

Immunohistochemical expression of CD4⁺ and CD8⁺ tumor-infiltrating lymphocytes in colorectal carcinoma

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Background/aim

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths in the world. The potential influences of tumor-infiltrating lymphocytes (TILs) in various neoplasms including CRC have been reported to reflect the antitumor immunity of the host. The aim of this study was to evaluate the expression of CD4⁺ and CD8⁺ TILs in CRC and to correlate their expression with patients' clinicopathological parameters.

Materials and methods

Paraffin blocks and clinicopathological data of 130 patients with CRC were obtained from private laboratories. The density of CD4 and CD8 tumor TILs was assessed immunohistochemically and evaluated by image analysis in 130 specimens of CRC. The CD4⁺ and CD8⁺ cell tumor infiltrate was classified into scanty, moderate, and abundant expression.

Results

Of the 130 CRC cases, 68 (66.2%) were adenocarcinoma and 44 (33.8%) were mucinous carcinoma, 114 (87.7%) were of grade II and 16 (12.3%) were of tumor grade III. CD4⁺ T cells expression was significantly correlated with the histopathological grade and Duke's classification. CD8⁺ T cells expression was significantly correlated with the Duke's classification.

Conclusion

Cancer colon progression is influenced by host immune response and the presence of TILs CD4⁺ and CD8⁺. Further studies are recommended to assess implication of these cells in CRC prognosis.

Keywords:

CD4⁺ cells, CD8⁺ cells, colorectal cancer, immunohistochemistry

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Introduction

The characteristics of cancer cells as well as the tumor microenvironment have been proposed to influence the biologic behavior of the tumors. Tumor-infiltrating lymphocytes (TILs), the primary immune response against cancer, were shown to reflect the immunity of the host and reflect the therapeutic outcome in patients with various types of tumors including lung and breast cancer [1,2].

Nowadays, TILs have been reported recently to attract attention as new biomarkers of colorectal cancer (CRC), the third most frequently malignancy worldwide. The incidence rates of colon cancer are higher in developed countries than in developing one. However, the mortality rate is higher in developing countries [3,4]. Approximately, one million new cases of CRC are diagnosed annually throughout the world, with more than half a million death per year [5].

Recently, during development of CRC, the role of immune system in recognizing altered cells and in underlying the mechanism capable of eliminating tumors even in advanced stage has been a growing research interest [6,7].

Generally, the immune system has evolved several mechanisms to protect the host against cancer. Each of them has to be undermined or evaded during cancer development to enable tumor outgrowth. Among these; CD4⁺ and CD8⁺ T lymphocytes contribute to tumor suppression [7].

CD8⁺ T lymphocytes are crucial component of tumor-specific immunity which attack tumor cells [8,9].

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However, the role of CD4⁺ T cells is more complicated, as CD4 helper T cells have a central role in maintaining anticancer immunity, whereas CD4 regulatory T cells (T regs) suppress anticancer immune response promoting tumor progression [10–12].

Although there have now been many studies suggesting a positive prognostic effect of CD4 and CD8, this still need to be verified in more studies. So the objective of this study was to evaluate the immunoexpression of both CD4 and CD8 in CRC.

Materials and methods

Specimens and ethical approval

We retrospectively obtained paraffin blocks and reviewed the database of 130 cases that underwent curative surgery for CRC from private laboratories. The patients had no other cancers or diseases, such as acute infection or diabetes, and they had not received previous radiotherapy or chemotherapy at the time of the surgery. This study was approved by the ethics committee of National Research Centre (Approval No. 16/308).

Histopathology

The histopathological characteristics were reviewed. The histological diagnosis was established and verified by two pathologists. Clinicopathologic data were retrospectively collected from medical records and are summarized.

Immunohistochemistry

The paraffin blocks were cut by microtome at 4 μ thickness. Sections were mounted on the charged glass slides. The slides were incubated at 37°C overnight for accurate adhesion of the section of the slide. Deparaffinization, rehydration, and epitope retrieval were performed in Pt link retrieval system (Dako, Copenhagen, Denmark).

The staining steps and incubation times were preprogrammed into the autostainer Link software (Dakoomnis, Copenhagen, Denmark). Diluted primary antibodies against CD4 and CD8 (Thermo Fisher Scientific, Runcorn, Cheshire, UK) and horseradish peroxidase labeled secondary antibody (Thermo Fisher Scientific) were used. For negative control, the primary antibody was replaced by normal mouse serum and human normal tonsil was used as positive control. Diaminobenzidine was used for color development and hematoxylin as counterstain.

