MEDDOCS Open Access Publisher

ISSN: 2637-4501

Annals of Gastroenterology and the Digestive System

Open Access | Research Article

# Vascular Endothelial Growth Factor- Receptor 2 and Inflammatory Bowel Disease – Angiogenesis within all Four Layers of Inflamed Intestinal Wall

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Received: May 05, 2020

Accepted: Jun 17, 2020

Published Online: Jun 19, 2020

Journal: Annals of Gastroenterology and the Digestive System

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/



**Abstract**

Inflammatory Bowel Disease (IBD) is defined as chronic idiopathic inflammation of the intestines which includes two gastrointestinal disorders of unknown etiology: Ulcerative Colitis (UC) and Crohn's Disease (CD). The nature of clinical symptoms persistence is connected with different factors, among others angiogenesis plays an important role in development of changes within bowel wall. Both macro- and microscopically examined bowels from patients suffering from acute phase of IBD revealed sometimes dramatically congested vessels with hemorrhagic foci. These changes are also connected with angiogenesis – the process, which plays crucial role in persistence of inflammation. Most authors examined mucosal samples taken during endoscopy, have tried to assess changes within the whole bowel wall thickness. To determine intensity of angiogenesis we used monoclonal antibody against Vascular Endothelial Growth Factor (VEGF) receptor R2 (VEGFR-2). We also compared obtained results with previously tested antibody against VEGF receptor 1.

## Introduction

Inflammatory Bowel Disease (IBD) is defined as chronic idiopathic inflammation of the intestines which includes two gastrointestinal disorders of unknown etiology: Ulcerative Colitis (UC) and Crohn's Disease (CD) [1]. The nature of clinical symptoms persistence is connected with different factors, among others angiogenesis plays an important role in development of changes within bowel wall [2-4]. Nonetheless, it is not clear whether this process is a pathological consequence or has a positive impact to resolve the inflammation. Study conducted on mice model of acute colitis suggested that inflammatory lymphangiogenesis may have pleiotropic effects at different stages of IBD [5]. Further trials on people are necessary. However, most papers concerning histopathological aspect of this topic are focused on bowel's mucosa investigations. We examined full-thickness intestinal samples using immunohistochemistry with antibody against VEGF receptor 2. According to our experience gathered during over thirty years of dealing with pathological findings in IBD [6,7], there is a striking pattern of vasocongestion within

**Cite this article:** Wejman J, Pyzlak M, Szymańska S, Ostrowska J, Tarnowski W. Vascular Endothelial Growth Factor- Receptor 2 and Inflammatory Bowel Disease – Angiogenesis within all Four Layers of Inflamed Intestinal Wall. Ann Gastroenterol Dig Sys. 2020; 3(1): 1019.

bowel wall both macro- and microscopically. Bowels of IBD patients are usually resected in acute phase of disease, therefore either fresh or clots of blood may be often found within intestines lumen. That finding raises the question if hyperemia with vasodilatation are the only causes of such findings.

## Material and Methods

We examined full thickness samples of intestines taken from patients suffering from ulcerative colitis, Crohn's disease and patients without IBD, as a control group. In order to compare our previous work concerning VEGFR1 [8] we examined the same set of cases. We collected 35 cases with ulcerative colitis: there were 15 women aged 14-57 and 20 men aged 30-70; mean age of CU patients was 39,55 years. The number of Crohns diseases patients was 37: 21 women aged 20-63 and 16 men aged 22-59 with mean age of all patients- 35,83. Control group (NO) consisted of 30 cases with no IBD. These were mostly intestines resected due to bowel cancer and diverticular disease but also a case of intestinal lipoma and bowel endometriosis. There were 11 women and 19 men with mean age 65,15.

All samples of bowels in the IBD group were resected due to complications of acute phase of the disease. All resected cases were assessed as grade 5 in UC according to grading scale for histological assessment of inflammation proposed by Geboes et al. [9]. Crohns disease activity was estimated as severe according to method showed in another Geboes's study [10].

All tissue specimens were collected during standard grossing procedures.

