Med Glas (Zenica) 2025;22(2)
https://doi.org/10.17392/1848-22-02

Med Glas (Zenica)

ORIGINAL ARTICLE

Prognostic significance of CD163 expression in colorectal cancer stroma: a retrospective cohort study

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ABSTRACT

Aim Colorectal cancer (CRC) ranks among the leading causes of cancer-related mortality worldwide, with significant incidence and mortality rates recorded in Kazakhstan. CRC poses a substantial healthcare burden, prompting investigations into novel prognostic markers. This study investigates the prognostic significance of CD163 expression in patients with colorectal cancer.

Methods This retrospective cohort study was conducted in Aktobe, Kazakhstan, utilizing tissue microarrays from 175 patients diagnosed with TNM (Tumour, Node, Metastasis) stage I–IV colon adenocarcinoma. CD163 expression was assessed through immunohistochemistry. Kaplan-Meier survival analysis was employed to estimate 5-year overall survival, defined as the interval from diagnosis to death or last follow-up. Cox proportional hazard models were used to evaluate the relationship between CD163 expression levels and survival outcomes.

Results High CD163 expression was linked to advanced disease stage of colorectal cancer ($p<0.001$), lymphovascular invasion ($p<0.001$), perineural invasion ($p<0.001$), and Collagen I>30%, A type ($p<0.001$). CD163 infiltration was a negative prognostic factor for colorectal cancer patients, with higher risks of death in the high CD163 expression group compared to the low CD163 group (HR=5.769, 95% CI 3.194 – 10.42; $p<0.001$).

Conclusion The findings highlight CD163 as a potential prognostic biomarker in CRC, warranting further investigation into its mechanistic role and therapeutic implications.

Keywords: adenocarcinoma, tumour biomarkers, immunohistochemistry, survival analysis, tumour microenvironment

INTRODUCTION

Colorectal cancer (CRC) ranks as the third most common malignancy and the second leading cause of cancer-related mortality globally, accounting for approximately 10% of all cancer cases and deaths (1). In 2020 alone, an estimated 1.93 million new CRC cases and 0.94 million deaths were reported worldwide (2). Kazakhstan reflects these trends, with 28,950 new CRC cases documented between 2009 and 2018 (3). Despite the introduction of national screening programs in 2011 targeting individuals aged 50 to 70, the disease continues to pose a significant public health challenge (4).

The prognosis in CRC varies widely and is influenced by a complex interplay of molecular and cellular factors (5). Recent research has increasingly focused on the tumour microenvironment (TME), particularly the stromal compartment, as a critical determinant of disease progression and patient outcomes (5,6). Among the components of the TME, tumour-associated macrophages (TAMs), identified by markers such as CD163, have garnered attention for their roles in immune regulation and tumour biology (7). CD163, an M2 macrophage marker, has been implicated in promoting tumour progression through immunosuppressive mechanisms and extracellular matrix remodelling (7).

Understanding the prognostic implications of CD163 expression in CRC is crucial for refining risk stratification and informing personalized therapeutic strategies. However, the existing literature on CD163 expression in CRC remains limited (8), particularly in regions like Aktobe, Kazakhstan, where environmental and industrial factors may contribute to unique disease patterns (9).

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| Submitted: 19. Jul. 2024. Revised: 12 Mar. 2025. Accepted: 13 Mar. 2025.

This retrospective cohort study aims to address this gap by analysing CD163 expression in CRC patients from Aktobe, exploring its associations with clinicopathological features and survival outcomes.

MATERIALS AND METHODS

Study design

This analysis is a part of the study that investigates the histological data of the colorectal cancer patients described elsewhere (10). This single-centre retrospective cohort investigation enrolled consecutively collected samples of resected colorectal cancer showing keloid-like collagen at the tumour's invasive edge using the exclusion criteria. The exclusion criteria were defined as follows: non-adenocarcinoma histological diagnosis, tumour-stroma ratio above 50%, desmoplastic reaction at the invasive tumour margin, classified as "mature" or myxoid stroma according to Ueno H. criteria (11), preoperative radiation therapy, patient death within 30 days post-surgery, prior malignancy within five years of colorectal cancer diagnosis (excluding basal cell carcinoma or cervical cancer in situ), refusal of treatment, severe clinical condition, or age over 90 years.