Image analysis immunoscore

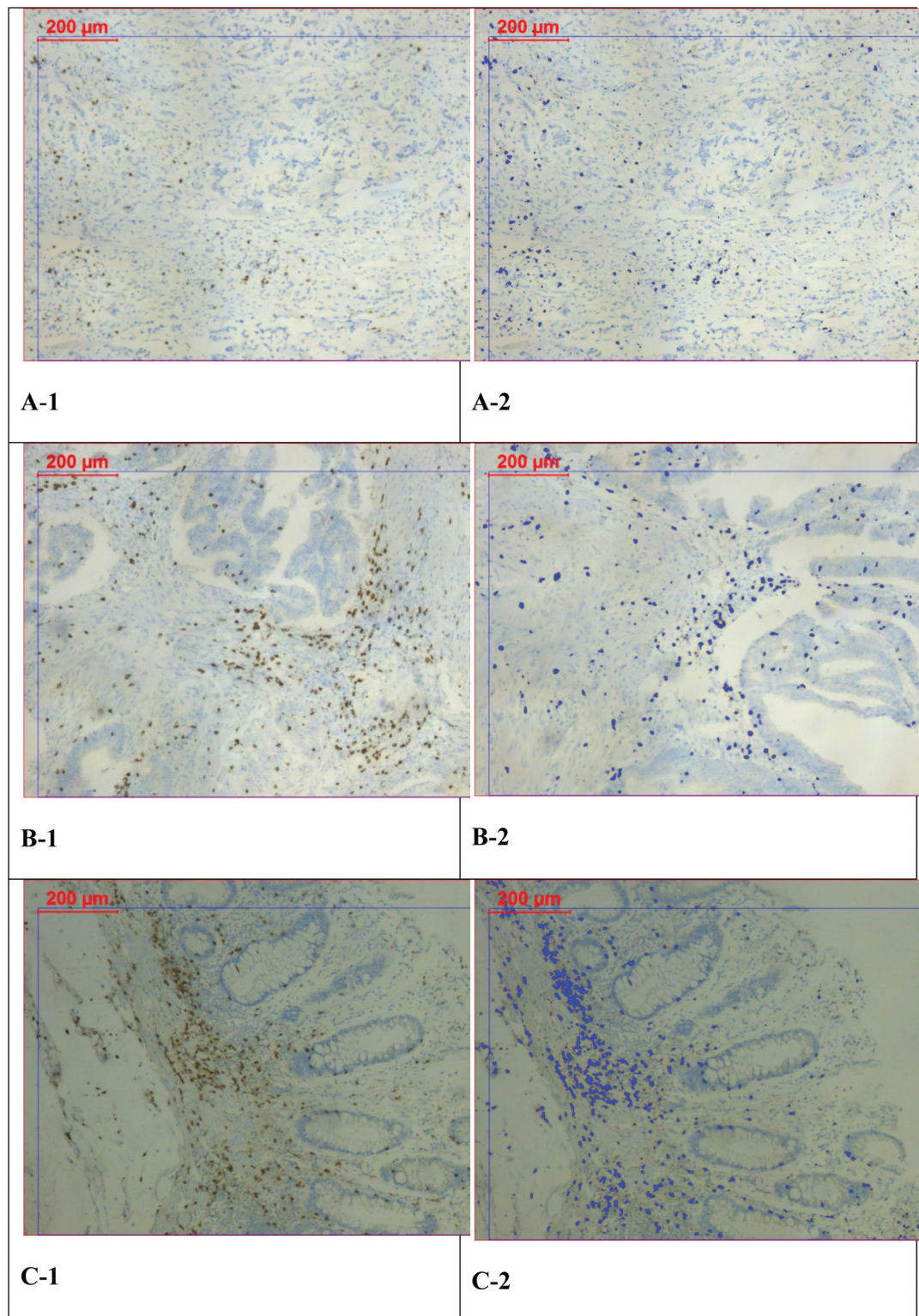
Immunostaining was visualized and photographed under a light microscope Olympus CX-41 with DP 12 Olympus digital camera (Olympus Optical Co. Ltd, Tokyo, Japan).