Tissue samples from the NO group were taken from regions that were in a distance of at least 15 cm from the tumor, endometriosis foci or inflamed diverticuli.

Tissue samples were fixed in formalin, embedded in paraffin, and sectioned into 4 micrometer sections, according to standard histopathology protocol.

One section from each case was stained routinely with hematoxylin & eosin to evaluate the extent of the disease or to confirm the lack of pathology (in the NO group). Other sections From the same sample were stained with immunohistochemical technique, using antibody against receptor 2 for vascular endothelial growth factor. Primary antibody VEGF Receptor 2 (D5B1) Rabbit mAb was obtained from Cell Signaling and used with dilution 1:400 with antigen retrieval at pH=9. Visualization was performed with En-Vision Flex visualization system (Dako). All obtained sections were scanned using Panoramic viewer by 3DHistech Company.

Each bowel section was divided into four layers according to histology: mucosa, submucosa, muscularis propria and subserosa. From each layer 10 randomized photos using 40x magnification were performed, so each case was photographed 40 times. Morphometric analysis was done using Leica Quantimet workstation along with ImageJ custom macros [11-13]. Number of stained vessels was counted independently by two experienced pathologists, with the use of computer assisted morphometry. Statistical analysis of the results (Mann-Whitney *U* test, Kruskall-Wallis test) was performed with the use of *R* programming language [14].

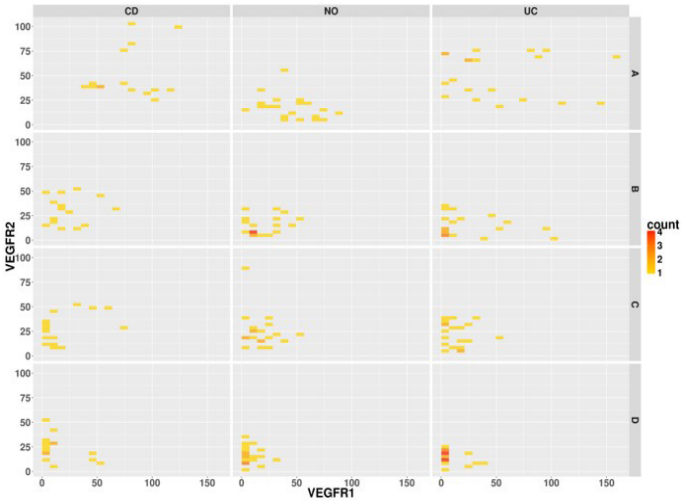


**Figure 1:** Number of VEGFR-1 and VEGFR-2 -positive vessels distribution according to disease and layer of the bowel wall. Color signifies number of patients with given values. (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

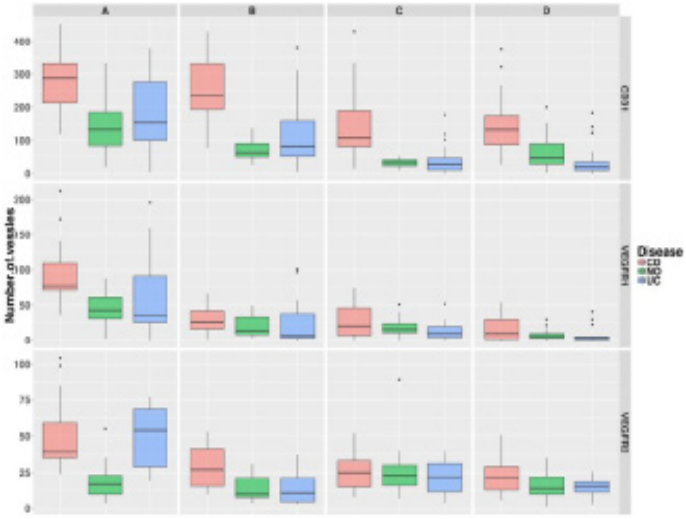


**Figure 2:** Chart showing CD31, VEGFR-1 and VEGFR-2 -positive vessels number according to type of disease and layer of the bowel wall. (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

