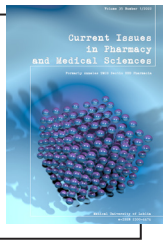
DOI: 10.2478/cipms-2023-0023 Curr. Issues Pharm. Med. Sci., Vol. 36, No. 3, Pages 136-139

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Perineural invasion in nearby tissue adjacent to colorectal carcinoma with CD166 stem cell marker expression

Mustafa Mohammed Ibraheem[1], Nawal Mustafa Abdullah[2*], Sawsan Salih Alharoon[3]

[1] Anatomy, Histology and Embryology, Al Mustansiriyah College of Medicine, Baghdad, Iraq
[2] Human Anatomy, University of Basrah, Al Zahraa College of Medicine, Iraq
[3] Pathology and Forensic Medicine, University of Basrah, Basrah Medical College, Iraq

ARTICLE INFO

Received 20 March 2022
Accepted 12 May 2023

Keywords:
perineural invasion,
colorectal carcinoma,
CD166 stem cell marker.

ABSTRACT

Pathological evaluation of colorectal cancer resection samples play a vital role in management and prognosis of postoperative cancer patients. Difficulties exist in the assessment and outcomes of these specimens for therapy protocol. PNI is a perineural invasion associated with increased mortality in many malignancies including colon cancer. In colorectal cancer (CRC) and nearby tissue, PNI evaluation as a potential prognostic indicator with the use of CD 166 stem cell marker remains to be clearly defined for providing a convenient information for future management and prognosis. The incidence and significance of histological neural invasion in nearby tissue was conducted in 52 patients with colorectal carcinoma operated on for the period from June 2017 to June 2020 retrospectively. Tumors were subjected to histopathological and immunohistochemical study (IHC) with CD166 stem cell marker for PNI in tissue adjacent to CRC. Data collected and analyzed, histopathological pictures was obtained and studied.

Outcomes showed that neural invasion was expressed by Cd166 stem cell marker as strong and severe in patients with stage B and C in tissues nearby tumor which reveals bad prognostic features.

Conclusions: Neurogenesis appeared to have a critical role in colorectal cancer progression. furthermore, current results indicated that neurogenesis functions as an independent predictor of outcomes for therapy protocol.

INTRODUCTION

The nervous system (NS) enhances development of tumor spread by controlling the metastatic cascades through release of many neural-related components via neurotrophins and neurotransmitters [1-3]. Perineural invasion (PNI) is an abnormal process of tumor invasion into perineural spaces by spreading along the peripheral nerves and nerve sheaths. This comes about through complex signaling between tumor cells, stromal cells and nerves (aka. neurotropic carcinomatous spread with neoplastic nerve involvement and inflammation) [4].

Colorectal cancer (CRC) is the third most deadly and fourth most commonly diagnosed cancer in the world [5,6]. Around 20% of all patients show metastatic disease at the time of diagnosis [7]. Different stem cell markers had been used to express colorectal carcinoma. Among these are CD166, which is an activated leukocyte cell adhesion molecule (ALCAM) and a trans membrane type-1 glycoprotein [8,9]. It is detectable in different tissues and cell types, including neuronal cells [8,9]. Its expression is documented to be significantly elevated in CRC [10].

Perineural invasion is associated with poor prognosis in several malignancies, but a wide variation in the reported prevalence of PNI in colon malignancies has been noticed. PNI is recognized as an important mode of regional spread, however, again variation in this is reported. Such variation in reportage and recognition possibly reflects differences in sampling, skill/diligence of histologic examination and in the characteristics of the cohorts studied. PNI appears to be underreported, with one study showing detection rates increasing from 0.5% on initial review, to 22% following expert review [11,12].

In operative CRC, an area near to the tumor will be left as normal adjacent to tumor (safe area), this is of apparently normal tissue, yet could express the IHC features of stem

* **Corresponding author**
e-mail: nawal.abdullah@uobasrah.edu.iq

cell neurotransmitters and neuropeptides with a direct action on cancer cell receptors [10,13].