Quantitative evaluation was performed by screening the sections and selecting at least five different fields with the high density of TILs. The density of CD4⁺ T cells and CD8⁺ T cells was determined using the Leica Qwin 500 image analysis system (LEICA Imaging Systems Ltd, Cambridge, England) which consists

Table 1 Clinicopathological features of colorectal carcinoma patients

Clinicopathological features	N=130 [n (%)]
Sex	
Male	56 (43.1)
Female	74 (56.9)
Age (years)	
<60	66 (50.8)
≥60	64 (49.2)
Tumor size	
<4.5	50 (38.5)
≥4.5	80 (61.5)
Tumor site	
Right	54 (41.5)
Left	44 (33.9)
Rectum	32 (24.6)
Tumor type	
Adenocarcinoma	86 (66.2)
Mucinous carcinoma	44 (33.8)
Tumor grade	
I	0 (0)
II	114 (87.7)
III	16 (12.3)
T classification	
T1	2 (1.5)
T2	10 (7.7)
T3	82 (63.1)
T4	36 (27.7)
N classification	
N0	56 (43.1)
N1	36 (27.7)
N2	38 (29.2)
M classification	
M0	114 (87.7)
M1	16 (12.3)
Stage	
I	10 (7.7)
II	44 (33.8)
III	60 (46.2)
IV	16 (12.3)
Duke's classification	
A	0
B	56 (43.1)
C	58 (44.6)
D	16 (12.3)

Figure 1



Immunohistochemical staining of CD4⁺ T cells in colorectal carcinoma and their binary images by Leica Qwin 500 image analysis system. (A1 and 2) Scanty expression, (B1 and 2) moderate expression, and (C1 and 2) abundant expression (CD4 immunohistochemical stain, Scale Bar: 200 μ m).

of Leica DM-LB microscope with JVC color video camera attached to a computer system. We selected five tumor fields with the highest expression and assessed the area percentage of the positive cells at high magnification ($\times 200$). We detected the positively

stained cells, which were masked automatically by a blue mask called binary image. We used the measure field software program that automatically measures the area percentage of the detected features in the binary image and then displays the results in a table form. The

Table 2 Immunostaining of CD4+ and CD8 T+ cells within tumor tissue

	Scanty	Moderate	Abundant
CD4 ⁺ T cells			
Number of patients	42	44	44
Range (%)	0.001–0.069	0.070–0.201	0.202–1.319
Mean±SD	0.026±0.021	0.126±0.046	0.565±0.316
CD8 ⁺ T cells			
Number of patients	44	42	44
Range (%)	0.039–0.0999	1.000–1.999	2.000–8.321
Mean±SD	0.531±0.267	1.488±0.323	3.638±1.665

immunoscore for CD4⁺ cells and CD8⁺ cells was evaluated according to Cho *et al.* [13] into scanty, moderate, and abundant expression.

Statistical analysis

Differences between groups were analyzed using the χ^2 -test. *P* values of less than 0.05 were considered to indicate statistical significance.

Results

The study population included 130 patients with CRC; there were 56 (43.1%) males and 74 (56.9%) females. The age of the patients ranged from 22 to 82 years, 66 (50.8%) of them were less than 60 years and 64 (49.2%) were greater than or equal to 60 years. Table 1 shows all the clinicopathological characteristics of the studied cases.

Immunoscore of CD4⁺ cells

A total of 42 cases showed scanty CD4⁺ T cells, with mean area percentage of 0.026±0.021 and range from 0.001 to 0.069%; 44 cases showed moderate CD4⁺ T cells, with mean area percentage of 0.126±0.046 and range from 0.070 to 0.201%; and 44 cases showed abundant CD4⁺ T cells, with mean area percentage of 0.565±0.316 and range from 0.202 to 1.319% (Fig. 1, Table 2).

CD4⁺ T cells expression was nonsignificantly correlated with all clinicopathological parameters, except the histopathological grade and Duke's classification, which were significantly correlated with CD4⁺ T cells expression (Table 3).

Immunoscore of CD8⁺ cells

A total of 44 cases showed scanty CD8⁺ T cells, with mean area percentage of 0.531±0.267 and range from 0.039 to 0.0999%; 42 cases showed moderate CD8⁺ T cells, with mean area percentage of 1.488±0.323 and range from 1.000 to 1.999%; and 44 cases showed abundant CD8⁺ T cells, with mean area percentage of 3.638±1.665 and range from 2.000 to 8.321% (Fig. 2, Table 2).

CD8⁺ T cells expression was nonsignificantly correlated with all clinicopathological parameters, except the Duke's classification, which was significantly correlated with CD8⁺ T cells expression (Table 4).

