY CHANGES IN ULCERATIVE COLITIS (UC) PATIENTS OVER TIME AND THE POTENTIAL IMPLICATIONS THESE ALTERATIONS MAY HAVE.*
MATERIAL AND METHODS*: WE RETROSPECTIVELY SEARCHED FOR UC PATIENTS WITH MULTIPLE PRESENTATIONS IN THE PATHOLOGY DEPARTMENT OF ELIAS UNIVERSITY EMERGENCY HOSPITAL IN THE LAST THREE YEARS AND IDENTIFIED 9 PATIENTS WITH COLONIC BIOPSIES TAKEN AT TWO OR THREE DIFFERENT TIMES IN THE EVOLUTION OF THE DISEASE. NECS WERE DETECTED BY IMMUNOHISTOCHEMISTRY USING CHROMOGRANIN A ANTIBODY. WE COUNTED THE NEC PER CRYPT AND EVALUATED THE PRESENCE OF LINEAR HYPERPLASIA.*
RESULTS*:WE IDENTIFIED A DEFINITE INCREASE IN NECS IN MOST UC PATIENTS, PARTICULARLY AT THE ONSET OF THE DISEASE, FOLLOWED BY A SIGNIFICANT DECREASE OVER TIME AS DEMONSTRATED BY THE MODERATE NEGATIVE CORRELATIONS DETECTED BY PEARSON'S TEST BETWEEN THE DURATION OF DISEASE AND BOTH THE MEAN (R_P=- 517, P=0.014) AND MAXIMUM NUMBER OF NECS PER CRYPT (R_P=- 557, P=0.007). LINEAR HYPERPLASIA IN THE HOTSPOT SHOWED A SIMILAR DECREASING TREND, BUT WITHOUT STATISTICAL SIGNIFICANCE ON OUR SAMPLE (R_P=- 388, P=0.074).*
CONCLUSIONS*: THERE IS AN INCREASE OF NECS IN UC PATIENTS, HALF OF THEM SHOWING LINEAR HYPERPLASIA. FINDINGS OF NEC HYPERPLASIA AT THE ONSET OF DISEASE COULD HELP WITH THE INITIAL HISTOLOGICAL DIFFERENTIAL DIAGNOSIS OF UC WHEN THE CLASSICAL HISTOLOGICAL ASPECTS ARE NOT PRESENT.*

KEY WORDS: ULCERATIVE COLITIS, NEUROENDOCRINE CELL HYPERPLASIA, CHROMOGRANIN A

INTRODUCTION

Ulcerative colitis (UC) is a chronic burdening disease that can lead to colectomy when the lesions are severe and do not respond to treatment. The main acknowledged

[1] "Elias" University Emergency Hospital, Pathology Department, Bucharest, Romania, anma.pop@gmail.com
[2] Associate Professor of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[3] Professor of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

histology changes induced by chronic inflammation in colonic and rectal mucosa in this disease are crypt distorsion, basal plasmacytosis, diffuse mucosal inflammatory infiltrate with lymphocytes, plasma cells and eosinophils[4]. All these changes may diminish or even completely resolve in some patients with the help of medication, but relapse usually occurs. Changes in neuroendocrine cells (NEC) numbers and distribution has been in focus recently and several studies have shown increased NECs in patients with ulcerative colitis and inflammatory bowel disease in general[5,6]. Increased numbers of Chromogranin A (CgA) positive cells in colonic crypts[7,8,9,10], NEC micronests[8,11] and NEC benign and malignant tumors[12,13,14] have been reported. However, to our knowledge, no study has focused on the NEC dynamic histological changes in the course of evolution of disease in UC patients, which could help predict clinical evolution.

The aim of this study was to determine how NEC histology changes in ulcerative colitis patients over time and the potential implications these alterations may have. Also, we intended to identify the histological parameters that would best describe these changes.