The samples were obtained from 883 patients who underwent full oncologic resection for TNM stage I – IV colon adenocarcinoma in Aktobe Oncology Medical Center, Kazakhstan between January 2001 and December 2019. Follow-up observations were conducted for all patients for a period of 5 years post-surgical resection. Complete follow-up data were obtained in 175 cases (Figure 1).

Prior to the commencement of the study, all samples were anonymized.

Patient clinical data were obtained from medical records within a comprehensive healthcare information platform. This information encompassed patient demographics such as gender, age, existing medical conditions, surgical dates, pre-surgery comorbidities, tumour localization in the colon, instances of recurrence, as well as the corresponding detection and treatment dates (Table 1).

Table 1. Association of CD163 expression with clinicopathological characteristics in colorectal cancer patients

Variable	Number of patients	No (%) of CD163 expression		p
		Low (N=87)	**High** (N=88)	
Cancer stage				<0.001
II	97	65 (74.8)	32 (36.4)	
III	56	19 (21.8)	37 (42.0)	
IV	22	3 (3.4)	19 (21.6)	
Lymphovascular invasion				<0.001
YES	60	18 (20.7)	42 (47.7)	
NO	148	69 (79.3)	46 (52.3)	
Perineural invasion				<0.001
YES	17	5 (5.7)	12 (13.7)	
NO	158	82 (94.3)	76 (86.3)	
collagen I≤30% A type				<0.001
YES	78	61 (78.2)	17 (21.8)	
NO	97	26 (26.8)	71 (73.2)	

low, <15% based on the median of the positive counts of CD163+ tumour-associated macrophages; high, >55% based on the median of the positive counts of CD163+ tumour-associated macrophages.