AIM

This study was performed to determine the degree of peri neural invasion (PNI) in tissue around or nearby colorectal carcinoma by using CD166 markers, and, subsequently, to evaluate its prognostic value.

MATERIAL AND METHODS

A retrospective study was conducted on fifty two patients with CRC of both gender, of age ranged between 20-68 years at Al Sader teaching hospital and two histopathological labs at Basrah locality, for the period between June 2017 to June 2020.

All specimens were graded and staged according to Broders and Dukes classification. The Immunohistochemical assays (IHC) were performed by CD166 mouse monoclonal antibody diagnostic kits (clone 8E12C7dil1:300Abcam:) for 30 minutes. Sections were subsequently incubated with secondary antibody for 10 minutes, followed by HRP streptavidin for 10 min, after which DAB were used as a chromogen, followed by slight hematoxylin counterstaining. The prepared slides were evaluated in terms of expression of CD166 stem cell marker. The cytoplasmic and membranous expression of CD166 marker in tissues were evaluated semi-quantitatively, i.e. the ratio of positive tumor cells to all tumor cells, as well as the intensity of staining. Negative control slides were produced by omitting the primary antibody. A positive control from colorectal adenocarcinoma patients were treated with anti-CD166 and included in each run of staining protocol.

According to literature review, the cut-off point of CD166 marker expression was assumed to be 50%. Positive stains with cell percentages above the cut-off point were considered over-expression and those below were considered as moderate or low expression [14].

Attention was directed to the presence of neural involvement, which can be differentiated from vascular invasion by discovery of RBC within the lumen of blood vessels or thrombi of tumor cells inside the lumen of the vein, or muscle destruction of the vein wall by tumor cells. Non-specific vascular changes were noted. These included dilatations, endothelial proliferations and aneurysms [15-17].

Neural invasion was classified as either endo neural dilation or perineural invasion, PNI was defined as tumor cells within any layer of the nerve sheath or tumor in the perineural space that involved at least one third of the nerve circumference.

After data collection, Statistical analysis was performed by using SPSS software V22. The significance of the neural invasion was demonstrated via histopathological images through digital camera (LIECA LAS). Dispensing with consent was requested for this study and was approved by the institutional ethics committee of the college and health directorate in Basrah.

RESULTS

Fifty two patients took part in the study – 32 (61.5%) men and 20 (38.4%) women. Mean age was 65 years. No important variations in age or tumor histological grades were found between those patients with or without neural invasion. From IHC analysis, CD166 expression in area nearby the tumor was cytoplasmic in 35 cases (67.1%), while both cytoplasmic and membranous expression was found in 17 patients (32.6%). The staining intensity ranged from severe and strong in 38 patients (73%) and moderate in 14 patients (26.9%).

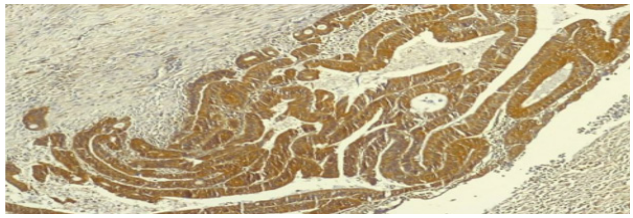


Figure 1. Cytoplasmic expression of CD166 in colorectal carcinoma (10×)