***Crohn's disease:***

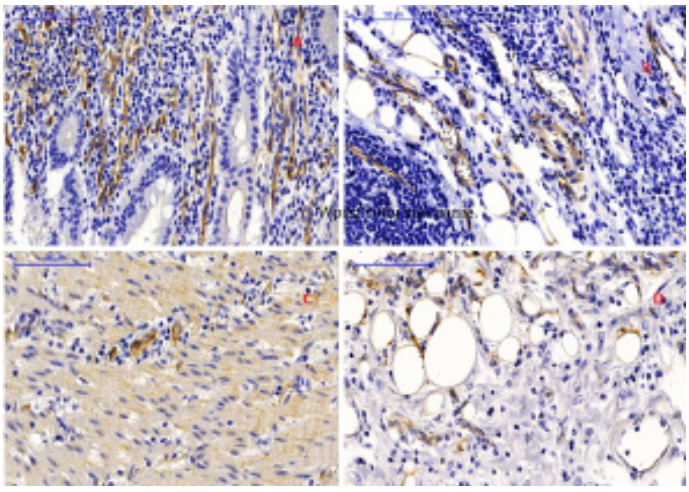

**Figure 3:** Microscopic view of four bowel layers from CD patient. (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa). All vessels presenting VEGFR-2 are brown stained. Magn. 20x. Blue measure marks 100µm.

Our study showed statistically significant ($p < 0.05$) differences in number of VEGFR-2 – positive vessels in the mucosa, between Crohn's disease and control (Table 1). The number of positive vessels stained with VEGFR2 was much bigger and was less than revealed with VEGFR1 as shown in the Figures 1 and 2.

**Table 1:** Comparison of VEGFR-2 positive vessels No between Crohn's disease and control (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

| Layer | | | | | p-value |
|---|---|---|---|---|---|
| A | St. | | | | <0,001 |
| | Group | Mean | Deviation | Valid N | |
| | CD | 49,79 | 25,32 | 30 | |
| | NO | 18,67 | 12,21 | 30 | |
| B | St. | | | | <0,001 |
| | Group | Mean | Deviation | Valid N | |
| | CD | 28,32 | 14,87 | 37 | |
| | NO | 14,33 | 8,68 | 30 | |
| c | St. | | | | 0,498 |
| | Group | Mean | Deviation | Valid N | |
| | CD | 26,63 | 14,07 | 37 | |
| | NO | 24,9 | 17,29 | 30 | |
| D | St. | | | | 0,0434 |
| | Group | Mean | Deviation | Valid N | |
| | CD | 23,53 | 12,09 | 37 | |
| | NO | 15,57 | 8,92 | 30 | |

Significant difference between CD and control group (Table 2) were also found within submucosa. There was no difference between R1 and R2 (Figures 1,2,3,5).

Muscularis layer revealed no differences between CD and control (Table 2) as well as between R1 and R2 VEGF receptors (Figures 1,2).

Differences between CD and control were detected within subserosa (Table 2), while no statistically differences between R1 and R2 (Figure 1,2).

**Table 2:** VEGF-R2 positive vessels in Crohn's disease group by layers (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

| Layer | Marker | Disease | Mean | St. Deviation | Valid N |
|---|---|---|---|---|---|
| A | VEGFR2 | CD | 49,79 | 25,32 | 37 |
| B | VEGFR2 | CD | 28,32 | 14,87 | 37 |
| C | VEGFR2 | CD | 26,63 | 14,07 | 37 |
| D | VEGFR2 | CD | 23,53 | 12,09 | 37 |

p-values for layer comparison:

