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ORIGINAL RESEARCH ARTICLE

Intra-tumoral tumor infiltrating Lymphocyte-T CD8+ and chemotherapy response in colorectal cancer: A prospective observational study

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ABSTRACT

Background: The immunotherapy approach to colorectal cancer is becoming one of the key approaches to colorectal cancer treatment. One of the components of immune responses is tumor-infiltrating lymphocyte CD8+ cells (TILs CD8+). While chemotherapy is one of the main treatments for colorectal cancer, we need to consider immunotherapy for advanced colorectal cancer. Thus, this study is aimed at finding the association between TIL CD8+ expression and chemotherapy response. **Methods:** This is a prospective cohort study with colorectal cancer patients in the Digestive Surgery division of a tertiary general hospital in West Java, Indonesia. An immunohistochemistry examination was used to evaluate the expression of TIL CD8+. The response evaluation criteria in solid tumors (RECIST) were used for the evaluation of chemotherapy response. **Results**: There were 53 research subjects included. There were 20 (37.7%) subjects with high expression of TILs CD8+; there were 30 (56.6%) subjects in stage III, followed by stage IV (17.32%) and stage II (6.11%). There were 18 subjects (34%) who showed progressive disease, 17 subjects (32.1%) showed partial response, and 16 subjects (30.2%) with high expression of TILs CD8+ showed partial chemotherapy response. The TILs CD8+ expression showed no significant relationship with age, sex, subtype, grade, or tumor location, but showed a significant relationship with stage and chemotherapy response ($P < 0.05$). **Conclusion**: High TILs CD8+ expression show a relationship with better chemotherapy response and a better prognosis based on disease stage in colorectal cancer patients.

Keywords: chemotherapy; colorectal cancer; immunohistochemistry; infiltrating lymphocytes; tumor-infiltrating lymphocytes

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1. Introduction

Colorectal cancer is a malignancy that affects the colon and rectum and causes approximately 135 thousand new cases each year in the United States. Colorectal cancer is also the second leading cause of death in the United States, with 50 thousand deaths estimated each year. The incidence rate is higher in developed countries than in other countries; in 2019, it was stated that the incidence rate reached 36 per 100.000 standard population[1–3]. In 2018, the Minister of Health in Indonesia released a report that showed that 8.6% of cancer incidence in Indonesia was caused by colorectal cancer, out of 348.809 total cases of all new cancer[4]. A study conducted by Dulskas et al., showed that there is an increasing survival rate in colorectal cancer patients, with the overall 5-year relative survival rate increasing from 37.9% in 1998–

2002 to 51.5% in 2008–2012. The increasing survival rate was probably possible due to the screening program that was implemented nationwide. The earlier the diagnosis, the better the treatment and survival options[5].

Colorectal cancer is marked by immune cell infiltration, which consists of granulocytes, lymphocytes, and macrophage subpopulations. Several studies have shown that the elevation of these immune cells in the tumor microenvironment leads to a better prognosis[6–9]. Anticancer immunity is one of the benefits of having immune response systems that are able to detect and destroy neoplastic cells[10,11]. Out of all, the most potent effectors come from cytotoxic CD8+ T cells[12,13]. Cytotoxic CD8+ T cells work by detecting foreign substances and directly killing malignant cells. The mechanism that leads to the death of neoplastic cells comes from the interaction between the T-cell receptor, a specific antigenic peptide on the surface of target cells served by human leukocyte antigen class I (HLA-I) or beta-2-microglobulin (β2m) complexes, and the cytotoxic CD8+ T cell itself [14]. They gathered on the surface of target cells so that immune synapse could occur. Once they create a synapse, the simultaneous event, the transduction cascade, happens, which allows cytotoxic T cells to function directly by exocytosis of cytotoxic granules containing perforin and granzymes into the target cells, which leads to malignant cell demolition, or indirectly through cytokine secretion, such as interferon (IFN) and tumor necrosis factor (TNF)[15,16]. According to Huh et al., it was found that tumor-infiltrating lymphocytes (TILs) are a strong stage-specific prognostic factor. In the study, it was stated that the TILs were a strong prognostic factor for stage III tumor patients, in which patients with a tumor microenvironment dense with TILs CD8+ cells showed a higher survival rate than those with sparse TILs CD8+ cells[17]. Another study conducted by Morris et al., showed that, by comparing the prognosis of patients treated with surgery alone and patients treated with 5-FU alone, the presence of TILs in patients treated with 5-FU had a better prognosis compared to patients treated by surgery alone. They also found that, by comparing patients with and without TILs while both types of patients were treated with 5-FU, patients with TILs obtained a higher survival rate than patients without TILs. Thus, the study suggested that TILs have predictive value for positive responses to 5-FU[18]. In correspondence to the previous study, Prall et al. found a significantly better prognosis with tumors with high densities of TILs CD8+ in patients treated with 5-FU compared to those with low TILs CD8+ densities[19].