Correlation between CD4⁺ and CD8⁺ T cells expression

Fourteen cases showed scanty CD4⁺ T cells and CD8⁺ T cells infiltration of tumor tissue. Twelve cases showed scanty CD4⁺ T cells and moderate CD8⁺ T cells infiltration of tumor tissue. Sixteen cases showed scanty CD4⁺ T cells and abundant CD8⁺ T cells infiltration of tumor tissue. Twelve cases showed moderate CD4⁺ T cells and scanty CD8⁺ T cells infiltration of tumor tissue. Sixteen cases showed moderate CD4⁺ T cells and CD8⁺ T cells infiltration of tumor tissue. Sixteen cases showed moderate CD4⁺ T cells and abundant CD8⁺ T cells infiltration of tumor tissue. Eighteen cases showed abundant CD4⁺ T cells and scanty CD8⁺ T cells infiltration of tumor tissue. Fourteen cases showed abundant CD4⁺ T cells and moderate CD8⁺ T cells infiltration of tumor tissue. Twelve cases showed abundant CD4⁺ T cells and CD8⁺ T cells infiltration of tumor tissue. There was a nonsignificant correlation between CD4+ and CD8+ T cell expression (Table 5).

Discussion

CRC is the third most common cause of cancer deaths in females and the fourth cancer-related mortality in males [14]. In the present study, immunohistochemistry was used to detect the expression of T-cell markers (CD4 and CD8) in 130 cases of CRC.

Previous studies showed that CD8⁺ T cells destroy large tumor masses *in vivo* and patients with abundant CD8⁺ T cells in either tumor nest or in the stroma had a better prognosis [13]. On the contrary, CD4⁺ T cells help prime CD8⁺ T cells responses and are essential for the maintenance of CD8⁺ T cell function by secreting cytokines required for CD8⁺ cell growth and

Table 3 Correlation between CD4 expression and clinicopathological features

	Scanty (N=42) [n (%)]	Moderate (N=44) [n (%)]	Abundant (N=44) [n (%)]	Total (N=130) [n (%)]
Sex				
Male	14 (25)	20 (35.7)	22 (39.3)	56 (100)
Female	28 (37.8)	24 (32.5)	22 (29.7)	74 (100)
Age (years)				
<60	10 (15.1)	26 (39.4)	30 (45.5)	66 (100)
≥60	32 (50)	18 (28.1)	14 (21.9)	64 (100)
Tumor size				
<4.5	8(16)	22(44)	20 (40)	50 (100)
≥4.5	34 (42.5)	22(27.5)	24 (30)	80 (100)
Tumor site				
Right	24 (44.5)	18 (33.3)	12 (22.2)	54 (100)
Left	12 (27.3)	18 (40.9)	14 (31.8)	44 (100)
Rectum	6 (18.7)	8 (25)	18 (56.3)	32 (100)
Tumor type				
Adenocarcinoma	32 (37.2)	32 (37.2)	22 (25.6)	86 (100)
Mucinous carcinoma	10 (22.7)	12 (27.3)	22 (50)	44 (100)
Tumor grade				
I	0	0	0	0
II	40 (35.1)	40 (35.1)	34 (29.8)	114 (100)
III	2 (12.5)	4 (25)	10 (62.5)	16 (100)
T classification				
T1	0	2 (100)	0	2 (100)
T2	4 (40)	2 (20)	4 (40)	10 (100)
T3	24 (29.3)	32 (39)	26 (31.7)	82 (100)
T4	14 (38.9)	8 (22.2)	14 (38.9)	36 (100)
N classification				
N0	18 (32.2)	20 (35.6)	18 (32.2)	56 (100)
N1	10 (27.8)	20 (55.6)	6 (16.6)	36 (100)
N2	14 (36.8)	4 (10.6)	20 (52.6)	38 (100)
M classification				
M0	38 (33.3)	40 (35.1)	36 (31.6)	114 (100)
M1	4 (25)	4 (25)	8 (50)	16 (100)
Stage				
I	4 (40)	2 (20)	4 (40)	10 (100)
II	14 (31.8)	18 (40.9)	12 (27.3)	44 (100)
III	20 (33.3)	20 (33.3)	20 (33.3)	60 (100)
IV	4 (25)	4 (25)	8 (50)	16 (100)
Duke's classification				
A	0	0	0	0
B	18 (32.2)	20 (35.6)	18 (32.2)	56 (100)
C	20 (34.5)	20 (34.5)	18 (31)	58 (100)
D	4 (25)	4 (25)	8 (50)	16 (100)