MATERIAL AND METHODS

We searched retrospectively for ulcerative colitis patients with multiple colonic biopsies taken at different moments in the evolution of the disease that were evaluated histologically in the pathology department of "Elias" University and Emergency Hospital in the last three years.

[4] Magro et al., "Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders," *J. Crohn's Colitis*, vol. 11, no. 6, pp. 649–670, 2017, doi: 10.1093/ecco-jcc/jjx008

[5] El-Salhy et al., "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel Disease," *World J. Gastroenterol.*, vol. 23, no. 28, pp. 5068–5085, 2017, doi: 10.3748/wjg.v23.i28.5068.

[6] Massironi et al., "Chromogranin A and other enteroendocrine markers in inflammatory bowel disease," *Neuropeptides*, vol. 58, pp. 127–134, 2016.

[7] Meianu et al., "P047 Neuroendocrine cells densities alterations in colonic mucosa of patients with inflammatory bowel disease," *J. Crohn's Colitis*, 2019.

[8] Wong, B. K. Larson, and D. Dhall, "Neuroendocrine proliferations in inflammatory bowel disease: differentiating neuroendocrine tumours from neuroendocrine cell micronests," *Histopathology*, vol. 74, no. 3. pp. 415–423, 2019.

[9] Gunawardene, Corfe, and Staton, "Classification and functions of enteroendocrine cells of the lower gastrointestinal tract," *Int. J. Exp. Pathol.*, vol. 92, no. 4, pp. 219–231, 2011.

[10] Nascimbeni et al., "Solitary microcarcinoid of the rectal stump in ulcerative colitis," *Neuroendocrinology*, vol. 81, no. 6, pp. 400–404, 2005.

[11] Kanada et al., "Microcarcinoid arising in patients with long-standing ulcerative colitis: histological analysis," *Human Pathology*, vol. 64. pp. 28–36, 2017.

[12] Derikx et al., "Is the prevalence of colonic neuroendocrine tumors increased in patients with inflammatory bowel disease?," *Int. J. Cancer*, vol. 139, no. 3, pp. 535–542, 2016.

[13] Shigaki *et al.*, "Immunohistochemical analysis of chromogranin A and p53 expressions in ulcerative colitis–associated neoplasia: neuroendocrine differentiation as an early event in the colitis-neoplasia sequence," *Hum. Pathol.*, vol. 44, no. 11, pp. 2393–2399, 2013.

[14] Costa *et al.*, "Adenocarcinoma is not always the diagnosis – colon neoplasia in patient with long-standing ulcerative colitis under long-term prednisone maintenance therapy," *Clin. Res. Hepatol. Gastroenterol.*, vol. 43, no. 4, pp. 362–364, 2019.

Histological examination

The slides were stained by hematoxylin and eosin. The diagnosis of ulcerative colitis was confirmed using the acknowledged histological criteria[15]. Mucosal inflammation was evaluated using two histological scoring systems – Nancy index and Geboes score.

Immunohistochemistry

Immunohistochemistry was performed on all slides using Cell Marque Chromogranin A (LK2H10) mouse monoclonal antibody, dilution 1:100, following the immunohistochemistry protocol for paraffin-embedded sections.

Analysis

We counted and described the distribution of CgA positive cells (manually and by computed analysis) within colonic cypts and evaluated their presence in the mucosal lamina propria.

The histological slides were either scanned using Aperio slide scanner or examined conventionally on the optical microscope. On the digital slides, we performed image analysis on the digital slides using QuPath software[16]. Each crypt was defined as a single annotation, then positive cell detection for DAB stain command was applied. The results were manually checked.

The cut-off for defining NEC hyperplasia was a count of ≥3.2 NEC/crypt[17,18]. Linear hyperplasia was defined as NEC hyperplasia consisting of 5 or more contiguous CgA positive cells.

A „hotspot“ was defined as the area of 10 crypts showing the maximum number of CgA positive cells on a slide.

