

Immunohistochemical expression of CD163 in colorectal carcinoma and its prognostic value

Noha N. Yassen^a, Marwa E. Shabana^a, Maha K. Desouky^b, Manal A. Badawi^a, Dalia M. Abouelfadl^a

^aDepartment of Pathology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, ^bDepartment of Anatomy, Faculty of Medicine, Minia University, Minia, Egypt

Correspondence to Noha N. Yassen, MD, Department of Pathology, Medical Research and Clinical Studies Institute, National Research Centre, Cairo, Egypt.
E-mail: noha.nazeeh@gmail.com

Received: 11 August 2022

Revised: 23 September 2022

Accepted: 3 October 2022

Published: 23 May 2023

Journal of The Arab Society for Medical Research 2023, 18:43–50

Background/aim

Colorectal cancer (CRC) is one of the most frequent cancers worldwide with one of the highest mortality rate. CD163 is a 130-KDa transmembrane protein, known to be involved in hemoglobin clearance by functioning as a receptor for hemoglobin-haptoglobin complex. Several studies have demonstrated that CD163 is expressed on some cancer cells, and its expression is associated with poor clinical prognosis. This study aimed to evaluate CD163 to assess the distribution of macrophages in invasive margins and intratumoral infiltration area, using computerized image analysis, to evaluate its prognostic value and its association with other clinicopathological characteristics in colorectal carcinoma.

Patients and methods

The present study enrolled 80 formalin-fixed paraffin-embedded CRC surgical specimens, obtained from the Department of Pathology of National Research Centre, Egypt, and examined for the expression of CD163 in CRCs by immunohistochemical techniques. The morphometric analysis was done on the invasive margins and intratumoral infiltration area in each slide.

Results

The study cases of colorectal carcinoma patients, 46 (57.5%) cases were diagnosed as adenocarcinoma and rest 34 (42.5%) cases were mucinous carcinoma. Twenty-two cases (22/80) showed a low intratumoral infiltration area and moderate invasive margin. CD163+ intratumoral infiltration significantly correlated with age, tumor site, tumor type, lymph node status, and metastasis. On the other hand, CD163+ invasive margins significantly correlated with tumor grade, tumor classification, metastatic status, tumor stage, and Duke's classification.

Conclusion

Invasive front of the tumor is the most suitable area for the evaluation of tumor-associated macrophages that is important in detecting prognostic prediction of colon cancer and its clinical outcome. So, it can be considered as an ideal prognostic marker in the treatment of colon cancer.

Keywords:

CD163, colorectal carcinoma, intratumoral infiltration, invasive margins

J Arab Soc Med Res 18:43–50

© 2023 Journal of The Arab Society for Medical Research
1687-4293

Introduction

Colorectal cancer (CRC) is one of the most frequent cancers worldwide with one of the highest mortality rate. CRC results in more than 600 000 deaths annually, with more increasing mortality rate [1]. In Egypt, colorectal carcinoma is the seventh most common cancer, representing 3.47% of male cancers and 3% of female cancers. Early diagnosis of CRC improves five-year survival to about 90% compared with 8% when diagnosed at an advanced stage of the disease [2,3]. Although patients with CRC are usually treated with surgical resection combined with chemotherapy, their prognosis is poor and so it is necessary to develop new strategies of treatment [4]. Consequently, there is increasing needs for improved prognostic and predictive markers in CRC that help guide treatment decision. Recent studies have

indicated that aggressiveness of the tumor and therapy resistance is affected with action between tumor cells and the microenvironment [5].

Cancers are not only formed of malignant cells, but they are formed of a complex environment of stromal cells, including vascular cells, macrophages, fibroblast, and inflammatory cells. There are many studies that confirmed that densities and localization of tumor-associated macrophages (TAM) are associated with poor clinical outcome in many types of solid cancers, such as bladder, renal, gastric, and prostate cancers.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

The presence of TAM in tumor stroma predicts worse prognosis, while its location in tumor islets indicates better CRC survival [6]. Some studies have confirmed that higher macrophage infiltration correlated with worse prognosis and advanced stage of the tumor [7].

Immunotherapy with immune checkpoint inhibitors has recently an important role in cancer treatment. The effect of immunotherapy can be improved by tumor-infiltrating lymphocytes; its function is regulated by M2 macrophages in the tumor microenvironment (TME) especially solid tumors [8].