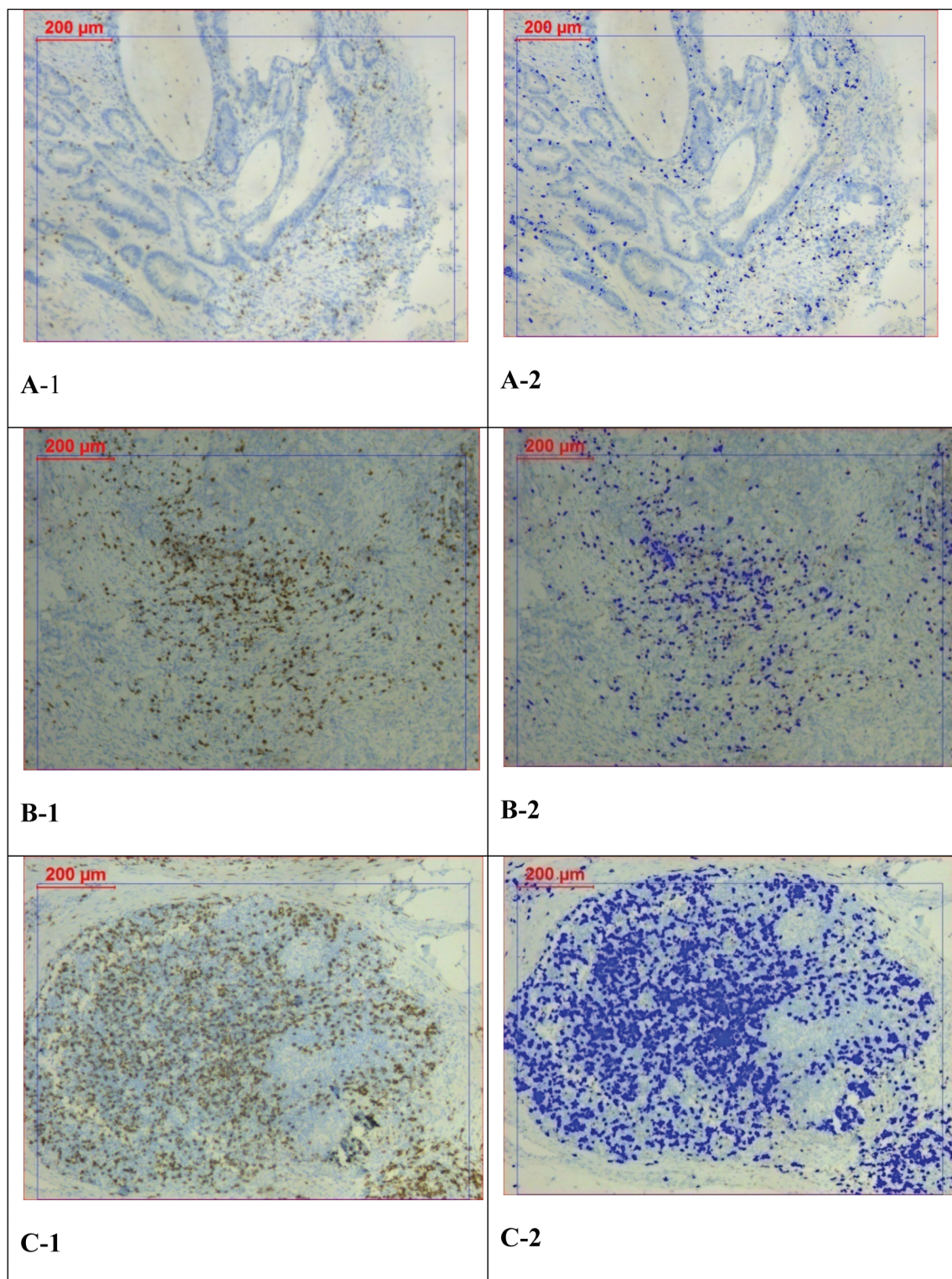
proliferation [15]. However, CD4⁺ TILs were classified into more subsets and the function of each one differs regarding antitumor immunity. T helper 1 (Th1) cells are a subset that activates CD8⁺ T cells, so they were reported to enhance antitumor immunity [16]. On the contrary, T helper 2 (Th 2) cells are another subset that seems to suppress antitumor immunity via the production of immunosuppressive cytokine interleukins-10 [17]. T helper 17 (Th 17) cells is the third subset that is reported by some authors to facilitate antitumor immune response. However, other authors showed that Th 17 cells may increase tumor growth through neoangiogenesis of the tumor [17].

Regulatory T cells (T reg) were shown to suppress antitumor immunity.