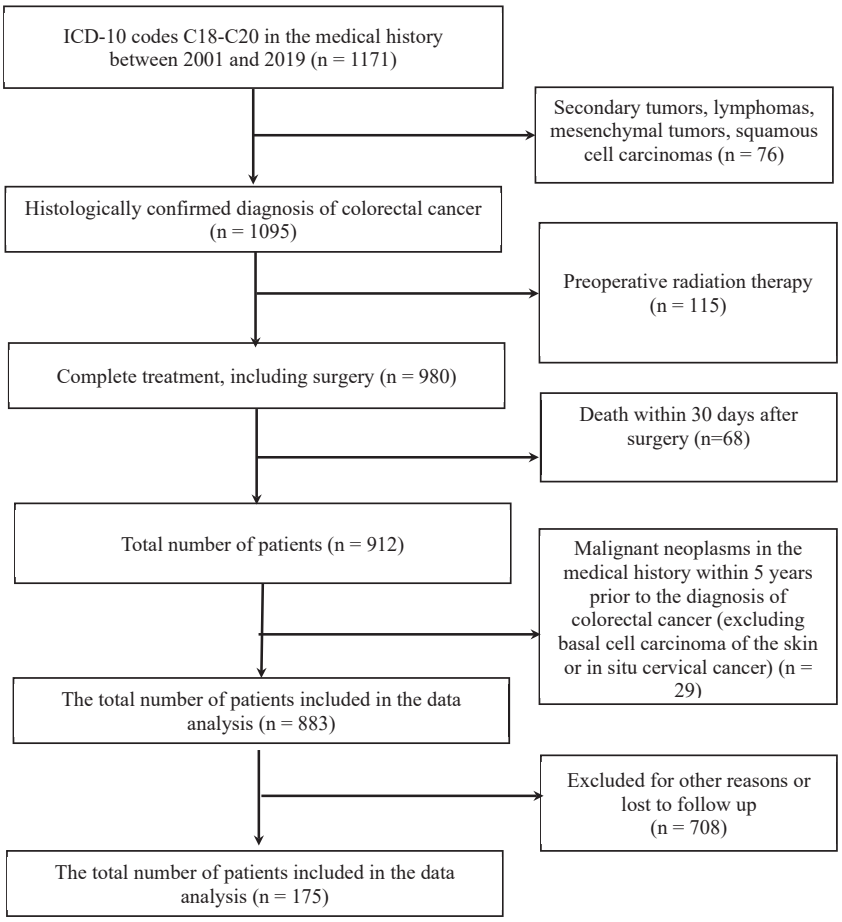

Figure 1. The flowchart for the inclusion of patients with colorectal cancer

Ethical approval was obtained from the Research Ethics Board of West Kazakhstan Marat Ospanov Medical University, with the study utilizing existing administrative healthcare databases. The study was granted a waiver for patient consent as it involved a retrospective analysis of deidentified tissue archives.

Methods

Histological examination. Before histological examination, tissue samples were fixed in 10% formalin at 4 °C for 24 hours. After fixation, they were washed with tap water and dehydrated through a graded series of ethanol concentrations (70%, 90%, 95%, and 100%). The dehydrated tissues were then cleared in xylene and embedded in paraffin. Tissue sections, 3 µm thick, were cut using a microtome and mounted on glass slides. The slides were subsequently deparaffinized and prepared for staining.

Hematoxylin and eosin staining procedure. The tissue sections were immersed in Mayer's hematoxylin for 15 minutes, followed by a 5-minute rinse in water. They were then stained with eosin for one minute.

Immunohistological evaluation. Adjacent to the hematoxylin and eosin-stained sections, sequential 2 µm-thick paraffin sections were prepared and mounted on glass slides. Tumour-infiltrating immune cells were identified via immunohistochemistry using the CONFIRM anti-CD163 (clone MRQ-26) monoclonal antibody (Roche Diagnostics, Germany). The primary antibody was diluted 1:200 in Ventana Antibody Diluent and incubated for 16 minutes at 37 °C. Detection was performed using the Ventana ultraView Universal DAB Detection Kit on the BenchMark IHC/ISH instrument (Ventana Benchmark, Tucson, AZ, USA), following the manufacturer's automated protocol. Lymph node tissues served as positive controls.

Tissue samples were fixed in 10% neutral buffered formalin, processed using standard methods, and embedded in paraffin. The 2 µm-thick sections were deparaffinized and hydrated prior to staining. Staining was conducted using the BenchMark ULTRA IHC/ISH automated immunostainer (Ventana Benchmark, Tucson, AZ, USA), adhering to the manufacturer's protocol with the appropriate visualization system. Following staining, slides were dehydrated and manually cover slipped. Mayer's hematoxylin was used for nuclear counterstaining, employing a commercial kit from Bio-Vitrum (Astana, Kazakhstan). After the immunohistochemical procedure, samples were thoroughly examined.

Immunostaining was considered positive if a distinct granular brown stain was observed in the cytoplasm and membranes. Each tissue sample included at least 50% tumour cells, adhering to established immunohistochemistry protocols. Positive staining was evaluated in ten separate high-power fields (20×objective). The extent of CD163-positive tumour-associated macrophage infiltration was calculated by averaging the counts from these fields. All tissues selected for the study underwent independent evaluation by two experienced pathologists (A.K. and A.Z.) without access to clinical information, and their results were averaged. The cut-off value was determined by selecting the median of the positive counts of CD163+ TAMs. The assessment of keloid-like collagen at the invasive margin of the primary tumour was conducted using hematoxylin and eosin, and Masson's trichrome staining described elsewhere (10).

Outcome variable and definitions. The primary endpoint of this study was overall survival. Overall survival was defined as the duration from the date of randomization to the date of death from any cause or the date of the last observation, with a maximum follow-up period of five years.