The principle of surgical management of colon cancer at an early stage is excision, both local and wide, without or with anastomosis. In advanced stages of disease, resection is the best option. Adjuvant therapy can be in the form of radiation, especially in rectal cancer, due to organ preservation or function considerations. Radiation for rectal cancer should be given in both resect-able and non-resect-able cases. A meta-analysis of large phase III clinical trials comparing surgery alone with the addition of 5FU adjuvant chemotherapy increased survival rates by 2.3 to 5.7%. The next development of this chemotherapy is a combination of leucovorin, which can modulate 5FU activity and reduce its toxicity. Chemically, fluorouracil, a fluorinated pyrimidine, is 5-fluoro-2,4(1H,3H) -pyrimidinedione. 5-Fluorouracil (5-FU) is a pyrimidine antimetabolite chemotherapeutic drug with a mechanism of action by inhibiting the methylation of deoxyuridylic acid to thymidylic acid by inhibiting the enzyme thymidylate synthase, resulting in a deficiency of thymine, thereby inhibiting the synthesis of deoxyribonucleic acid (DNA), and at a smaller level, it can inhibit the formation of ribonucleic acid (RNA), which is important in cell division and growth. Adjuvant chemotherapy is generally given at stage II with high risk. Current guidelines recommend the administration of 5FU-Leucovorin chemotherapy and an oxaliplatin-based regimen as standard adjuvant therapy. Tumor resection can be performed in stage IV[20]. According to several studies, the expression of TILs CD8+ in the tumor microenvironment and specific immune checkpoint expression, expressed on the surface of the immune system itself, aid in determining which treatment may be more suitable for patients based on the tumor characteristics and efficacy. Thus, data regarding the correlation between TILs CD8+ expression and chemotherapy response is needed to consider treatment options and guide for individualized therapy. This study is aimed at finding the association between TIL CD8+ expression and chemotherapy response.

2. Methods

2.1. Study design and setting

This research is a prospective cohort observational study. This study was held in a tertiary general hospital in West Java, Indonesia, and follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[21]. This study used data taken from the results of anatomic pathology and medical records of all colorectal cancer cases that came to the digestive surgery polyclinic and the emergency room of a tertiary general hospital in West Java, Indonesia. The research subjects in this study were colorectal cancer patients in the Digestive Surgery division of a tertiary general hospital in West Java, Indonesia, from March 2022 to February 2023. There were 177 patients who completed chemotherapy, and 165 met the inclusion criteria. The sample required for this study was 53 patients. Using the manual simple random method, the 177 patients were sorted based on the medical record number and randomly selected based on the number three sequel, and 53 patients were selected as shown in **Figure 1**. The inclusion criteria for this study are colorectal cancer patients with complete registry data, including pathology examinations; patients who are over 18 years old; and patients who had complete chemotherapy in the same hospital, and who have agreed to be the subject of this study. We excluded colorectal patients who had a history of discontinuation of chemotherapy and stage I colorectal cancer. The examination of TILs CD8+ was carried out by the immunohistochemistry (IHC) method of paraffin blocks using a biopsy or surgical sample from colorectal cancer patient's tissue. The hospital's ethical committee approved this study with No. Ethical Approval LB.02.01/X.6.5/08/2022. Every research participant signed an informed consent form before participating in the study. Univariate statistical tests were used to find the relationship between the expression of TILs CD8+ and chemotherapy response using Chi Square, and a P-value less than 0.05 was considered significant.