In the present study, we used quantitative evaluation of area percentage of the positive cells to determine the density of CD4⁺ and CD8⁺ T cells in cases of CRC by immunohistochemistry. The immunoscore for CD4⁺ cells and CD8⁺ cells was evaluated according to Cho *et al.* [13] into scanty, moderate and abundant expression.

Our results showed scanty CD4⁺ T cells in 42 cases, with mean area percentage of 0.026±0.021. However,

Figure 2



Immunohistochemical staining of CD8⁺ T cells in colorectal carcinoma and their binary images by Leica Qwin 500 image analysis system. (A1 and 2) Scanty expression, (B1 and 2) moderate expression, and (C1 and 2) abundant expression (CD8 immunohistochemical stain, Scale Bar: 200 μm).

44 cases showed moderate expression with mean area percentage of 0.126 ± 0.046 , and 44 cases showed abundant expression of CD4⁺ cells with mean area percentage of 0.565 ± 0.316 within the tumor tissue. On the other hand 44 cases showed scanty CD8⁺ T cells

expression with mean area percentage of 0.531 ± 0.267 , 42 cases showed moderate expression with mean area percentage of 1.488 ± 0.323 and 44 cases showed abundant CD8⁺ cells expression with mean area percentage of 3.638 ± 1.665 . These results were

Table 4 Correlation between CD8 expression and clinicopathological features

	Scanty (N=44) [n (%)]	Moderate (N=42) [n (%)]	Abundant (N=44) [n (%)]	Total (N=130) [n (%)]
Sex				
Male	22 (39.3)	18 (32.1)	16 (28.6)	56 (100)
Female	22 (29.7)	24 (32.4)	28 (37.9)	74 (100)
Age (years)				
<60	22 (33.3)	26 (39.4)	18 (27.3)	66 (100)
≥60	22 (34.4)	16 (25)	26 (40.6)	64 (100)
Tumor size				
<4.5	24 (48)	10 (20)	16 (32)	50 (100)
≥4.5	20 (25)	32 (40)	28 (35)	80 (100)
Tumor site				
Right	18 (33.3)	22 (40.8)	14 (25.9)	54 (100)
Left	14 (31.8)	18 (40.9)	12 (27.3)	44 (100)
Rectum	12 (37.5)	2 (6.2)	18 (56.3)	32 (100)
Tumor type				
Adenocarcinoma	26 (30.2)	28 (32.6)	32 (37.2)	86 (100)
Mucinous	18 (40.9)	14 (31.8)	12 (27.3)	44 (100)
Tumor grade				
I	0	0	0	0
II	38 (33.3)	36 (31.6)	40 (35.1)	114 (100)
III	6 (37.5)	6 (37.5)	4 (25)	16 (100)
T classification				
T1	0	0	2 (100)	2 (100)
T2	4 (40)	2 (20)	4 (40)	10 (100)
T3	28 (34.1)	24 (29.3)	30 (36.6)	82 (100)
T4	12 (33.3)	16 (44.5)	8 (22.2)	36 (100)
N classification				
N0	20 (35.7)	20 (35.7)	16 (28.6)	56 (100)
N1	10 (27.8)	12 (33.3)	14 (38.9)	36 (100)
N2	14 (36.8)	10 (26.4)	14 (36.8)	38 (100)
M classification				
M0	40 (35.1)	36 (31.6)	38 (33.3)	114 (100)
M1	4 (25)	6 (37.5)	6 (37.5)	16 (100)
Stage				
I	4 (40)	2 (20)	4 (40)	10 (100)
II	16 (36.4)	16 (36.4)	12 (27.2)	44 (100)
III	20 (33.3)	18 (30)	22 (36.7)	60 (100)
IV	4 (25)	6 (37.5)	6 (37.5)	16 (100)
Duke's classification				
A	0	0	0	0
B	20 (35.7)	20 (35.7)	16 (28.6)	56 (100)
C	20 (34.5)	16 (27.6)	22 (37.9)	58 (100)
D	4 (25)	6 (37.5)	6 (37.5)	16 (100)

Table 5 Correlation between CD4⁺ and CD8⁺ T-cells in patients with colorectal carcinoma

CD4 ⁺ T cells	CD8 ⁺ T cells [n (%)]			
	Scanty	Moderate	Abundant	Total
Scanty	14 (31.8)	12 (27.3)	18 (40.9)	44 (100)
Moderate	12 (28.6)	16 (38.1)	14 (33.3)	42 (100)
Abundant	16 (36.4)	16 (36.4)	12 (27.2)	44 (100)
Total	42 (100)	44 (100)	44 (100)	130 (100)

comparable with those of other studies; however, the methodology for evaluating the densities of TILs has not been standardized.