Statistical analysis

Descriptive statistics were employed for data presentation. The $\chi 2$ test was performed to compare relative frequency distributions of categorical variables between the groups. The Kaplan-Meier method was employed to calculate 5-year overall survival, measured from the time of diagnosis to death or last follow-up visit. Cox proportional hazards model was used to determine the risk ratios of explanatory variables for overall survival. A $p< 0.05$ was considered statistically significant.

RESULTS

Figure 2 presents representative immunohistochemical staining of tumour-infiltrating CD163-positive immune cells. Panel (A) demonstrates low expression of CD163-positive immune cells, characterized by sparse distribution and minimal staining intensity. In contrast, panel (B) exhibits high expression of CD163-positive immune cells, with a dense infiltration and strong immunoreactivity.

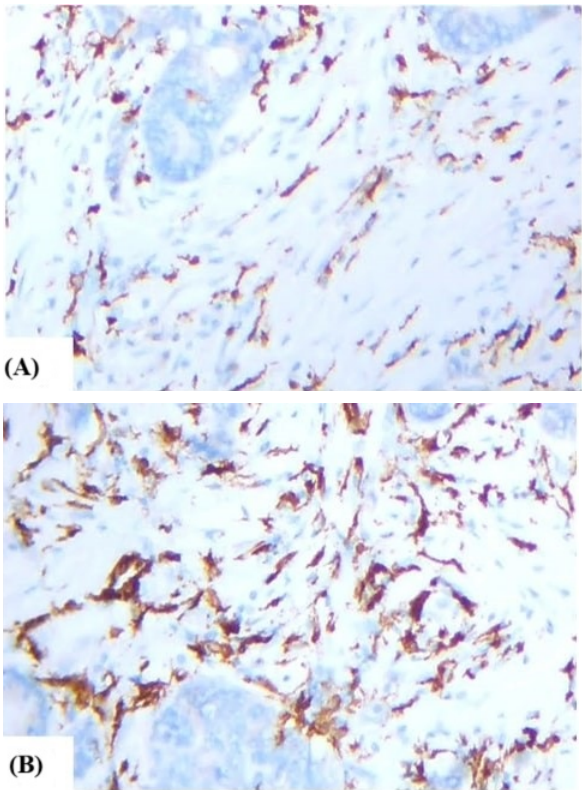

Figure 2. Representative immunohistochemistry staining of tumour-infiltrating CD163-positive immune cells (A) low expression of CD163-positive immune cells (B) high expression of CD163-positive immune cells (×400)

High CD163 expression was associated with advanced stages of colorectal cancer ($p<0.001$), lymphovascular invasion ($p<0.001$), perineural invasion ($p<0.001$), and Collagen I ≤30%, A type ($p<0.001$) (Table 1).

The overall survival of patients in the high CD163 expression group was lower than in the low CD163 group ($p<0.001$). CD163 infiltration was a negative prognostic factor for colorectal cancer patients, with higher risks of death in the high