Macrophages are plastic cells that respond and adapt to external stimuli; they are present in TME as one of the most abundant cells which function in response to signals of the microenvironment either stromal or tumor cells [9]. Recently, the most known models of macrophages are M1 which are activated macrophages. They can promote antitumor immune response and wound healing. M2 is an alternatively activated macrophage, which is anti-inflammatory, plays an important role in promoting tumor growth, lymphangiogenesis, angiogenesis, migration, and intravasation of tumor cells, thus leading to suppression of antitumor immune response [8,10].

CD163 is a 130-KDa transmembrane protein, known to be involved in hemoglobin clearance by functioning as a receptor for the hemoglobin-heptoglobin complex [8]. Now, CD163 is widely used as a marker for macrophages and monocytes. Several studies have demonstrated that CD163 is expressed on some cancer cells, and its expression is associated with poor clinical prognosis as in breast cancer and bladder cancer [11]. However, the monocyte-macrophage marker CD163 has not been widely investigated in CRC patients [12]. Shabo *et al.* [13] suggested that CD163 expression in cancer cells is caused by cell fusion between macrophages and cancer cells.

Considering all the above points, the present study is designed to set up the value of CD163 to assess macrophage distribution in invasive margins and intratumoral infiltration area, using computerized image analysis, to evaluate its prognostic value and its association with other clinicopathological characteristics in colorectal carcinoma.

Patients and methods

Specimens and study design

Paraffin blocks from 80 CRC cases were retrospectively obtained from the Department of Pathology of

National Research Centre, Egypt, and database of cases were reviewed. Cases who underwent surgery were included in this study, and patients who had adjuvant therapy or chemotherapy were excluded. Two sections each of 4 µm thickness were cut from paraffin blocks using a Leica RM 2155 microtome. One section was stained with hematoxylin and eosin for histopathological evaluation and grading. The other section was mounted on positively charged glass slides for immunohistochemical staining.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study was approved by the Ethics Committee of National Research Center, Cairo, Egypt, under approval number 16/308.

Histopathology

The histopathological characteristic was reviewed and diagnosis was verified by two pathologists. Clinicopathological data were retrospectively collected from records and summarized.

Immunohistochemistry

CD163 marker is used as a specific marker to detect M2 macrophages (function). The paraffin blocks were cut by microtome at 4 µm thickness. Sections were mounted on positively charged slides. The slides were incubated at 37°C overnight for accurate adhesion of the section on the slide. Tissues were deparaffinized; hydrated endogenous peroxidase activity was blocked with 3% methanol in hydrogen peroxidase for 10 min. Following antigen retrieval in a water bath at 98°C with Tris EDTA, pH9 (CD163 : 40 min), the primary antibody was incubated CD163 : 30 min (1 : 100) at room temperature. After washing, the labeled polymer secondary antibody was added to the slide, and peroxidase activity was detected using diaminobenzidine-tetrahydrochloride liquid plus substrate chromogen system. The reaction was stopped with distilled water, and the sections were counterstained with hematoxylin and mounted on Richard-Allan Scientific mounting medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA). For positive control, normal human tonsil was used, and for negative control, primary antibody was replaced by normal mouse serum. Following immunohistochemistry, the slides were visualized and photographed using the light microscope Olympus CX41 with DB 12 Olympus digital camera (Olympus Optical Corp., Ltd, Tokyo, Japan).

Image analysis immunoscore

The morphometric analysis was performed at the Department of Pathology, National Research Center, using a Leica Qwin 500 Image Analyzer (Leica Imaging Systems Ltd, Cambridge, UK). Morphometric analysis was carried out on CD163 immunostained slides. The slides to be examined were placed on the stage of the microscope. The light source was set to the required level. Successful adjustment of illumination was checked for on the monitor.

The density of CD163 was determined by quantitative evaluation by screening the sections and selecting at least five different fields with high cellular areas. Morphometric analysis was done on the invasive margins and intratumoral infiltration area in each slide stained with CD163 using objective lens with a magnification of $\times 200$. The positive stained cells were masked automatically by a blue mask, which is known as the binary image. We use a software program to measure the area percentage of the detected features in the binary image automatically in a frame area of $3905.5 \mu\text{m}^2$, and the results appeared in a table form. The area percentage of each of CD163 positively stained cells was determined as an area per field in micrometer square using the interactive measurement software of the system. Overlapping nuclei were not measured. Any area of necrosis as well as processing-related artifacts were erased.