Yasuda *et al.* [18] showed that the number of CD4⁺ was greater than or equal to 78 in 17 of 40 cases of CRCs and less than 78 in 23 cases, whereas CD8⁺ density was greater than or equal to 54 in 17 cases and less than 54 in 23 cases. Chirica *et al.* [19] studied CD4⁺ lymphocytes present in cancer colon and compared their numbers with those in neighboring unaffected mucosa. They showed that the percentage of CD4⁺ was higher in the tumor compared with unaffected mucosa. In support with this finding, Jasim *et al.* [20] showed similar results.

CD8⁺ T cells are cytotoxic T cells that play a critical role in attacking tumor cells with evidence that their

densities were associated with long-term survival in various malignant tumors [17,21]. Furthermore, it was reported that CD8⁺ T lymphocytes density was associated with the responses to radiotherapy and chemotherapy [22,23].

Our study showed that CD4 and CD8 lymphocytes infiltrate within the tumor tissue in direct contact with tumor cells which are often referred to as TILs. According to Guidoboni *et al.* [24], the presence of CD8⁺ T cells in stromal and peritumoral tissue was not correlated with better prognosis, whereas CD8⁺ T cells infiltrated within cancer cell nests were associated with good survival, independently of pathologic stage. Our results showed that no significant correlation between CD4⁺ and CD8⁺ cell infiltration of the tumor tissue with any of clinicopathologic parameters, except for significant correlation between histopathologic grade and CD4⁺ lymphocyte infiltration. There was also significant correlation between CD4⁺ and CD8⁺ cell infiltration of the tumor tissue and dukes classification. Similar results were reported by Matsutani *et al.* [25] who showed no significant association of CD8⁺ T cells with any of the clinicopathological except significant association of the density of CD8⁺ T cells and lymph node metastasis.

It has been reported TILs have a crucial effect on the tumor progression and clinical outcome in various types of cancer such as colorectal, esophageal, non-small cell lung cancer, bladder cancer and melanoma [26–30].

In conclusion, data accumulated support the hypothesis that cancer colon progress is influenced by the host immune response and the presence of TILs (CD4⁺ and CD8⁺). Further studies are recommended to assess implication of TILs in CRC prognosis.

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Conflicts of interest

There are no conflicts of interest.