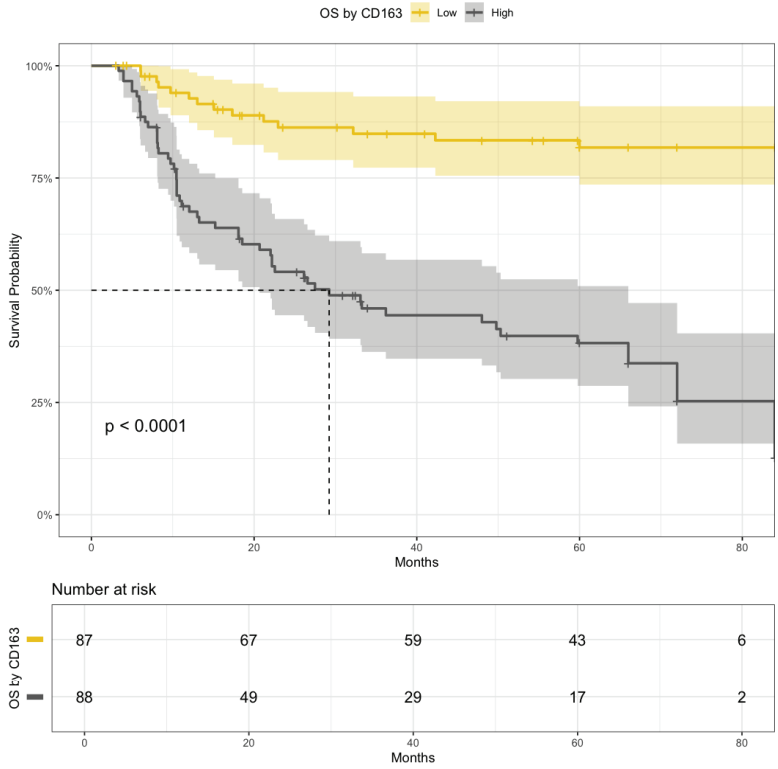

Figure 3. Kaplan-Meier survival curves for overall survival (OS) stratified by CD163 expression

CD163 expression group compared to the low CD163 group (HR=5.769, 95% CI 3.194 – 10.42; p<0.001) (Figure 3).

DISCUSSION

The prognostic significance of CD163 expressions in colorectal cancer patients was explored in this study. High CD163 expression was associated with advanced stages of colorectal cancer, lymphovascular invasion, perineural invasion, and Collagen I>30%, A type. CD163 is a marker of M2 macrophages, which have been implicated in promoting tumour growth and metastasis (12). Our findings suggest that high CD163 expression is a negative prognostic factor in colorectal cancer, consistent with previous studies showing that M2 macrophages promote tumour progression in various cancers (13,14).

Previous research has demonstrated a significant association between higher stromal content in CRC patients and reduced overall survival (15). TAMs significantly influence tumour progression by secreting cytokines and chemokines, thereby coordinating with inflammatory mechanisms to promote tumour development, invasion, metastasis, immunosuppression, angiogenesis, and drug resistance (16–18). In this study, high CD163 expression was associated with poor survival, supporting previous research results.

Overall, our study provides insights into the prognostic significance of immune cell markers in colorectal cancer and highlights the potential role of the immune microenvironment in shaping clinical outcomes. Further research is needed to elucidate the precise mechanisms underlying the effects of immune cells on colorectal cancer progression and to explore potential therapeutic strategies targeting the immune microenvironment.

The study has some limitations. The study's retrospective design, relying on archival data from a single oncology centre in Kazakhstan and small sample size, may limit the generalizability of its findings to broader populations. The exclusion of patients with incomplete follow-up data, as well as those over 90 years of age or with prior malignancies, could introduce selection bias, potentially affecting the study's external validity. Moreover, the observational nature of the study precludes the establishment of causality between CD163 expression and patient outcomes.

In conclusion, our study identifies CD163 infiltration as an independent prognostic indicator and a significant risk factor for mortality in colorectal cancer patients. These results underscore the prognostic value of immune cell markers in colorectal cancer and emphasize the critical role of the immune microenvironment in influencing clinical outcomes. To deepen our understanding, further research is warranted to elucidate the mechanisms by which immune cells impact colorectal cancer progression. Additionally, targeting immune cell markers presents a promising therapeutic avenue for enhancing patient outcomes in colorectal cancer.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