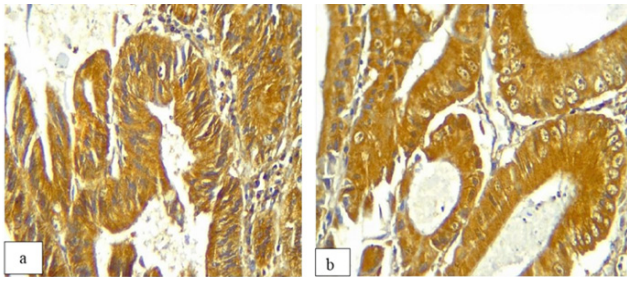


Figure 2. Cytoplasmic expression of CD166 immunostain in CRC (40×). a and b

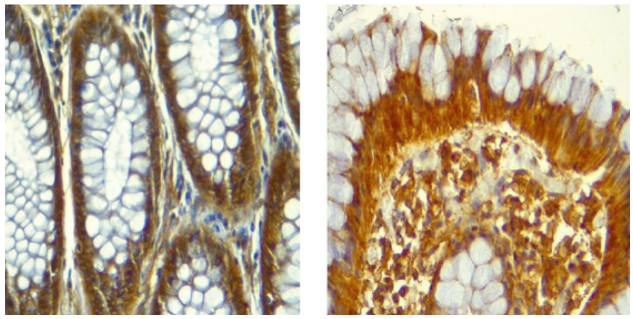
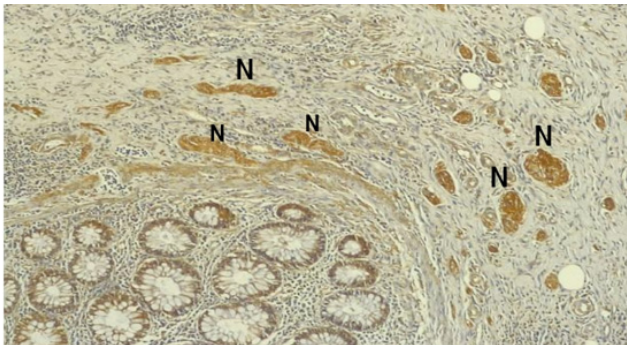


Figure 3. Cytoplasmic and membranous expression of CD166 in normal adjacent area to CRC (40×). a and b



N - nerve

Figure 4. A CD166 immunostain highlights the nerve in normal adjacent area to colorectal adenocarcinoma (10×)

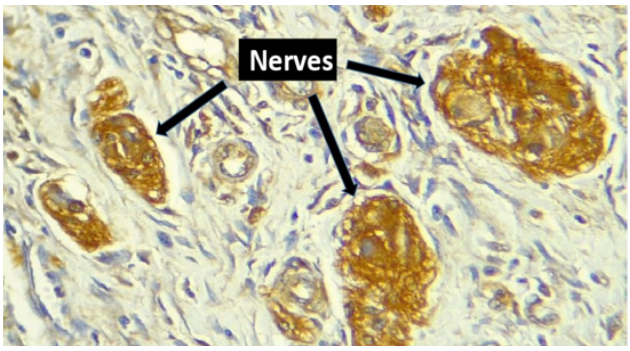
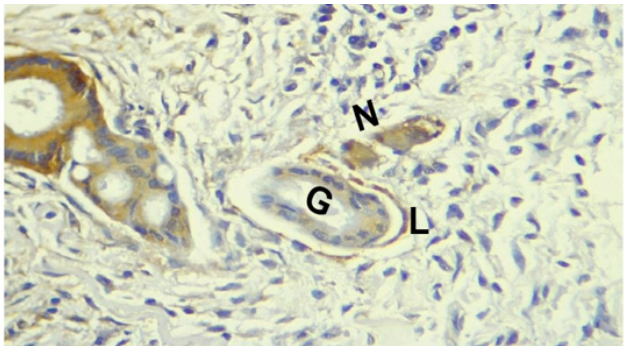


Figure 5. A CD166 immunostain highlights the nerve bundles in colorectal adenocarcinoma (40×)



N - nerve

Figure 6. A CD166 immunostain shows lymphatic vessel (L) invesion by adenocarcinomatous gland (G) (40×)

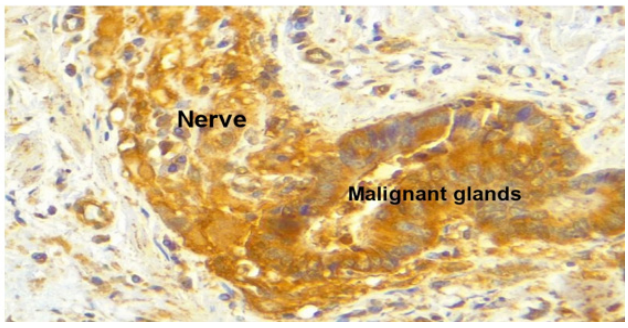


Figure 7. Perineural invasion is highlighted by a CD166 immunostain in colorectal adenocarcinoma (40×)