| | A | B | C |
|---|---|---|---|
| B | 0,012 | - | - |
| C | 0,0016 | 0,77 | - |
| D | <0,001 | 0,365 | 0,5491 |

### *Ulcerative colitis:*

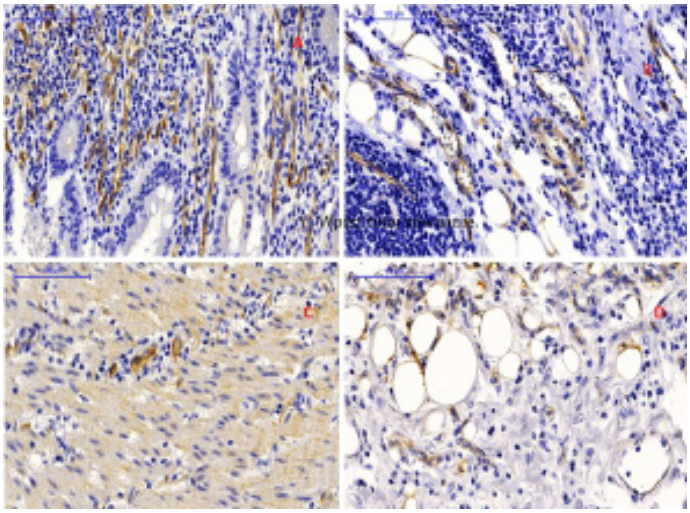

**Figure 4:** Microscopic view of four bowel layers from UC patient. (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa). All vessels presenting VEGFR-2 are brown stained. Magn. 20x. Blue measure marks 100µm.

There was statistically significant difference between UC and control with $p<0,001$ (Table 3), whereas reaction with R1 was no valued within mucosal layer (Figures 1, 2).

**Table 3:** Comparison of VEGFR-2 positive vessels No between ulcerative colitis and control (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

| Layer | | | | | p-value |
|---|---|---|---|---|---|
| A | Disease.gr | | St. | | <0,001 |
| | p | Mean | Deviation | Valid N | |
| | NO | 18,67 | 12,21 | 30 | |
| | UC | 50 | 21,71 | 35 | |
| B | Disease.gr | | St. | | 0,855 |
| | p | Mean | Deviation | Valid N | |
| | NO | 14,33 | 8,68 | 30 | |
| | UC | 14,55 | 10,63 | 35 | |
| c | Disease.gr | | St. | | 0,754 |
| | p | Mean | Deviation | Valid N | |
| | NO | 24,9 | 17,29 | 30 | |
| | UC | 21,95 | 12,45 | 35 | |
| D | Disease.gr | | St. | | 0,948 |
| | p | Mean | Deviation | Valid N | |
| | NO | 15,57 | 8,92 | 30 | |
| | UC | 14,8 | 6,21 | 35 | |

Submucosa, muscularis propria and subserosa not show any significant differences between UC and control (Table 3, Figures 4, 5) as well as within R1 and R2 (Figures 1, 2).

**Table 4:** VEGF-R2 positive vessels in ulcerative colitis group by layers (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa). *Control group (NO):*

| Layer | Marker | Disease | Mean | St. Deviation | Valid N |
|---|---|---|---|---|---|
| A | VEGFR2 | UC | 50 | 21,71 | 35 |
| B | VEGFR2 | UC | 14,55 | 10,63 | 35 |
| C | VEGFR2 | UC | 21,95 | 12,45 | 35 |
| D | VEGFR2 | UC | 14,8 | 6,21 | 35 |

p-values for layer comparison:

| | A | B | C |
|---|---|---|---|
| **B** | <0,001 | - | - |
| **C** | <0,001 | 0,0562 | - |
| **D** | <0,001 | 0,4897 | 0,083 |

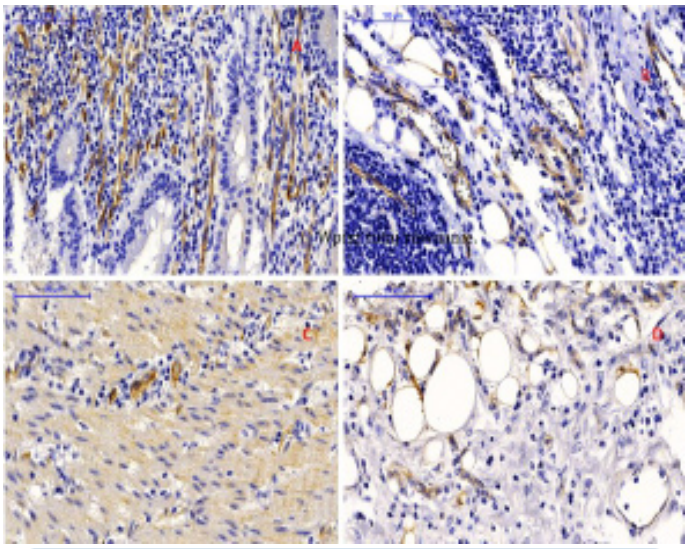

**Figure 5:** Microscopic view of four bowel layers from control group patient. (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