Statistical tests were done using IBM SPSS 20 software.

RESULTS

Patients

We identified 9 patients, 5 with two presentations, and 4 patients with three presentations respectively, corresponding to 22 cases (28 histological slides). The relevant demografic, clinical and endoscopical parameters, along with histological scores of inflammation activity are presented in Table1.

The majority of the patients had moderate or severe lesions at the first presentation, and a slight decrease of both endoscopical and histological scores of inflammation at further examinations. They also had a rather extensive colonic involvement (either left colonic or pancolonic).

Most of the patients were under conventional treatment, and 1, and 2 respectively were under biological therapy at the first and second presentations.

---

[15] Magro et al., “Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders,” *J. Crohn’s Colitis*, vol. 11, no. 6, pp. 649–670, 2017, doi: 10.1093/ecco-jcc/jjx008

[16] Bankhead *et al.*, “QuPath: Open source software for digital pathology image analysis,” *Sci. Rep.*, vol. 7, no. 1, p. 16878, 2017.

[17] Wong, B. K. Larson, and D. Dhall, “Neuroendocrine proliferations in inflammatory bowel disease: differentiating neuroendocrine tumours from neuroendocrine cell micronests,” *Histopathology*, vol. 74, no. 3. pp. 415–423, 2019.

[18] Nascimbeni et al., “Solitary microcarcinoid of the rectal stump in ulcerative colitis,” *Neuroendocrinology*, vol. 81, no. 6, pp. 400–404, 2005.

Upon colonoscopy, biopsies were taken mainly from the areas with the most severe lesions. 28.5% were labeled as originating from the sigmoid, 28.5% from the rectum, 7.14% from the descending colon, 3.57% from the transverse colon, and 3.57% from the ceacum. For 25% of the biopsies the segment of origin was not specified.

Table 1. Clinical, endoscopical and histological aspects of the patients.

	Presentation 1	**Presentation 2**	**Presentation 3**
Age (median)	30 [18, 56]	31 [19, 57]	37 [28, 58]
Gender	7 males, 2 females	7 males, 2 females	3 males, 1 female
Duration of disease (median)	9 weeks [1, 73]	22 weeks [11, 94]	13 weeks [4, 83]
Montreal classification		3 E2, 6 E3	
Mayo partial score	1 Mayo 1 3 Mayo 2 5 Mayo 3	1 Mayo 0 6 Mayo 2 2 Mayo 3	 3 Mayo 2 1 Mayo 3
Treatment			
5-ASA	6	5	1
Azathioprine	2	3	0
Corticoids	4	4	1
Biological agents	1	2	1
Histological inflammation			
Nancy Index<2	1	3	1
Nancy Index ≥3	8	6	3
Geboes grade <3.2	0	2	0
Geboes grade ≥3.2	9	7	4

Histological aspects

Although nitrocellulose paper was not routinely used by colonoscopists, most of the biopsies were oriented. Nevertheless, 22.72% of the histological slides had at least partial lack of orientation.

All the colonic biopsies taken at the first presentation in our department showed either mild or marked architectural alterations of the crypts. At the second presentation, 44.44% of the patients showed normalization of the crypt architecture, but still had increased mucosal inflammatory infiltrates of various levels. At the third presentation, only one patient showed normal crypts, the others showing marked crypt distortion.

Immunohistochemical staining for CgA

NEC hyperplasia, defined as a mean of ≥3.2 NECs/crypt, was detected in 68.18% of the colonic biopsies. The mean density of NECs was 4.18 CgA positive cells/crypt. Linear hyperplasia was present in 54.55% of all biopsies (Figures 1-4) .

On the slides we evaluated, there were no proliferations of NECs within the lamina propria on multiple sections.

ISSN-P: 2247-4455 / ISSN-E: 2285-9632

Figure 1. Chromogranin A immunohistochemical stain, 5x. The crypt architecture is distorted in this hotspot and both single-cell and linear neuroendocrine hyperplasia is present, mainly in the lower half of the crypts. Arrows indicate linear hyperplasia.