Statistical methods

All data were collected, revised, coded, and entered into the Statistical Package for the Social Sciences (IBM SPSS), version 23.1 New Orchard Road, Armonk, New York 10504-1722, United States. The quantitative data were presented as mean, SD, and ranges when parametric and median. Also, qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done using the χ^2 test. *P* values of less than 0.05 were considered to indicate statistical significance.

Results

The study population included 80 CRC patients: there were 36 (45%) males and 44 (55%) females. The age of the patients ranged from 22 to 82 years, 40 (50%) of them were less than 60 years. Tumor size ranged from 2 to 11 cm; 52 (65%) of them were ≥ 4.5 cm. According to their sites, 34 (42.5%) were in the right colon, 26 (32.5%) in the left colon, and 20 (25%) were located in the rectum. Forty-six cases were diagnosed as adenocarcinoma and the rest 34 cases were

Table 1 Clinicopathological features of colorectal carcinoma patients

Clinicopathological features	Number of patients (N=80) [n (%)]
Sex	
Male	36 (45)
Female	44 (55)
Age (years)	
<60	40 (50)
≥ 60	40 (50)
Tumor size	
<4.5	28 (35)
≥ 4.5	52 (65)
Tumor site	
Right	34 (42.5)
Left	26 (32.5)
Rectum	20 (25)
Tumor type	
Adenocarcinoma	46 (57.5)
Mucinous	34 (42.5)
Tumor grade	
I	0
II	74 (92.5)
III	6 (7.5)
T-classification	
T1	2 (2.5)
T2	6 (7.5)
T3	52 (65)
T4	20 (25)
N-classification	
N0	34 (42.5)
N1	20 (25)
N2	26 (32.5)
M-classification	
M0	72 (90)
M1	8 (10)
Stage	
I	6 (7.5)
II	26 (32.5)
III	40 (50)
IV	8 (10)
Duke's classification	
A	0
B	34 (42.5)
C	38 (47.5)
D	8 (10)

diagnosed as mucinous carcinoma. Table 1 shows all the clinicopathological characteristics of the studied cases.

Immunoscore of CD163+ at invasive margins and intratumoral infiltration area

Fifty-four cases showed low intratumoral infiltration of CD163+ cells, with mean area percent of 2.75 ± 3.21 and ranging from 0.31 to 4%. Twelve cases showed moderate intratumoral infiltration CD163+ cells

with a mean area percentage of 4.72 ± 1.3 and ranging from 4.1 to 7.9%. Fourteen cases showed high intratumoral infiltration of CD163+ cells with mean area percentage of 11.44 ± 2.83 and ranging from 8 to 15.27% (Table 2).

On the other hand, regarding invasive margins of CD163+ cells, 32 cases showed low expression, with mean area percentage of 1.35 ± 0.69 and ranging from 0.45 to 1.5%. Thirty-six cases showed moderate expression with mean area percentage of 2.68 ± 1.34

Table 2 Immunostaining of CD163+ intratumoral infiltration and invasive margin located cells within the tumor tissue

	Low	Moderate	High
CD163+ intratumoral			
Number of patients	54	12	14
Range (%)	0.31–4	4.1–7.9	8–15.27
Mean $\pm$ SD	2.38 $\pm$ 3.22	4.72 $\pm$ 1.30	11.44 $\pm$ 2.83
CD163+ invasive margin			
Number of patients	32	36	12
Range (%)	0.45–1.5	1.6–3.9	4–7.68
Mean $\pm$ SD	1.35 $\pm$ 0.69	2.68 $\pm$ 1.34	5.47 $\pm$ 1.42

Table 3 CD163+ intratumoral infiltration and clinicopathological features of cases