Author contribution statement

Conceptualization: N.I., Y.I., and A.K. Data curation: N.I., Y.I., and A.Z. Formal analysis: N.I., Y.I. and A.K. Methodology: N.I., A.Z. and A.K. Project administration: N.I., B.B., N.K, and Z.S. Resources: N.I., A.I., A.T., and D.A.Z. Software, N.I., Y.I., A.K., A.Z.; M.A.A, and A.B.Z. Supervision: N.I. and Y.I.; A.K., A.Z., N.K., and Z.S. Investigation: N.K., Z.S., A.I., A.T. and D.A.Z. Validation, N.I., Y.I., M.A. and A.Z. Visualization, A.T. and D.Z. Writing, original draft, N.I., M.A., and A.Z. Writing, review and editing, N.I., Y.I., A.K., A.Z., B.B., N.K., Z.S., A.I., A.T., D.Z. and A.Z. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. World Health Organisation. Fact Sheets. 2023. Colorectal cancer. https://www.who.int/news-room/fact-sheets/detail/colorectal-cancer (accessed April 16, 2024)
2. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol 2021; 14(10):101174.
3. Mauyenova D, Zhadykova Y, Khozhayev A, Turebayev D, Kulmirzayeva D, Urazova S, et al. Trends of colorectal cancer incidence in Kazakhstan. Asian Pac J Cancer Prev 2021; 22(10):3405–14.
4. Kaidarova D, Zhylkaydarova A, Ukolova Y, Jumanov A. The results of colorectal cancer screening in the Republic of Kazakhstan during the period from 2013 to 2018. J. Clin. Oncol 2019; 37:e13074.
5. Larionova I, Tuguzbaeva G, Ponomaryova A, Stakheyeva M, Cherdyntseva N, Pavlov V, et al. Tumor-associated macrophages in human breast, colorectal, lung, ovarian and prostate cancers. Front Oncol 2020; 10:566511.
6. Ma S, Zhao Y, Liu X, Sun Zhang A, Zhang H, Hu G, et al. CD163 as a potential biomarker in colorectal cancer for tumor microenvironment and cancer prognosis: a Swedish study from tissue microarrays to big data analyses. Cancers (Basel) 2022; 14(24):6166–80.
7. Xue T, Yan K, Cai Y, Sun J, Chen Z, Chen X, et al. Prognostic significance of CD163+ tumor-associated macrophages in colorectal cancer. World J Surg Oncol 2021; 19(1):186–92.
8. Krijgsman D, De Vries NL, Andersen MN, Skovbo A, Tollenaar RAEM, Møller HJ, et al. 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(16):5925.
9. Kenessary D, Kenessary A, Adilgireiuly Z, Akzholova N, Erzhanova A, Dosmukhametov A, et al. Air pollution in Kazakhstan and its health risk assessment. Ann Glob Health 2019; 85(1).
10. Imanbayev NM, Iztleuov YM, Kamyshanskiy YK, Zhumasheva A V. Diagnostic and prognostic significance of keloid-like collagen remodeling patterns in the extracellular matrix of colorectal cancer. Pathol Oncol Res 2024; 30:1611789–99.
11. Ueno H, Kajiwara Y, Ajioka Y, Sugai T, Sekine S, Ishiguro M, et al. Histopathological atlas of desmoplastic reaction characterization in colorectal cancer. Jpn J Clin Oncol 2021; 51(6):1004–12.
12. Shabo I, Olsson H, Sun X, Svanvik J. Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. Int J Cancer 2009; 125(8):1826–31.
13. Liu J, Geng X, Hou J, Wu G. New insights into M1/M2 macrophages: key modulators in cancer progression. Cancer Cell Int 2021; 21:1–7.
14. Xu Y, Wang X, Liu L, Wang J, Wu J, Sun C. Role of macrophages in tumor progression and therapy. Int J Oncol 2022; 60(5):1–19.
15. Huijbers A, Tollenaar R, v Pelt GW, Zeestraten ECM, Dutton S, McConkey CC, et al. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Ann. Oncol 2013; 24(1):179–85.
16. Zhong X, Chen B, Yang Z. The Role of Tumor-Associated Macrophages in Colorectal Carcinoma Progression. Cell Physiol Biochem 2018; 45(1):356–65.
17. Wang H, Tian T, Zhang J. Tumor-Associated Macrophages (TAMs) in Colorectal Cancer (CRC): From Mechanism to Therapy and Prognosis. Int J Mol Sci 2021;22(16):8470–80.
18. Hou S, Zhao Y, Chen J, Lin Y, Qi X. Tumor-associated macrophages in colorectal cancer metastasis: molecular insights and translational perspectives. J Transl Med 2024; 22(1):1–14.