All vessels presenting VEGFR-2 are brown stained. Magn. 20x. Blue measure marks 100µm. Number of vessels stained with VGFR1 was significantly much higher than those with R2 receptor (Figures 1, 2).

Other layers of bowels walls not show any differences.

**Table 5:** VEGF-R2 positive vessels in control group by layers (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

| Layer | Marker | Disease | Mean | St. Deviation | Valid N |
|---|---|---|---|---|---|
| A | VEGFR2 | NO | 18,67 | 12,21 | 30 |
| B | VEGFR2 | NO | 14,33 | 8,68 | 30 |
| C | VEGFR2 | NO | 24,9 | 17,29 | 30 |
| D | VEGFR2 | NO | 15,57 | 8,92 | 30 |

p-values for layer comparison:

| | A | B | C |
|---|---|---|---|
| **B** | 0,23626 | - | - |
| **C** | 0,12142 | 0,00947 | - |
| **D** | 0,52883 | 0,56211 | 0,02502 |

***CD vs UC:***

While using antibody against R2 receptor we could show statistically significant differences in number of vessels between CD and UC within submucosa and subserosa (Table 6). No differences were observed in R1 staining as well as between those two receptors (Figures 1,2). One could explain this phenomenon bearing in mind broad range of vessels number between bowel layers (Figure 1).

There are no differences with other layers stained with R2.

On the other hand VEGFR1 did not discriminate CD and UC in any bowel layers (Figures 1,2).

While comparing particular layers within control group R2 revealed differences between submucosa and muscular layer as well as between muscular layer and subserosa (Table 5, 6).

Both IBD diseases revealed significant differences only between mucosa and other layers (Tables 2,4).

**Table 6:** Comparison of VEGFR-2 positive vessels number between Crohn's disease and ulcerative colitis (A-mucosa, B- submucosa, C – muscularis propria; D – subserosa).

| Layer | | | | | p-value |
|---|---|---|---|---|---|
| A | Disease.gr | | St. | | 0,811 |
| | p | Mean | Deviation | Valid N | |
| | CD | 49,79 | 25,32 | 37 | |
| | UC | 50 | 21,71 | 35 | |
| B | Disease.gr | | St. | | 0,00328 |
| | p | Mean | Deviation | Valid N | |
| | CD | 28,32 | 14,87 | 37 | |
| | UC | 14,55 | 10,63 | 35 | |
| c | Disease.gr | | St. | | 0,376 |
| | p | Mean | Deviation | Valid N | |
| | CD | 26,63 | 14,07 | 37 | |
| | UC | 21,95 | 12,45 | 35 | |
| D | Disease.gr | | St. | | 0,0211 |
| | p | Mean | Deviation | Valid N | |
| | CD | 23,53 | 12,09 | 37 | |
| | UC | 14,8 | 6,21 | 35 | |

## Discussion

Authors mostly examined VEGF or its receptors in blood serum or within mucosal samples obtained from endoscopic examinations. The main goal of our study was to determine intensity of angiogenesis across the full bowel wall thickness using tissue antibody against VEGF receptor R2 (VEGFR-2), taken from patients suffering from IBD.