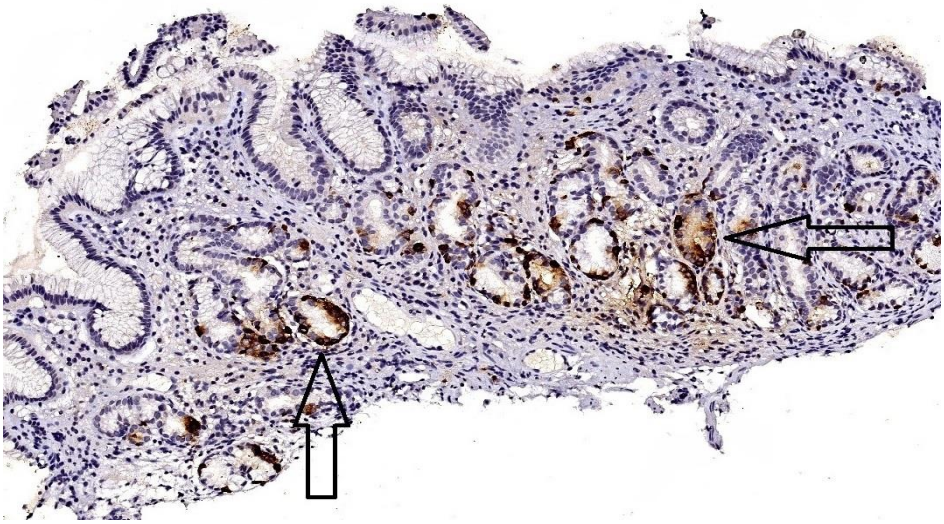


Figure 2. Chromogranin A immunohistochemical stain, 5x. Crypts show ramifications and loss of orientation. Neuroendocrine cell hyperplasia can be seen also in the upper half of the crypts (linear hyperplasia is highlighted by arrows).