	Low (N=54)	Moderate (N=12)	High (N=14)	Total (N=80) [n (%)]	P value
Sex					
Male	28	2	6	36 (45)	0.150
Female	26	10	8	44 (55)	
Age (years)					
<60	22	8	10	40 (50)	0.040*
≤60	32	4	4	40 (50)	
Tumor size					
<4.5	26	0	2	28 (35)	0.070*
≥4.5	28	12	12	52 (65)	
Tumor site					
Right	18	8	8	34 (42.5)	0.001*
Left	20	2	4	26 (32.5)	
Rectum	16	2	2	20 (25)	
Tumor type					
Adenocarcinoma	32	6	8	46 (57.5)	0.020*
Mucinous	22	6	6	34 (42.5)	
Tumor grade					
I	0	0	0	0	0.500
II	50	12	12	74 (92.5)	
III	4	0	2	6 (7.5)	
T-classification					
T1	2	0	0	2 (2.5)	0.220
T2	6	0	0	6 (7.5)	
T3	34	8	10	52 (65)	
T4	12	4	4	20 (25)	
N-classification					
N0	20	8	6	34 (42.5)	0.001*
N1	16	2	2	20 (25)	
N2	18	2	6	26 (32.5)	
M-classification					
M0	50	10	12	72 (90)	0.050*
M1	4	2	2	8 (10)	
Stage					
I	4	2	0	6 (7.5)	0.110
II	18	4	4	26 (32.5)	
III	28	4	8	40 (50)	
IV	4	2	2	8 (10)	
Duke's classification					
A	0	0	0	0	0.230
B	22	6	6	34 (42.5)	
C	28	4	6	38 (47.5)	
D	4	2	2	8 (10)	

*Significant difference at P value less than 0.05 using the χ^2 test.

and ranging from 1.6 to 3.9%. Twelve cases were highly expressed of invasive margins CD163+ cells with mean area percentage of 5.47 ± 1.42 and ranging from 4 to 7.68% (Table 2).

CD163+ intratumoral infiltration of expressed cells significantly correlated with age, tumor site, tumor type, lymph node status, and metastasis (Table 3). On the other hand, CD163+ invasive margins of expressed cells was significantly correlated with tumor grade, tumor classification, metastatic status, tumor stage, and Duke's classification (Table 4).

Comparison between intratumoral infiltration and marginal CD163+ expressed cells

Twenty-four cases (24/80) showed low expression of CD163+ cells in both intratumoral infiltration area and invasive margin. Twenty-two cases (22/80) showed low intratumoral infiltration area and moderate invasive margin. Eight cases (8/80) showed low intratumoral infiltration area but high at the invasive margin (Table 5).

Examination of all specimens under light microscope showed high CD163+ at the invasive margin than

Table 4 CD163+ invasive margin and clinicopathological features of cases

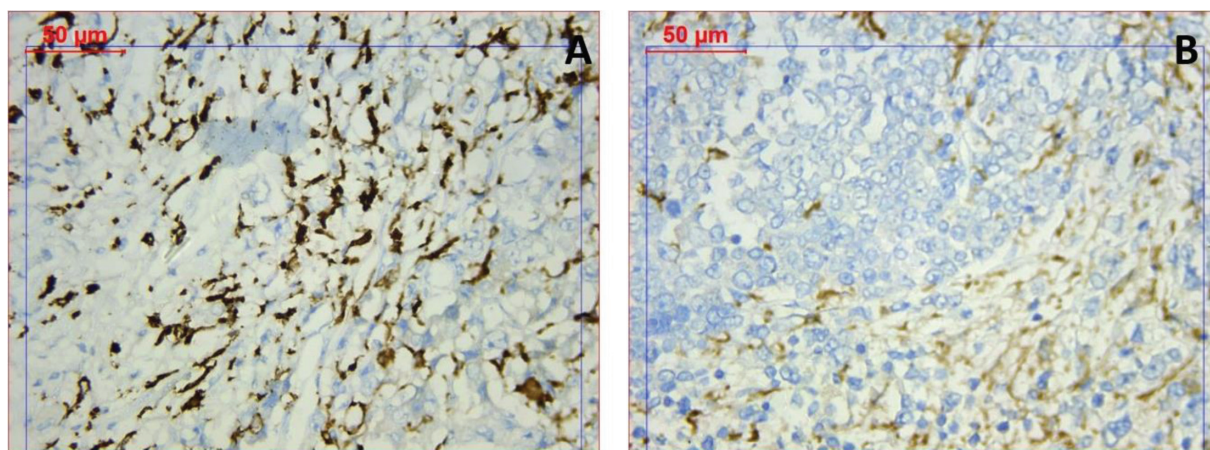
	Low (N=32)	Moderate (N=36)	High (N=12)	Total (N=80) [n (%)]	P value
Sex					
Male	12	20	4	36 (45)	0.670
Female	20	16	8	44 (55)	
Age (years)					
<60	16	16	8	40 (50)	0.150
≥60	16	20	4	40 (50)	
Tumor size					
<4.5	16	10	2	28 (35)	0.240
≥4.5	16	26	10	52 (65)	
Tumor site					
Right	12	16	6	34 (42.5)	0.530
Left	8	12	6	26 (32.5)	
Rectum	12	8	0	20 (25)	
Tumor type					
Adenocarcinoma	20	18	8	46 (57.5)	0.500
Mucinous	12	18	4	34 (42.5)	
Tumor grade					
I	0	0	0	0	0.007*
II	28	34	12	74 (92.5)	
III	4	2	0	6 (7.5)	
T-classification					
T1	2	0	0	2 (2.5)	0.018*
T2	4	2	0	6 (7.5)	
T3	20	26	6	52 (65)	
T4	6	8	6	20 (25)	
N-classification					
N0	14	18	2	34 (42.5)	0.640
N1	10	10	0	20 (25)	
N2	8	8	10	26 (32.5)	
M-classification					
M0	30	34	8	72 (90)	0.050*
M1	2	2	4	8 (10)	
Stage					
I	4	2	0	6 (7.5)	0.040*
II	10	14	2	26 (32.5)	
III	16	18	6	40 (50)	
IV	2	2	4	8 (10)	
Duke's classification					
A	0	0	0	0	0.038*
B	14	18	2	34 (42.5)	
C	16	16	6	38 (47.5)	
D	2	2	4	8 (10)	