Based on the increased angiogenesis which favours carcinogenesis some authors postulated this phenomenon in IBD. In 2006 Danese et al. stated that angiogenesis was a novel component of inflammatory bowel disease pathogenesis [3]. Until then more than a hundred reports of the angiogenesis in IBD have been published. Some authors also stressed that angiogenesis in IBD is accompanied by expression of thrombospondin. Punekar et al. examined murine model of DSS-

induced colitis. They found that Thrombospondin-1- deficient mice presented more active inflammation [15]. Alkim et al. revealed that both VEGF and Thrombospondin-1 (TSP-1) were increased in active phase of IBD [16]. Zak et al. [17] noticed that TSP-1 may decrease angiogenesis by reducing the levels of pro-angiogenic factors and inducing apoptosis in epithelial cells . However, in our previous work, this thesis was not supported [8].

Angiogenesis in IBD could be a target for drugs used in therapeutic efforts for UC and CD cure. In 1979 Folkman et al. published their excellent work presented efforts of capillary endothelial cells in long-term cultures. They stated that, "tumor angiogenesis takes place at the level of capillary endothelium, not aortic endothelium. Then these new cloned capillary endothelial cells may serve as a quantitative in vitro assay system to detect stimulators as well as inhibitors of angiogenesis" [18]. Next step to understand the role of angiogenesis and for the treatment was connected also with culture of Human Intestinal microvascular Endothelial cells (HIVEC) [19]. The concept of HIVEC was the base of therapeutic properties of Thalidomide. Rafiee et al. noticed that, ability of endothelial cells to migrate and form capillary- like structures is was critical in growth factor-induced angiogenesis. The antiangiogenic effect of thalidomide on VEGF-induced HIMEC activation was evident by its inhibitory affect on cell migration, growth, proliferation, in vitro capillary tube formation, and Akt phosphorylation [20]. The effectiveness of thalidomide treatment cause clinical remission in 51.5% cases [21]. Infliximab is also in use as antiangiogenic drug [22,23].

Angiogenesis is the process of new blood vessel development from existing vessels and running through several steps with vasodilation and increased permeability induced by vascular endothelial growth factor (VEGF) in the beginning [24]. Vascular endothelial growth factors (VEGFs)—VEGF-A, -B, -C, and -D, and PIGF (placental growth factor)—are a family of homodimeric proteins. VEGF-A is generally referred as VEGF; it is the major angiogenic factor [24]. VEGF induces angiogenesis by promoting endothelial cell migration, proliferation (capillary sprouting), and formation of the vascular lumen. VEGFs also induce vascular dilation and increased vascular permeability [24]. VEGFs bind to a family of receptor tyrosine kinases (VEGFR-1, -2, and -3); VEGFR-2 is highly expressed in endothelium and is the most important for angiogenesis [24]. Zhou et al. in paper concerning wounds healing stated that activation of VEGFR-2 was the major reason for mitogenic, angiogenic and permeability-enhancing effects, abnormal lymphangiogenesis, promoting cell adhesion and migration by VEGF-A [25]. Scaldaferri et al. in their series of examined IBD tissues found no differences between control and IBD concerning VEGFR-1, which is in accordance with our own experience. Whereas VEGFR-2 expression was strongly up-regulated in actively inflamed CD and UC comparing with control mucosa - the finding we could confirmed in our study [26, 8]. However mentioned authors compared mucosal reactions in their figures pointed mostly submucosal vessels. We concluded, that biopsy containing submucosa and stained with R2 could be useful in recognizing CD -see results.

Kapsoritakis et al. examined level of VEGF in plasma and serum patients with IBD and also performed immunohistochemistry in tissue samples. They not find VEGF expression within muscular layer and intestinal epithelium was negative in CD, while a cytoplasmic reactivity was noted in UC and normal controls [27]. VEGF serum levels were significantly higher in IBD patients compared to controls- according to Kopanakis [28]. The level of serum VEGF patients with IBD could be modified by using exclusive enteral nutrition [29].

Comprehensive data of definitions, diagnosing and management of UC and CD are published by European Crohn and Colitis Organization (ECCO) [30-33].

## Conclusions

There are some differences within expression of VGFR2 while comparing ulcerative colitis and Crohn's disease. Within mucosa UC vs. control group differences are significant, whereas staining against R1 show no such distinction.