References

- 1 Liu H, Zhang T, Ye J, Li H, Wu B, Huang X, Hou J. Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small lung cancer. *Cancer Immunol Immunother* 2012; 61:1849–1856.
- 2 Wang K, Xu J, Zhang T, Xue D. Tumor-infiltrating lymphocytes in breast cancer predict the response to chemotherapy and survival outcome: a meta-analysis. *Oncotarget* 2016; 7:44288–44298.
- 3 Ferlay J, Soerjomataram I, Dikshit R, Mathers C, Rebelo M. Cancer incidence and mortality worldwide sources, methods and major patterns in GLOBOCAN 2012. *Int J cancer* 2015; 136:E359–E386.
- 4 Kim Y, Bae M, Li G, Cho Y, Kang H. Image analyzer-based assessment of tumor-infiltrating T cell subsets and their prognostic values in colorectal carcinomas. *PLoS One* 2015; 10:e0122183.
- 5 De Vries L, Swets M, Vahrmeijer L, Hokland M, Kuppen J. The immunogenetics of colorectal cancer in relation to tumor development and treatment. *Int J Mol Sci* 2016; 17:197.
- 6 Tougeron D, Fauquembergue E, Latouche B. Immune response and colorectal cancer. *Bulletin du cancer* 2013; 100:283–294.
- 7 Ostroumov D, Fekete-Drimusz N, Saborowski M, Kuhenl F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. *Cell Mol Life Sci* 2018; 75:689–713.
- 8 Smyth J, Dunn P, Schreiber D. Cancer immunosurveillance and immunoeediting: the role of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol* 2006; 90:1–50.
- 9 Mahmoud M, Paish C, Powe G, Macmillan D, Grainge J, Lee H, *et al.* Tumor-infiltrating CD8⁺ lymphocytes predict clinical outcome in breast cancer. *J Clin Oncol* 2011; 29:1949–1955.
- 10 Gobert M, Treilleux I, Bendriss-Vermare N, Bachelot T, Goddard-Leon S, Arfi V, *et al.* Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res* 2009; 69:2000–2009.
- 11 Zamarron F, Chen W. Dual roles of immune cells and their factors in cancer development and progression. *Int J Biol Sci* 2011; 7:651–658.
- 12 Gu-Trantein C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, *et al.* CD4⁺ follicular helper T cell infiltration predict breast cancer survival. *J Clin Invest* 2013; 123:2873–2892.
- 13 Cho Y, Miyamoto M, Koto K, Fukunaga A, Shichinohe T, Kawarada Y, *et al.* CD4⁺ and CD8⁺ T cells cooperate to improve prognosis of patients with esophageal squamous cell carcinoma. *Cancer Res* 2003; 63:1555–1559.
- 14 Vlado A, Alexander S, Kent EK, Peter JW, Bjorn LDMB, Mladjan P, *et al.* Significance of infectious agents in colorectal cancer development. *J Cancer* 2013; 4:227–240.
- 15 Donia M, Hansen M, Sendrup SL, Iversen TZ, Ellebaek E, Andersen MH, *et al.* Methods to improve adoptive T-cell therapy for melanoma: IFN-gamma enhances anticancer responses of cell products for infusion. *J Invest Dermatol* 2012; 10:545–552.
- 16 Xie Y, Akpinarli A, Maris C, Hipkiss EL, Lane M, Kwon EK, *et al.* Native tumor specific Cd4(+) T cells differentiated in vivo eradicate established melanoma. *J Exp Med* 2010; 207:651–667.
- 17 Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumors: impact on clinical outcome. *Nat Rev Cancer* 2012; 12:298–306.
- 18 Yasuda K, Nirei T, Sunami E, Nagawa H, Kitayama J. Density of CD4(+) and CD8(+) T lymphocytes in biopsy samples can be a predictor of pathological response to chemoradiotherapy(CRT) for rectal cancer. *Radiat Oncol* 2011; 6:49.
- 19 Chirica M, Le Bourhis L, Lehmann- Che J, Chardiny V, Bouhidel F, Foulboeuf L, *et al.* Phenotypic analysis of T cells infiltrating colon cancers: correlations with oncogenetic status. *Oncoimmunology* 2015; 4:8.
- 20 Jasim S, Dawood S, Ali M. Immunohistochemical expression of FOXP3 +CD4+CD25 regulatory T cells suppress anti-tumor immune responses in patients with colorectal cancer. *Int J Adv Res Biol Sci* 2015; 2:30–38.
- 21 Nobel F, Mellows T, McCormick Matthews H, Bateman C, Harris S, Underwood J, *et al.* Tumor infiltrating lymphocytes correlate with improved survival in patients with oesophageal adenocarcinoma. *Cancer Immunol Immunother* 2016; 65:651–662.
- 22 Shinto E, Hase K, Hashiguchi Y, Sekizawa A, Ueno H, Shikina A, *et al.* CD8 + and FOXP3+ tumor-infiltrating T cells before and after chemoradiotherapy for rectal cancer. *Ann Surg Oncol* 2014; 21:S414–S421.

- 23 Teng F, Mu D, Meng X, Kong L, Zhu H, Liuu S, *et al.* Tumor infiltrating lymphocytes (TILs) before and after neoadjuvant chemoradiotherapy and its clinical utility for rectal cancer. *Am J Cancer Res* 2015; 5:2064–2074.
- 24 Guidoboni M, Gafa R, Viel A. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *Am J Pathol* 2001; 159:297–304.
- 25 Matsutani S, Shibutani M, Maeda K, Nagahara H, Fukuoka T, Iseki Y, *et al.* Verification of the methodology for evaluating tumor-infiltrating lymphocytes in colorectal cancer. *Oncotarget* 2018; 9:15180–15197.
- 26 Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, *et al.* In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clinoncl* 2009; 27:5944–5951.
- 27 Horne D, Jack R, Gray T, Siegfried M, Wilson O, Yousem A, *et al.* Increased levels of tumor-infiltrating lymphocytes are associated with improved recurrence-free survival in stage 1A non-small-cell lung cancer. *J Surg Res* 2011; 171:1–5.
- 28 Angell H, Galon J. From the immune contexture to the immunoscore: the role of prognostic and predictive immune markers in cancer. *Curr Immunol* 2013; 25:261–267.
- 29 Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, *et al.* Toward the introduction of the 'immunoscore' in the classification of malignant tumours. *J Pathol* 2014; 232:199–209.
- 30 Azimi F, Scolyer A, Rumcheva P, Moncrieff M, Murali R, McCarthy W, *et al.* Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol* 2012; 30:2678–2683.