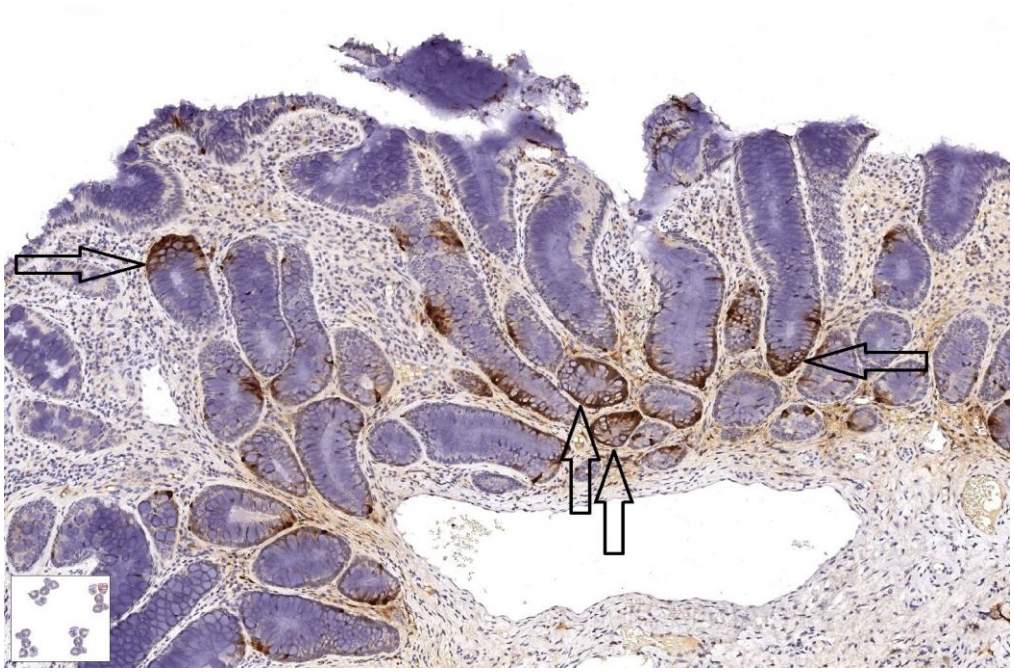
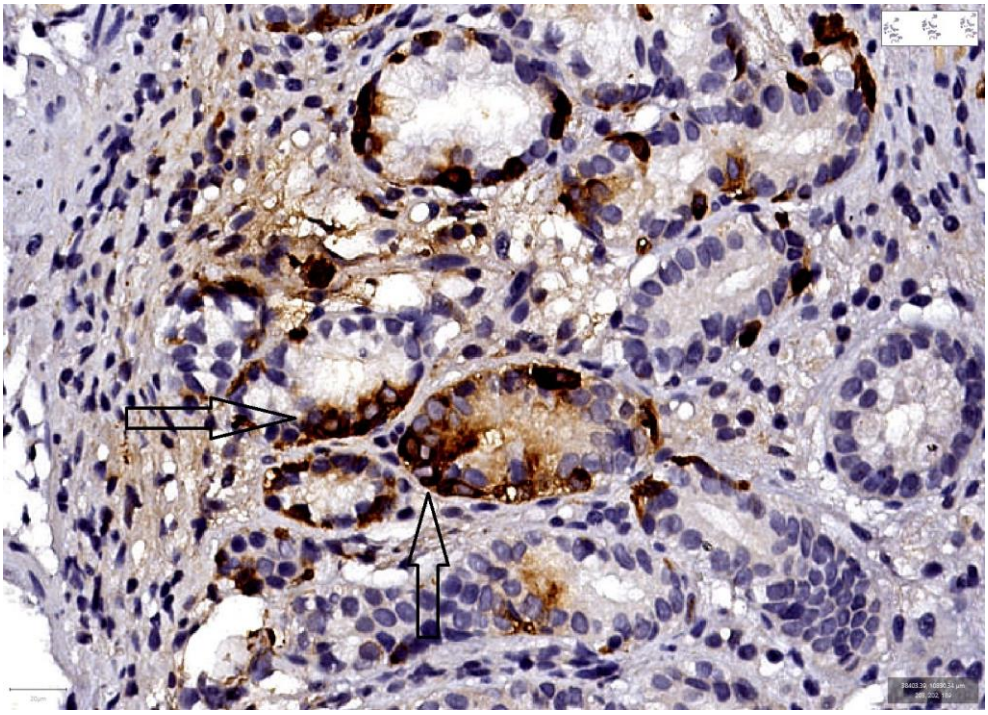


Figure 3. Chromogranin A immunohistochemical stain, 40x. Strong immunohistochemical stain is seen in hyperplastic neuroendocrine cells within colonic crypts (arrows indicate linear hyperplasia).



NEC changes with disease duration

All but one patient (88.88%) had NEC hyperplasia at least at one moment in the evolution of disease.

We observed a decrease over time in the NECs per crypt in the examined ulcerative colitis patients (Figures 1 and 2).

There was a statistically significant, moderate negative correlation by Pearson's test between both the mean and maximum number of NECs per crypt and duration of disease (Table 2).