According to Dukes classification, From total number of cases, 15 patients had stage A with no neural invasion (0%), while 17 cases had stage B with nerve invasion in 8 cases (47%), 20 patients had Stage C with 11 cases with neural invasion (55%). Nerve involvement in most patients was in the form of endoneural involvement as in Table 1. Vascular involvement was found along with perineural invasion in the form of extramural and intramural vein involvement, both large and small vessels were invaded (Fig. 8).

Table 1. Neural invasion in relation to Dukes classification

Parameters			No	No	No
			Dukes classification		
	Age	Sex M\F	Stage A	Stage B	Stage C
	18-68	32\20 61\38%	15 (28.8%)	17 32.6%	20 38.8%
Number			15	17	20
Neural Invasion			0	8	11
% of invasion			0%	47%	55%

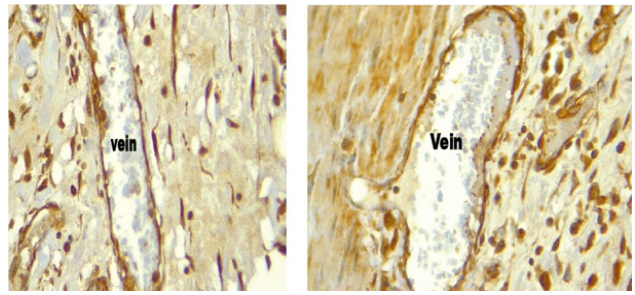


Figure 8. Expression of CD 166 immunostain in the wall of the vein in normal adjacent area to colorectal adenocarcinoma (40×). a and b

DISCUSSION

In spite of hard work to progress early detection and treatment for colorectal carcinoma, over one-third of all patients die yearly from this disease [17]. Up to now, adjuvant radiotherapy and chemotherapy have not been shown to be of great value in the management of this problem [18]. More criteria such as the presence of vascular and/or neural invasion may identify subgroups of patients who are suitable for adjuvant therapy in the scheduled clinical trials. The search for vascular and neural invasion using extra histologic procedures and new recent tissue stains, may gain valuable data for the management of patients with CRC [9,10].

Seefeld and Bargen were the first (since Cruvilhier in 1842) to study neural invasion in colon cancer, recognizing it in 30% of their patients. It is known that survival rates and incidence of metastases are much lower than when neural invasion was not found. In those patients with both vascular and neural invasion, prognosis is the worst [19]. Previous work has documented that the peripheral nerves are potentially important elements of the tumor microenvironment and the tumor stromal interactions, and play a critical role in tumor development and progression. Matrix renovation, Angiogenesis and inflammation are examples of the dynamic stromal methods essential in the tumor micro environment and tumor development [20-26].

Many cancer-related adhesion molecules are involved in cancer pathophysiology. Among these are CD166 stem cell markers in both normal and diseased tissues. The tumor innervation is attributed to the ability of cancer cells to attract normal nerve fibers through secretion of signallin [1]. Recent studies has demonstrated that CD166 stem cell malfunction, overexpression, or loss of expression may contribute to the break of tumor cells and therefore to local invasion and neutrotropic malignant spread of the nerves and progression of the tumor [27-29], and play a critical role in tumor development and progression. Matrix renovation, Angiogenesis and inflammation are examples of the dynamic stromal methods essential in the tumor micro environment and tumor development [30,31].

Most patients covered by our study had severe expression of CD166 and thus poor outcome in colon cancer, and the outcome of our work reinforces other studies that focussed on the PNI as a marker for more aggressive tumor phenotypes and poor prognosis in several malignancies - commonly head and neck and prostate cancers [32,33]. Furthermore, such articles report that tumor microenvironment

initiate a feedback loop with the nervous system, enabling the growth of primary and secondary tumors, and that metastasis is initiated when unhinged tumor cells alter to a nearby site microenvironment by increasing cellular plasticity and stemness due to the action of proteolytic enzymes such as matrix metalloproteinase [34]. These MMPs are initiated by neural factors and neurotransmitters which are overexpressed in tumors [35].