*Significant difference at P value less than 0.05 using the χ^2 test.

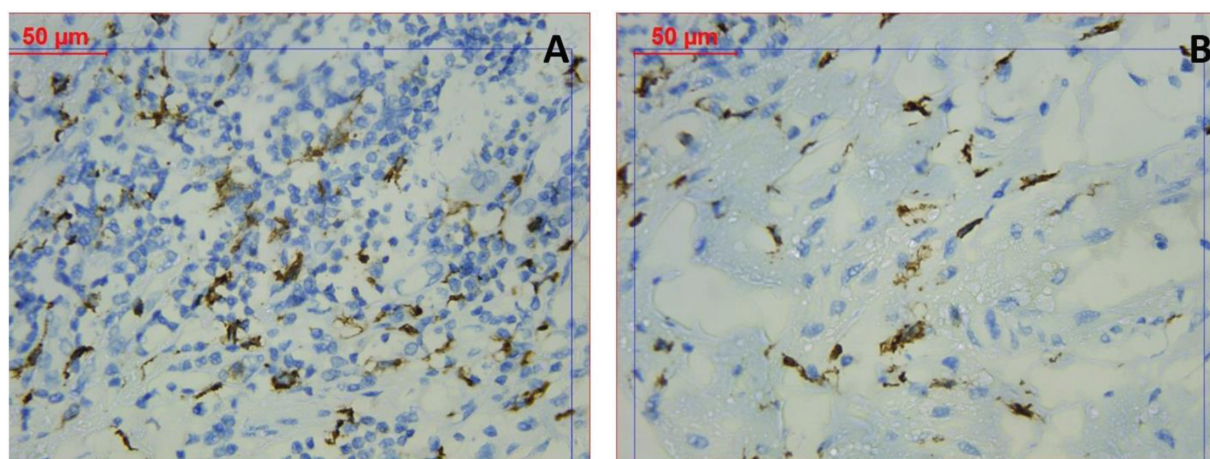
Table 5 Comparison between CD163 intratumoral and CD163 invasive margin located cells in the tumor tissue

CD163 intratumoral	CD163 invasive margin	Low [n (%)]	Moderate [n (%)]	High [n (%)]	Total [n (%)]	P value
Low		24 (75)	4 (12.5)	4 (12.5)	32 (100)	0.043*
Moderate		22 (61)	6 (16.7)	8 (22.3)	36 (100)	
High		8 (66.6)	2 (16.7)	2 (16.7)	12 (100)	
Total		54 (100)	12 (100)	14 (100)	80 (100)	

*Significant difference at *P* less than 0.05 using the χ^2 test.

Figure 1

Immunohistochemical staining of CD163+ macrophages in colorectal carcinoma: (a) high infiltration in the invasive margin, (b) low infiltration of intratumoral cells (original magnification $\times 200$).