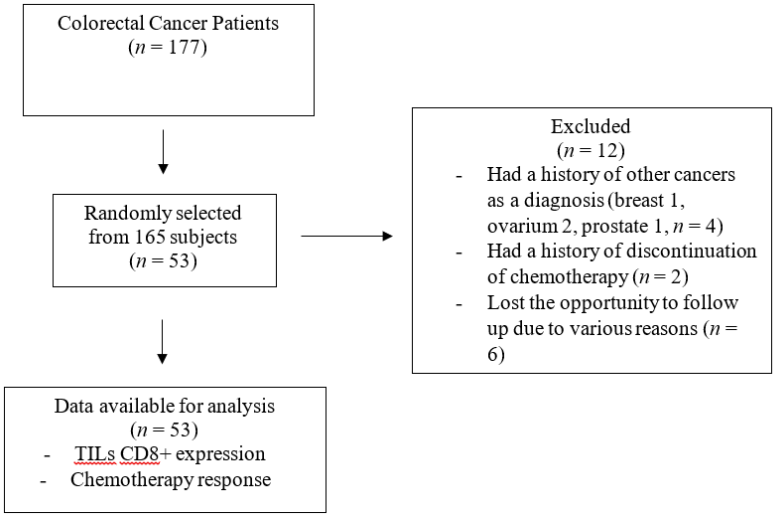

Figure 1. The flowchart of the study selection process.

2.2. TILs CD8+ expression using IHC analysis

Hematoxylin and eosin (HE) staining was used to examine tissue samples for TILs CD8+ expression. Immunohistochemical staining using the labelled streptavidin-biotin immunoperoxide complex method. The primary antibody used was anti-CD8. The 4 micrometer-thick cut preparation is placed on an object of glass that has been coated and left in an incubator at 38 °C overnight. Then, we deparaffinize it by dipping it in xylene; next, to rehydrate, we immerse the preparation in an ethanol solution. To open the epitope on the sample through the antigen retrieval process, we use ethylenediamine tetra acetic acid (EDTA) solution in a decloaking chamber at 96 °C for 20 minutes. When finished, we turn off the decloaking chamber and let it cool, then mark the sample using a Pap pen. We then drop the peroxidase solution into methanol and incubate

it. We then drop each preparation with antibodies and counterstain them. Evaluation of CD8+ T cell immune expression was carried out semi-quantitatively using an Olympus CX-21 light microscope. The assessment was carried out without knowing the patient's clinicopathological data. Assessment of CD8+ lymphocyte cells in the initial examination using 100× magnification and then using 400× magnification. Evaluation was carried out in five large fields randomly. Positive assessment of CD8+ T cell immune expression on the membrane and part of the cell cytoplasm is brown. Positive control of CD8+ T cell immune expression using liver tissue. The cut-off value for CD8+ T cell expression used in this study was 75%; the intensity and percentage of staining of CD8+ were interpreted as negative (less than 5%), weak staining (between 5%–75%), and strong or intense straining (>75%)[22]. The evaluation criteria for CD8+ T cell immunochemotherapy were calculated and divided into "high" and "low" as shown in **Figure 2**.

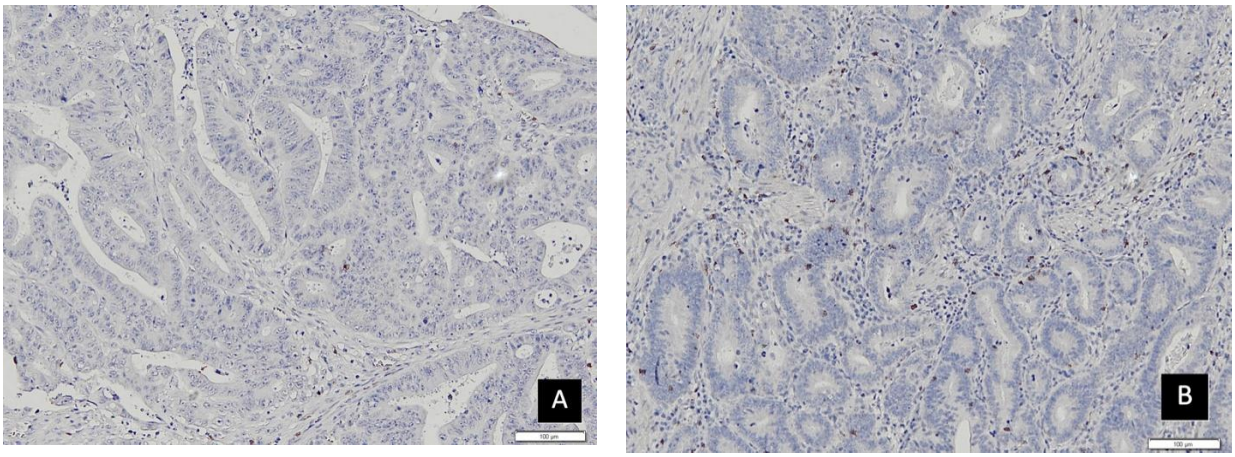

Figure 2. Assessment of TILs CD8+ expression with immunohistochemistry: **(A)**. Normal or low TIL CD8+ expression (0–75%); **(B)**. Positive TIL CD8+ expression (> 75%).