ISSN-P: 2247-4455 / ISSN-E: 2285-9632

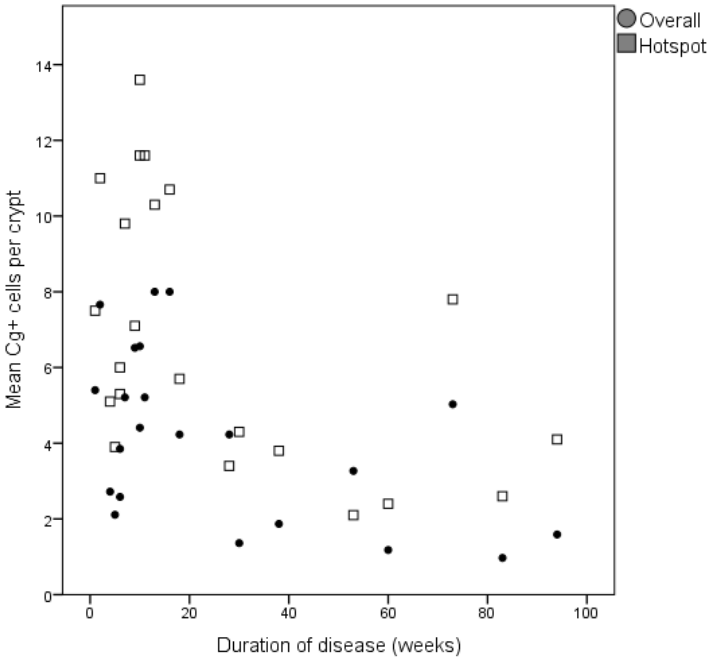


Figure 1. Dynamics of the mean NECs per crypt with duration of disease

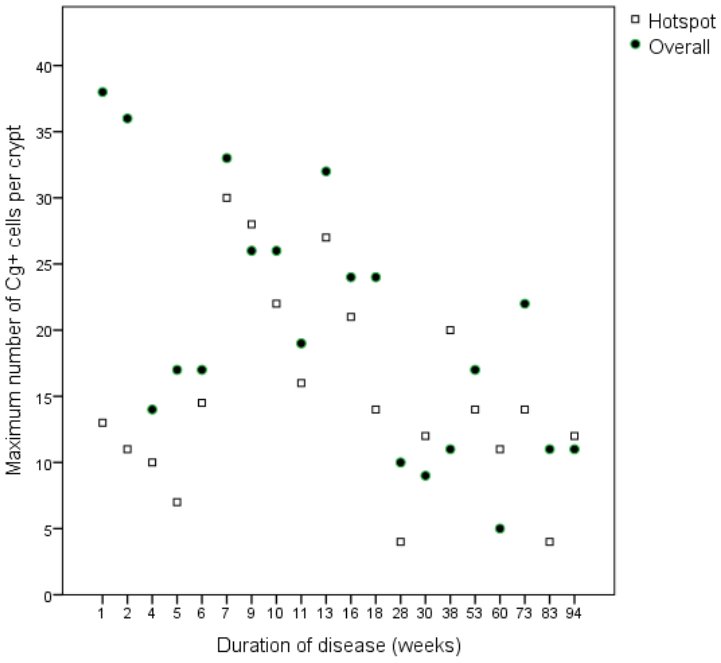


Figure 2. Dynamics of the maximum numbers of NECs per crypt with duration of disease

On the other hand, the mean number of crypts with linear hyperplasia of NECs on the biopsies was more constant over time (Figure 3), as opposed to the mean number of single-cell NEC hyperplasia showed above (Figure 1), which dramatically decreased with disease duration.

However, most of the crypts showing linear hyperplasia were clustered in the hotspots. The number of crypts with linear hyperplasia in the hotspot appears to decrease as

well with disease evolution over time, but is not statistically significant in the patients we studied (p= 0.074), possibly because of the reduced sample (Table 2).