Figure 2

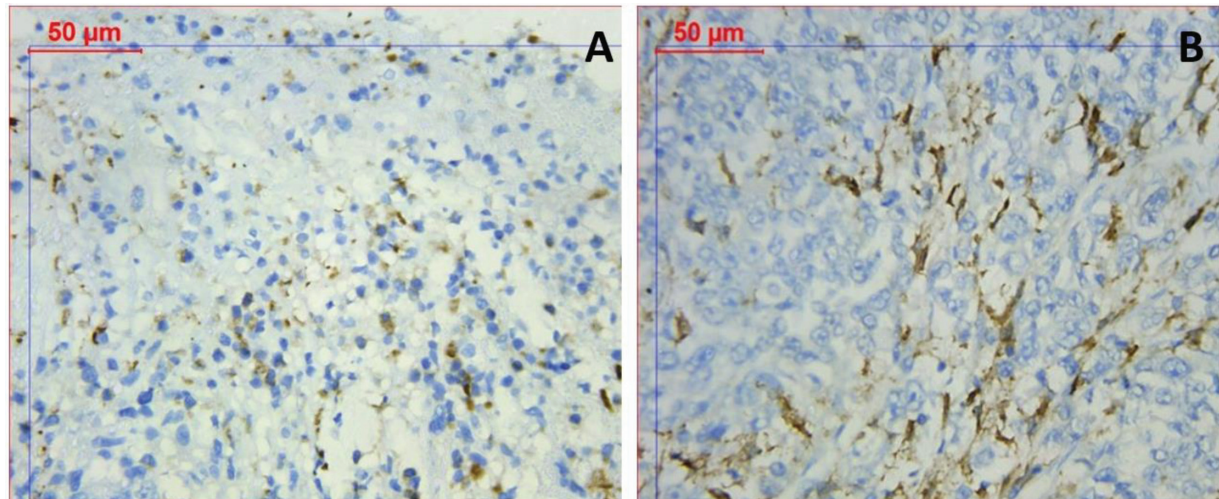
Immunohistochemical staining of CD163+ macrophages in colorectal carcinoma showing (a) moderate infiltration in the invasive margin and (b) low intratumoral infiltration (original magnification $\times 200$).

intratumoral invasion (Fig. 1) and moderate CD163+ at both sites: the invasive margin and intratumoral invasion (Fig. 2), while low CD163+ at the invasive margin than intratumoral invasion (Fig. 3), and finally the CD163+ expression is moderate at the invasive margin with high intratumoral invasion (Fig. 4).

Discussion

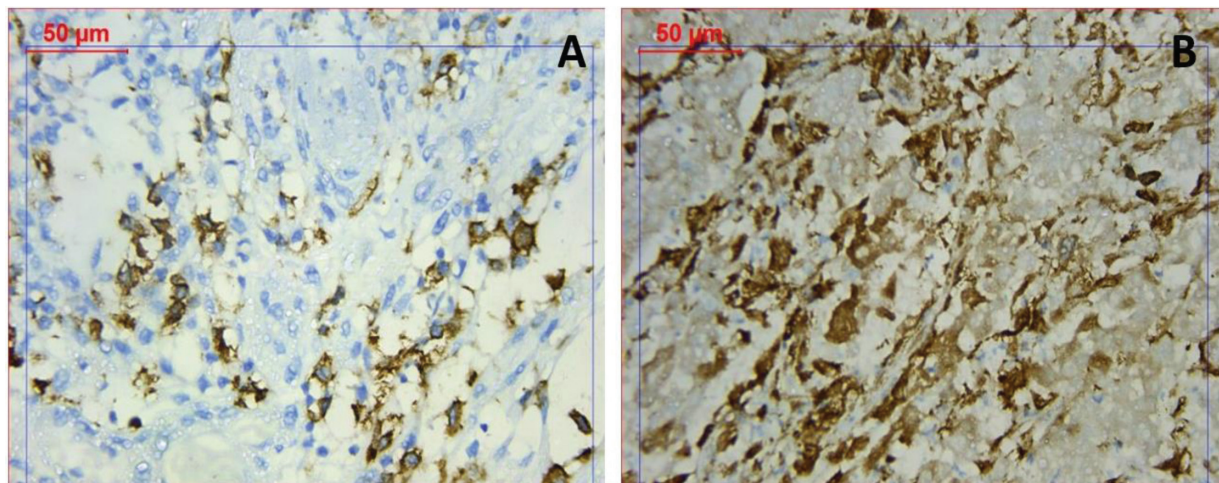
CRC is the third primary cause of death in the world. TME, which includes many factors such as fibroblast, blood vessels, immune cells, paracrine glands, and extracellular matrix, plays a vital role in tumor progression, so it becomes an important aspect in

Figure 3



Immunohistochemical staining of CD163+ macrophages in colorectal carcinoma showing (a) low infiltration invasive margin, (b) moderate intratumoral infiltration (original magnification $\times 200$).