2.3. Chemotherapy response evaluation using RECIST

There were three chemotherapy regimens used in this study. First, the fluorouracil and leucovorin (de Gramont) regimen, with 2 hours of 200 mg/m^2 leucovorin and a 400 mg/m^2 bolus injection dosage of fluorouracil, was continued with a 600 mg/m^2 infusion of fluorouracil for 22 hours. The sequence is repeated for two days and given every two weeks. Second, the fluorouracil, leucovorin, and oxaliplatin (Folfox-6) regimen, with 2 hours of 400 mg/m^2 leucovorin and oxaliplatin 100 mg/m^2 on day 1, followed by a bolus of 400 mg/m^2 fluorouracil and a 46-hour infusion of 2.4–3 g/m^2 fluorouracil, given every two weeks. Third, the Capecitabine regimen, with an oral dose of 1250 mg/m^2 given twice daily for 14 days, is off for one week[23,24].

The evaluation of chemotherapy responses was measured using the response evaluation criteria in solid tumors (RECIST). To date, the RECIST based on anatomic measurements of tumor size has been most widely used for imaging tumors in drug trials or in clinical practice. The RECIST criterion uses the longest diameter (unidimensional). These criteria provide a standardized assessment of tumor response in terms of progression-free survival (PFS), which is considered an acceptable surrogate end point for overall survival (OS)[25,26]. The comparation of size was taken from pre-chemotherapy and post-chemotherapy abdominal computed tomography scan (abdominal CT-scan) as shown in **Figures 3–6**.

2.4. Statistical analysis

The data that has been collected is processed and computerized; numerical data is presented as mean and standard deviation. Categorical data is presented as percentages. The comparison of the variables was based on the chi2 test performed using the SPSS 26 software (SPSS Inc., Chicago, IL, USA). The *P*-value < 0.05 indicates a significant relationship.

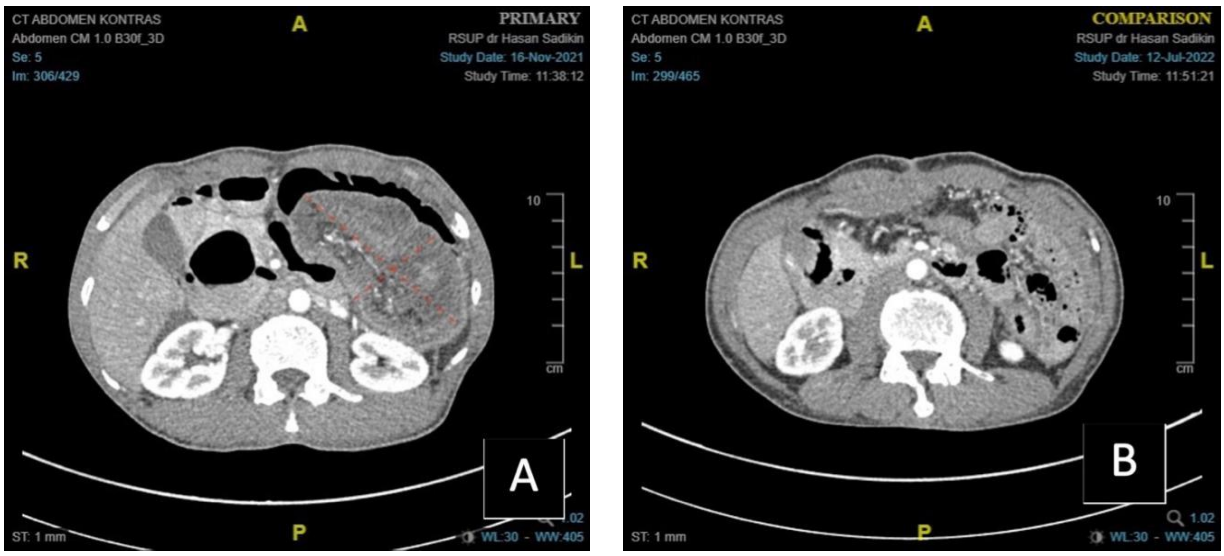

Figure 3. Complete response to chemotherapy. **(A)** The size of the solid lesion in the intraluminal descending colon and the narrowing of the lumen with thickening of the surrounding wall. **(B)** No residual or mass residue is seen.