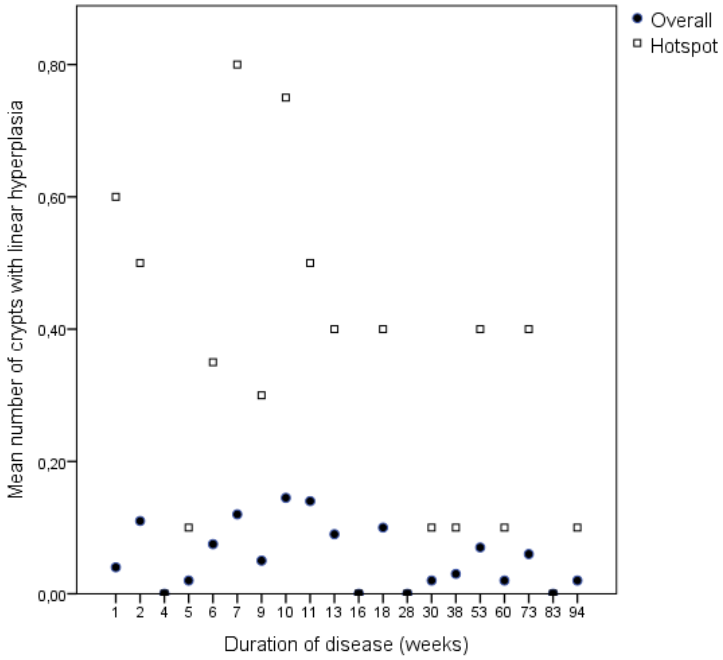


Figure 3. Dynamics of the mean number of crypts with linear hyperplasia NECs with duration of disease

Table 2. Pearson's correlation tests between duration of disease and histological variables

Variable	r_p	p value
Mean number of NECs per crypt overall	-.517	.014
Mean number of NECs per crypt in hotspot	-.518	.014
Maximum number of NECs cells per crypt overall	-.557	.007
Maximum number of NECs per crypt in hotspot	-.355	.105
Mean number of crypts showing linear hyperplasia of NECs overall	-.376	.084
Number of crypts showing linear hyperplasia of NECs in hotspot	-.388	.074

Discussions

In this study we identified a definite increase in NECs in most ulcerative colitis patients, a finding that confirms previous studies mentioned in the introduction section. This increase particularly manifests at the onset of the disease and is followed by a decrease over time, with the increase of disease duration.

We observed that changes in the hotspot reflect the overall changes on the histological sections. Thus, we believe that examining the immunohistochemical staining for CgA in the hotspot is reliable for detecting NEC hyperplasia, both single-cell and linear. This approach appears less time consuming than assessing NEC numbers/crypt on the whole slide and was not considered by other studies in the literature, to our knowledge.

We also found that the mean and maximum numbers of NECs per crypt are reliable histological parameters for evaluating NEC hyperplasia. Assessing the maximum number per crypt on the histological slide also seems more practical and less time consuming.

Linear hyperplasia is also a very helpful tool, though not statistically significant in our sample possibly because of its small size, especially because it is very easy to identify on the immunohistochemical stain. Since linear hyperplasia was most often clustered, using the hotspot method could be the easiest and most efficient way to detect it.

Findings of NEC hyperplasia at the onset of disease could help with the initial histological diagnosis, as these changes were not reported in infectious or ischemic colitis in the literature. This is a new direction we would like to follow in a future study.

CONCLUSIONS

In our study, neuroendocrine cells generally showed increased numbers in ulcerative colitis patients, similar to previous data in the literature. We showed that the mean and maximum number of Chromogranin A positive cells per crypt can be reliably used for assessing the level of NEC hyperplasia.

Also, we found that neuroendocrine cell linear hyperplasia is a striking feature in many patients with UC and this histological parameter, when present, highlights the area with the most numerous neuroendocrine cells (the hotspot).

The presence of neuroendocrine cell hyperplasia at the onset of ulcerative colitis could be a promising feature in the histological differential diagnosis and deserves future attention.

ACKNOWLEDGEMENTS

All authors equally contributed in the research and drafting of this paper.

All authors report no potential conflict of interest.