Figure 4



Immunohistochemical staining of CD163+ macrophages in colorectal carcinoma showing (a) moderate infiltration at the invasive margin and (b) high intratumoral infiltration (original magnification $\times 200$).

oncology research. In particular, innate immune cells present in the microenvironment participates in several stages of tumor progression, and TAMs have an important role in tumorigenesis by supporting tumor progression, angiogenesis, invasion, and metastasis [14].

TAMs are one of the most dynamic cells in CRC that are associated with cancer development. The plasticity of macrophages determines the polarization state and the function of macrophages differentiate with its phenotype and tumor type. M1 is related to the early stage of the tumor that can induce inflammatory response and phagocytosis, while M2 polarization inhibits antitumor immune response with changes in the TME. In most human cancers, a large number of TAM are significantly related to poor disease prognosis [15].

CD163, a macrophage-associated molecule, has been suggested to be a predictive biomarker in patients with solid tumors. So, it has great potential to be a therapeutic target for solid tumor treatment [8]. Kwak *et al.* [7] used the M2 phenotype-associated macrophages to conduct an immunoquantitative analysis of advanced CRC using CD163+ macrophages in primary lesions.

TAMs are distributed in different anatomical locations in colon cancer, either in the center of the tumor or invasive front of the tumor. This different location may include variation in different biological and prognostic characteristics [16].

In the present study, we used quantitative evaluation of area percentage of the positive cells to determine the

density of CD163 in intratumoral infiltration area and invasive margin site in cases of CRC by immunohistochemistry.

Some previous studies have proved the correlation of M2 macrophages with clinicopathological parameters on different anatomical localizations [16]. In our study, the central expression of CD163-positive cells had significant correlation with age of the patient, site, and type of cancer. Moreover, CD163 immune expression was associated with lymphatic invasion and metastasis. This also was confirmed by Kwak *et al.* [7], who showed that CD163 expression in the CRC tissue was related to the degree of lymphatic metastasis and poor survival. Also, with our line of results [14] it has been shown that M2 macrophages is present in high concentrations at the invasive margin, but also fewer was found in the intratumoral area. So, this leads to remodeling of lymphatic networks, thus helping the invasion of tumor cells and promoting metastasis. In our study, we found that increased CD163+ macrophages expression at the invasive site of the tumor significantly correlates with metastatic status of the tumor, Duke's classification, tumor grade, and stage. This is in agreement with Kwak *et al.* [17] and Ding *et al.* [18], who confirmed that CD163+ TAM infiltration at the invasive front of the tumor is significantly related to poor prognosis of CRC patients and may play a role in increased invasion and spread of the tumor. On the opposite side [19], Huang and colleagues had proved that there was no relation between CD163 expression and both grade and stage of cancer. Algars *et al.* [20] showed that interstitial infiltration of CD163+ TAMs in colorectal carcinoma was associated with significant improvement in survival rate and may have a antitumor role. On the opposite side, Shabo *et al.* [13] proved that macrophage density of the stroma was related to poor survival.