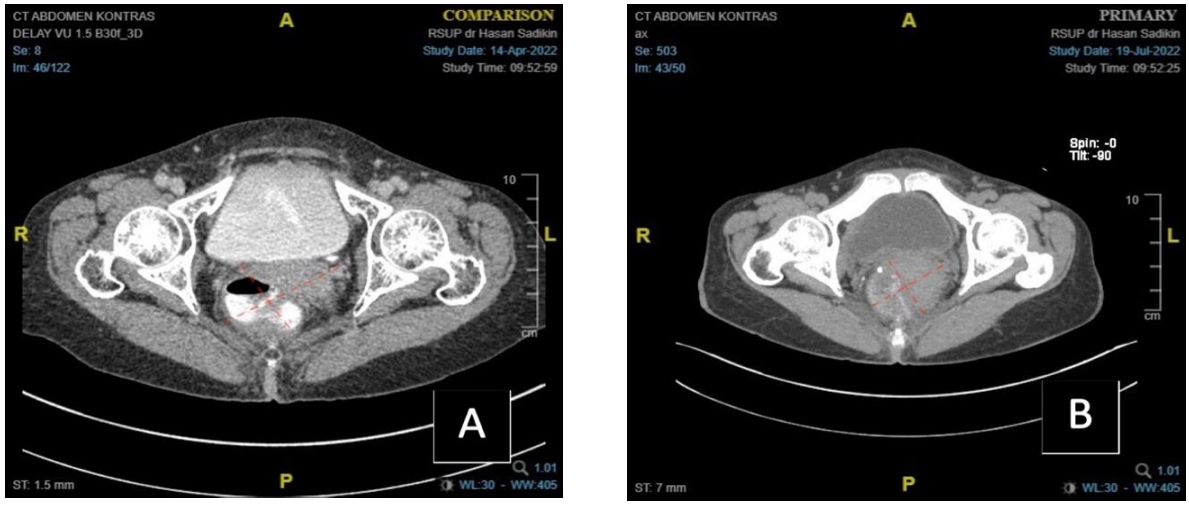

Figure 4. Partial response to chemotherapy. **(A)** There is wall thickening coupled with an irregular narrowing of the lumen of the sigmoid colon distal to the anus. **(B)** The size of the solid lesion from the intraluminal sigmoid colon to the proximal rectum was slightly reduced, coupled with thickening of the surrounding wall.

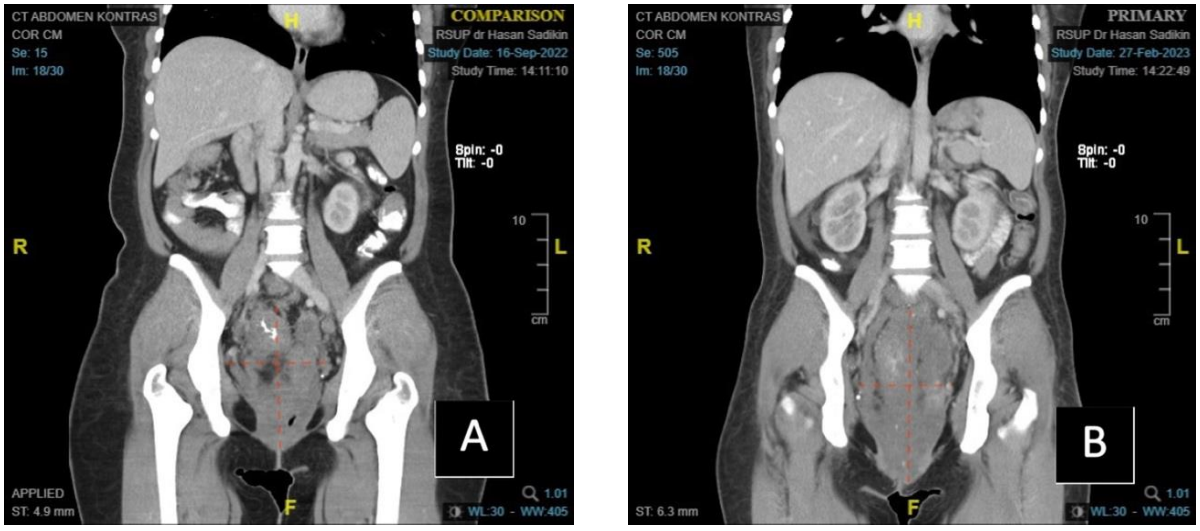

Figure 5. Progressive response to chemotherapy. **(A)** Irregular wall thickening with narrowing of the lumen in the area distal of the sigmoid colon to the medial rectum that appears to invade the posterior wall of the uterine corpuscle **(B)** Pushing of the small intestine superiorly, pushed caecum to the right lateral, invasion of the ascending and descending colon, invasion of the rectum and posterior wall of the uterus inferiorly by the residual colorectal mass (compared to previously seen increases in size by half).