REFERENCES

1. **Costa, J. M.; Rodrigues, A.P.; Fernandes, D.; Costeira, F.; Vieira, F.; Goncalves, R.; Teixeira, M.; Soares, J.B;** "Adenocarcinoma is not always the diagnosis – colon neoplasia in patient with long-standing ulcerative colitis under long-term prednisone maintenance therapy," *Clin. Res. Hepatol. Gastroenterol.*, vol. 43, no. 4, pp. 362–364, 2019;
2. **Derikx, Lauranne A.A.P.; Vierdag, Wouter-Michiel A.M.; Kievit, Wietske; Bosch, Steven; Hoentjen, Frank; and Nagtegaal, Iris D**; "Is the prevalence of colonic neuroendocrine tumors increased in patients with inflammatory bowel disease?," *Int. J. Cancer*, vol. 139, no. 3, pp. 535–542, 2016;
3. **El-Salhy, Magdy; Hausken, Trygve;** "Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel Disease," *World J. Gastroenterol*., vol. 23, no. 28, pp. 5068–5085, 2017, doi: 10.3748/wjg.v23.i28.5068;
4. **Gunawardene, Ashok R.; Corfe, Bernard M.; and Staton, Carolyn A;** "Classification and functions of enteroendocrine cells of the lower gastrointestinal tract," *Int. J. Exp. Pathol.*, vol. 92, no. 4, pp. 219–231, 2011;
5. **Kanada, Sachie; Sugita, Akira; Mikami, Tetuo; Ohashi, Kenichi; and Hayashi, Hiroyuki;** "Microcarcinoid arising in patients with long-standing ulcerative colitis: histological analysis," *Human Pathology*, vol. 64. pp. 28–36, 2017;
6. **Magro, Fernando; Gionchetti, Paolo; Eliakim, Rami; Ardizzone, Sandro; Armuzzi, Alessandro; Acosta, Manuel; Burisch, Johan; Gecse, Krisztina; Hart, Ailsa; Hindryckx, Pieter; Langner, Cord; Limdi, Jimmy; Pellino, Gianluca; Zagórowicz, Edyta; Raine, Tim; Harbord, Marcus; and Rieder, Florian;** "Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders," *J. Crohn's Colitis*, vol. 11, no. 6, pp. 649–670, 2017, doi: 10.1093/ecco-jcc/jjx008;
7. **Massironi, Sara; Zilli, Alessandra; Cavalcoli, Federica; Conte, Dario; Peracchi, Maddalena;** "Chromogranin A and other enteroendocrine markers in inflammatory bowel disease," *Neuropeptides*, vol. 58, pp. 127–134, 2016;
8. **Meianu, Corina; Preda, Carmen; Becheanu, Gabriel; Ciora, Cosmin; Proca, Doina; Dumbrava, Mona; Lupu, Alexandru; Diculescu. Mircea;** "Neuroendocrine cells alterations in colonic mucosa of patients with inflammatory bowel disease", *Research and Science Today*. 2019, Issue 1:195-204;
9. **Nascimbeni, R.; Villanacci, Vincenzo; Di Fabio, F.; Gavazzi, E.; Fellegara, G.; and Rindi, Guido;** "Solitary microcarcinoid of the rectal stump in ulcerative colitis," *Neuroendocrinology*, vol. 81, no. 6, pp. 400–404, 2005;
10. **Shigaki, Kotaro; Mitomi, H; Fujimori, T; Ichikawa, K; Tomita, S; Imura, J; Fujii; S, Itabashi, M ; Kameoka, S; Sahara, R; Takenoshita, S;** "Immunohistochemical analysis of chromogranin A and p53 expressions in ulcerative colitis–associated neoplasia: neuroendocrine differentiation as an early event in the colitis-neoplasia sequence," *Hum. Pathol.*, vol. 44, no. 11, pp. 2393–2399, 2013;
11. **Wong, Mary; Larson, Brent K.; and Dhall, Deepti;** "Neuroendocrine proliferations in inflammatory bowel disease: differentiating neuroendocrine tumours from neuroendocrine cell micronests," *Histopathology*, vol. 74, no. 3. pp. 415–423, 2019.