Conclusion

These results showed that invasive front of the tumor is the most suitable area for the evaluation of M2 TAM that is important in detecting prognostic prediction of colon cancer and its clinical outcome. Therefore, the presence of CD163+ at the invasive front is considered an ideal prognostic marker in the treatment of colon cancer.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin* 2018; 68:394–424.
- Ibrahim AS, Khaled HM, Mikail NN, Barka H, Kamel H. Cancer incidence in Egypt. Results of the national population-based cancer registry program. *J Cancer Epidemiol* 2014; 2014:437971.
- Metwally I, Shetiwy M, Elalfy A, Abouzid A, Saleh S, Hamdy M. Epidemiology and survival of colon cancer among Egyptians: a retrospective study. *J Colorectal* 2018; 38:24–29.
- Gollins S, Sebag-Montefiore D. Neoadjuvant treatment strategies for locally advanced rectal cancer. *Cancer Oncol* 2016; 28:146–151.
- Korehisa S, Oki E, Limori M, Nakaji Y, Sacki H, Oda Y, *et al.* Clinical significance of programmed cell death-ligand 1 expression and immune microenvironment at the invasive front of colorectal cancer with high microsatellite instability. *Int J Cancer* 2018; 142:882–883.
- Guo I, Yan Y, Guo Q, Zhang M, Zhang J, Goltzman D. Tumor associated macrophages induce the expression of FOX Q1 to promote epithelial-mesenchymal transition and metastasis in gastric cancer cells. *Oncol Rep* 2017; 38:2003–2010.
- Kwak Y, Koh J, Kim DW, Lee HS. Immunscore encompassing CD3+ and CD8+ T cell densities in distant metastasis is a robust prognostic marker for advanced colorectal cancer. *Oncotarget* 2016; 7:81778–81790.
- Ding D, Yao Y, Yang C, Zhang S. Identification of mannose receptor and CD163 as novel biomarkers for colorectal cancer. *Cancer Biomark* 2018; 21:689–700.
- Huang Z, Yin Y, Yao S, Hu Y, Feng Y, Li M, *et al.* The immune microenvironment confers chemoresistance of colorectal cancer through macrophages derived IL-6. *Clin Cancer Res* 2017; 23:7375–7387.
- Kikuchi T, Mimura K, Ashizawa M, Okayama H, Endo E, Saito K, *et al.* Characterization of tumor-infiltrating immune cells in relation to microbata in colorectal cancer. *Cancer Immunol Immunother* 2020; 69:2–32.
- Chaoya M, Hasita H, Koji O, Takenobu N, Sohsunke Y, Shohei K, *et al.* CD163 positive cancer cells have potentially associated with malignant potential in clear cell renal cell carcinoma. *Med Mol Morphol* 2018; 51:13–20.
- Daniëlle K, Natasja L, Morten N, Anni S, Holger J, Marianne H Peter J. CD163 as a biomarker in colorectal cancer: the expression on circulating monocytes and tumor-associated macrophages, and the soluble form in the blood. *Int J Mol Sci.* 2020; 21:5925.
- Shabo I, Olsson H, Wegman P, Gunnorsson C, Lindstrom A, Andersson H. Macrophages triates in cancer cells are induced by macrophages cancer cell fusion and can not be explained by cellular interaction. *BMC Cancer* 2015; 15:922.
- Wang Y, Wang J, Liu J, Wang Y, Shi Z, Chen Y, *et al.* A study of correlation between M2 macrophages and lymph node metastasis in colorectal carcinoma. *World J Surg Oncol* 2021; 19:91.
- Liu C, Yao Z, Wang J, Zhang W, Yang Y, Zhang Y, *et al.* Macrophages derived CCL5 facilitates immune escape of colorectal cancer cells via the P65/STAT3-CSNS-PD-L1 pathway. *Cell Death Differ* 2020; 27:1765–1781.
- Xu G, Jiang L, Ye C, Qin G, Luo Z, Mo Y, Chen J. The ratio of CD86+/CD163+ macrophages predicts post operative recurrence in colorectal cancer. *Front Immunol* 2021; 12:724429.
- Yang C, Wei C, Wang S, Shi D, Zhang C, Lin X, *et al.* Elevated CD163 (+)/CD68 (+) ratio at tumor invasive front is closely associated with aggressive phenotype and poor prognosis in colorectal cancer. *Int J Biol Sci* 2019; 15:984–998.
- Wei C, Yang C, Wang S, Shi D, Zhang C, Lin X, Xiong B. M2 macrophages confer resistance to 5-fluorouracil in colorectal cancer through the activation of CCL22/PI3K/AKT signaling. *Oncotarget Ther* 2019; 12:3051–3063.
- Rey I, Putra A, Lindarto D, Yusuf F. Relation between CD163 tumor associated macrophages and colorectal cancer stem cell marker. *Open Access Maced J Med Sci* 2021; 9(B):1381–1386.
- Algars A, Irjala H, Vaitinen S, Huhtinen H, Sundstrom J, Salmi M, *et al.* Type and location of tumor infiltrating macrophages and lymphatic vessels predict survival of colorectal patients. *Int J Cancer* 2012; 131:864–873.