Unfortunately, we not revealed any significant difference in expression of R2 within mucosa patients suffering for UC and CD, thus such immunostaining could not be used as a marker of differentiation in shallow endoscopic biopsy. Deeper biopsy containing submucosa could be helpful, but as results revealed, this needs further investigations.

The answer for the title question of our work is: angiogenesis remains in deeper layers of bowel wall and these fact should always be kept in mind concerning clinical course and treatment of the disease.

As we revealed above shallow endoscopic biopsy containing only mucosa is sufficient to assess angiogenetic condition within suffering bowel, which may be useful to collect group of patients who may benefits from receiving anti-angiogenic drugs. This may be also useful for monitoring such a treatment.

The assessment of angiogenesis could not unequivocally discriminate ulcerative colitis and Crohn's disease in endoscopic biopsy samples. In such cases concerning also troubles and doubts with classic histopathological patterns we recommend term in use as inflammatory bowel disease unclassified- IBDU.

## References


1. Cecilia MFP, Amy EN, Grant NS, Patrick EL, Peter GI. Gastrointestinal Pathology: An Atlas and Text, Lippincott Williams & Wilkins. 2008.
2. Alkim C, Alkim H, Koksal AR, Boga S, Sen I. Angiogenesis in Inflammatory Bowel Disease, Int J Inflam, 2015; 2015: 970890.
3. Danese S, Sans M, de la Motte C, Graziani C, West G, Phillips MH, Pola R, Rutella S, Willis J, Gasbarrini A, Fiocchi C. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis.. Gastroenterology. 2006; 130: 2060–2073.
4. Koutroubakis IE, Tsiolakidou G, Karmiris K, Kouroumalis EA. Role of angiogenesis in inflammatory bowel disease, Inflammatory Bowel Diseases. 2006; 12: 515–523.
5. Wang XL, Zhao J, Qin L, Qiao M Promoting inflammatory lymphangiogenesis by vascular endothelial growth factor-C (VEGF-C) aggravated intestinal inflammation in mice with experimental acute colitis. Braz J Med Biol Res. 2016; 49: e4738.
6. Wejman J. Acute fulminant ulcerative colitis. A case report. Patol Pol. 1986; 37: 191-194. Polish
7. Wejman J, Bielecki K, Ostrowska J, Baczuk L, Perkowska-Ptasińska A, Tarnowski W. Pathological analysis of lesions within intestines resected due to ulcerative colitis. Pol J Pathol. 2006; 57: 113-116.
8. Wejman J, Pyzlak M, Szukiewicz D, Jarosz D, Tarnowski W, Szewczyk G. Thrombospondin and VEGF-R: is there a correlation in inflammatory bowel disease?. Mediators Inflamm. 2013;

2013: 908259.

9. Geboes K, Riddell R, Ost B. Jensfelt, T. Persson, and R. Lofberg. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis, Gut. 2000; 47: 404–409.

10. K. Geboes. Is histology useful for the assessment of the efficacy of immunosuppressive agents in IBD and if so, how should it be applied? Acta Gastro-Enterologica Belgica. 2004; 67: 285–289.

11. ImageJ, Rasband WS, U.S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2012.

12. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis, Nature Methods. 2012; 9: 671–675.

13. Abramoff MD, Magalhaes PJ, Ram SJ. Image Processing with Image, Biophotonics International. 2004; 11: 36–42.

14. R Core Team. A Language and Environment For Statistical Computing, Vienna, Austria. 2012.

15. Punekar S, Zak S, Kalter VG, Dobransky L, Punekar I, Lawler JW, Gutierrez LS. Thrombospondin 1 and its mimetic peptide ABT-510 decrease angiogenesis and inflammation in a murine model of inflammatory bowel disease. Pathobiology. 2008; 75: 9-21.

16. Alkim C, Sakiz D, Alkim H, Livaoglu A, Kendir T, Demirsoy H, Erdem L, Akbayir N, Sokmen M. Thrombospondin-1 and VEGF in inflammatory bowel disease. Libyan J Med. 2012; 7.