Current study in our locality agree with others that when neural invasion was detected, the neurogenesis functions as an independent predictor of antagonist, hence works as an indication of poor survival and recurrence in colorectal cancer.

AUTHORS' CONTRIBUTIONS

In accordance with blinded peer-review rules, data will be provided in a separated file.

ORCID iDs

Mustafa Mohammad Ibraheem
https://orcid.org/0000-0002-0572-2593
Nawal Mustafa Abdullah
https://orcid.org/0000-0003-2455-3534

REFERENCES

1. Albo D, Akay CL, Marshall CL, Wilks JA, Verstovsek G, Liu H, et al. Neurogenesis in colorectal cancer is a marker of aggressive tumor behavior and poor outcomes. *Cancer.* 2011;117(21):4834-45.
2. Seifert P, Spitznas M. Tumours may be innervated. *Virchows Arch.* 2001;438:228-31.
3. Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner M-J. SEER Survival Monograph: Cancer Survival Among Adults: U.S. SEER Program, 1988-2001, Patient and Tumor Characteristics. National Cancer Institute, SEER Program. *NIH Pub.* No. 07-6215. Bethesda; 2007.
4. Beard CJ, Chen MH, Cote K, Loffredo M, Renshaw AA, Hurwitz M, et al. Perineural invasion is associated with increased relapse after external beam radiotherapy for men with low-risk prostate cancer and may be a marker for occult, high-grade cancer. *Int J Radiat Oncol Biol Phys.* 2004;58:19-24.
5. Dawson MD, Kirsch R, Messenger D, Driman D. A review of current challenges in colorectal cancer reporting heather. *Arch Pathol Lab Med.* 1992;143(7):869-882.
6. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011;61(2):69-90.
7. Rozen P, Fireman Z. Early detection of colorectal tumors in high risk groups(Abstr). *Harefuah.* 1981;5:214.
8. Levin TG, Powell AE, Davies PS, Silk AD, Dismuke AD, Anderson EC, et al. Characterization of the intestinal cancer stem cell marker CD166 in the human and mouse gastrointestinal tract. *Gastroenterology.* 2010;139:2072-82.
9. Liebig C, Ayala G, Wilks J, Verstovsek G, Liu H, Agarwal N, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J Clin Oncol.* 2009;27(31):5131-7.
10. Yang Y, Huang X, Sun J, Gao P, Song Y, Chen X, et al. Prognostic value of perinrural invasion in CRC: a meta-analysis. *J Gastrointest Surg.* 2015;19(6):1113-22.
11. Krasna MJ, Flancbaum L, Cody RP, Shneibaum S, Ben Ari G. Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. *Cancer.* 1988;61:1018-23.
12. Knijn N, Mogk SC, Teerenstra S, Nagtegaal ID. Perineural invasion is a strong prognostic factor in colorectal cancer: A systematic review. *Am J Surg Pathol.* 2016;40(1):103-12.
13. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prze Gastroenterol.* 2019;14(2):89-103.
14. Zlobec I, Steele R, Terracciano L, et alSelecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. *J Clin Pathol.* 2007;60:1112-6.
15. Brown CE, Warren S. Visceral metastasis from rectal carcinoma. *Surg Gynecol Obstet.* 1938;66:611-21.
16. Bums FJ, Pfaff J. Vascular invasion in carcinoma of the colon and rectum. *Am J Surg.* 1956;92(5):704-9.
17. *Cancer Facts & Figures 2008*. American Cancer Society, Atlanta; 2008.
18. Bentzen SM, Balslev I, Pedersen M, Teglbjaerg PS, Hanberg-Sørensen F, Bone J, et al. Time to loco-regional recurrence after resection of Dukes' B and C colorectal cancer with or without adjuvant postoperative radiotherapy. A multivariate regression analysis. *Br J Cancer.* 1992;65(1):102-7.