17. Zak S, Treven J, Nash N, Gutierrez LS. Lack of thrombospondin-1 increases angiogenesis in a model of chronic inflammatory bowel disease. Int J Colorectal Dis. 2008; 23: 297-304.

18. Folkman J, Haudenschild CC, Zetter BR. Long-term culture of capillary endothelial cells. Proc Natl Acad Sci U S A. 1979; 76: 5217-5221.

19. Haraldsen G1, Rugtveit J, Kvale D, Scholz T, Muller WA, Hovig T, Brandtzaeg P. Isolation and longterm culture of human intestinal microvascular endothelial cells. Gut. 1995; 37: 225-234.

20. Rafiee P, Stein DJ, Nelson VM, Otterson MF, Shaker R, Binion DG. Thalidomide inhibits inflammatory and angiogenic activation of human intestinal microvascular endothelial cells (HIMEC), Am J Physiol Gastrointest Liver Physiol. 2010; 298: G167-176.

21. Bramuzzo M1, Ventura A, Martelossi S, Lazzerini M. Thalidomide for inflammatory bowel disease: Systematic review. Medicine (Baltimore). 2016 ; 95: e4239.

22. Rutella S, Fiorino G, Vetrano S, Correale C, Spinelli A, Pagano N, Arena V, Maggiano N, Repici A, Malesci A, Danese S. Infliximab therapy inhibits inflammation-induced angiogenesis in the mucosa of patients with Crohn's disease. Am J Gastroenterol. 2011; 106: 762-770.

23. Altorjay I, Veréb Z, Serfozo Z, Bacskai I, Bátori R, Erdodi F, Udvardy M, Sipka S, Lányi Á, Rajnavölgyi É, Palatka K. Anti-TNF-alpha antibody (infliximab) therapy supports the recovery of eNOS and VEGFR2 protein expression in endothelial cells Int J Immunopathol Pharmacol. 2011; 24: 323-335

24. Robbins and Cotran pathologic basis of disease, Ninth Edition ISBN: 978-1-4557-2613-4, [edited by] Vinay Kumar, Abul K. Abbas, Jon C. Aster ; by Saunders, Philadelphia, PA. 2015.

25. Zhou K, Mac Y, Brogan MS. Chronic and non-healing wounds: The story of vascular endothelial growth factor, Medical Hypotheses. 2015; 85: 399–404.

26. Scaldaferri F, Vetrano S, Sans M, et al. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 2009; 136 :585–595.

27. Kapsoritakis A, Sfiridaki A, Maltezos E, Simopoulos K, Giatromanolaki A, Sivridis E, Koukourakis MI. Vascular endothelial growth factor in inflammatory bowel disease.. Int J Colorectal Dis. 2003; 18: 418-422.

28. Kopanakis N, Saiti A, D'Avgerinos E, Masselou K, Simiri M, Mandaraka A, Vasiliadis G, Katergiannakis V. Serum VEGF and bFGF in patients with inflammatory bowel diseases. Ann Ital Chir. 2014; 85: 203-206.

29. Wedrychowicz A, Kowalska-Duplaga K, Jedynak-Wasowicz U, Pieczarkowski S, Sladek M, Tomasik P, Fyderek K. J Serum concentrations of VEGF and TGF-β1 during exclusive enteral nutrition in IBD. Wedrychowicz A, Kowalska-Duplaga K, Jedynak-Wasowicz U, Pieczarkowski S, Sladek M, Tomasik P, Fyderek K. J Pediatr Gastroenterol Nutr. 2011; 53: 150-155.

30. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, et al. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management. J Crohns Colitis. 2017; 11: 3-25.

31. Gionchetti P, Dignass A, Danese S, Magro Dias FJ, Rogler G, et al. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 2: Surgical Management and Special Situations. J Crohns Colitis. 2017; 11: 135-149.

32. Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. J Crohns Colitis. 2012; 6: 965-90.

33. Dignass A, Lindsay JO, Sturm A, Windsor A, Colombel JF, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. J Crohns Colitis. 2012; 6: 991-1030.