19. Seefeld PH, Bargen JH. The spread of cancer of the rectum: Invasion of the lymphatics, veins and nerves. *Ann Surg.* 1943;1(18):76-89.
20. Fagan JJ, Collins B, Barnes L, D'Amico F, Myers EN, Johnson JT. Perineural invasion in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg.* 1998;124:637-40.
21. Goepfert H, Dichtel WJ, Medina JE, Lindberg RD, Luna MD. Perineural invasion in squamous cell skin carcinoma of the head and neck. *Am J Surg.* 1984;148:542-7.
22. O'brien CJ, Lahr CJ, Soong S-J, Gandour MJ, Jones JM, Urist MM, et al. Surgical treatment of early-stage carcinoma of the oral tongue – would adjuvant treatment be beneficial? *Head Neck. 1*986;8:401-8.
23. Beard CJ, Chen MH, Cote K, Loffredo M, Renshaw AA, Hurwitz M, D'Amico AV. Perineural invasion is associated with increased relapse after external beam radiotherapy for men with low-risk prostate cancer and may be a marker for occult, high-grade cancer. *Int J Radiat Oncol Biol Phys.* 2004;58:19-24.
24. Ichiro H, Wataru K, Koichiro O, Shun K, Koichi S, Hiroshi K, et al. Perineural invasion in pancreatic cancer. *Pancreas.* 2002;24(1):15-25.
25. Takahashi T, Ishikura H, Motohara T, Okushiba S, Dohke M, Katoh H. Perineural invasion by ductal adenocarcinoma of the pancreas. *J Surg Oncol. 1*997;65:164-70.
26. Okada Y, Eibl G, Guha S, Duffy JP, Reber HA, Hines OJ. Nerve growth factor stimulates MMP-2 expression and activity and increases invasion by human pancreatic cancer cells. *Clin Exp Metastasis.* 2004;21:285-92.
27. Chedotal A, Kerjan G, Moreau-Fauvarque C. The brain within the tumor: New roles for axon guidance molecules in cancers. *Cell Death Differ.* 2005;12:1044-56.
28. Cornell RJ, Rowley D, Wheeler T, Ali N, Ayala G. Neuroepithelial interactions in prostate cancer enhanced in the presence of prostatic stroma. *Urology.* 2003;61(4):870-5.
29. Ayala GE, Wheeler TM, Shine HD, Schmelz M, Frolov A, Chakraborty S, et al. In vitro dorsal root ganglia and human prostate cell line interaction: Redefining perineural invasion in prostate cancer. *Prostate.* 2001;49:213-23.
30. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer. *Cancer.* 2009;115:3379-91.
31. Kurtz KA, Hoffman HT, Zimmerman MB, Robinson RA. Perineural and vascular invasion in oral cavity squamous carcinoma: Increased incidence on re-review of slides and by using immunohistochemical enhancement. *Arch Pathol Lab Med.* 2005;129(3):354-9.
32. Rubin MA, Mucci NR, Manley S, Sanda M, Cushenberry E, Strawderman M, et al. Predictors of Gleason pattern 4/5 prostate cancer on prostatectomy specimens: Can high grade tumor be predicted preoperatively? *J Urol.* 2001;165(1):114-8.
33. de la Taille A, Katz A, Bagiella E, Olsson C, O'toole K, Rubin M. et al. Perineural invasion on prostate needle biopsy: An independent predictor of final pathologic stage. *Urology.* 1999;54:1039-43.
34. Soo KC, Carter RL, O'Brien CJ, Bar L, Bliss JM, Shaw HJ. Prognostic implications of perineural spread in squamous carcinomas of the head and neck. *Laryngoscope.* 1986;96(10):1145-8.
35. Visioli A, Giani F, Trivieri N, Pracella R, Miccinilli E, Cariglia MG, et al. Stemness underpinning all steps of human colorectal carcinoma defines the core of effective therapeutic strategies. *EBio Medicine.* 2019;44:346-60.