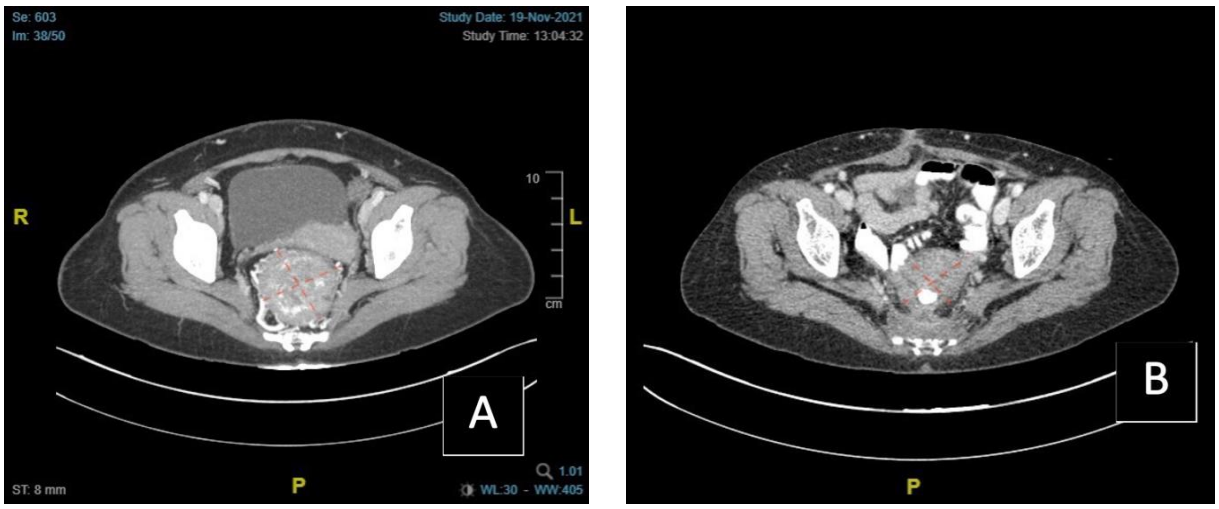

Figure 6. Stable disease response to chemotherapy. **(A)** Rectal malignancy extending inferiorly to the anus, superior to the distal sigmoid, as well as anterior prostatic infiltration **(B)** No increase in size compared to image A.

3. Results

3.1. Subject characteristics

There were 53 research subjects, and the characteristics of the research subjects are shown in **Table 1**. There were 30 female and 23 male patients. The mean age was 57.45 years ± 12.81, and 77.4% of patients were older than 50. Most of the patients showed adenocarcinoma histologically (92.5%), and 79.2% showed well-differentiated tumors. There were 36 (67.9%) rectal cancer patients and 17 (32.1%) colon cancer patients. High TILs CD8+ expression was shown in 20 (37.7%) patients. The fluorouracil-based chemotherapy regimen was shown in 45 (84.9%). The majority of the chemotherapy responses were progressive disease and partial responses (34% and 32.1%, respectively).

Table 1. Characteristics of research subjects.

Variable		**Proportion (%)**
Age	Mean	57.45 ± 12.81 years
	Median	59 years
Age	<50	12 (22.6%)
	≥50	41 (77.4%)
Sex	Male	23 (43.4%)
	Female	30 (56.6%)
Histologic	Adenocarcinoma	49 (92.5%)
	Signet ring cell	2 (3.8%)
	Mucinous adenocarcinoma	2 (3.8%)
Grade	Well diff	42 (79.2%)
	Moderately diff	5 (9.4%)
	Poorly diff	2 (3.8%)
	Specific	4 (7.5%)
Tumor location	Caecum	2 (3.8%)
	Ascending	4 (7.5%)
	Transverse	4 (7.5%)
	Descending	2 (3.8%)
	Sigmoid	5 (9.4%)

Table 1. (*Continued*).

Variable		Proportion (%)
	Rectum	36 (67.9%)
Tumor location	Colon	17 (32.1%)
	Rectum	36 (67.9%)
Stage	II	6 (11.3%)
	III	30 (56.6%)
	IV	17 (32.1%)
Metastases	Liver	12 (22.6%)
	Lung	1 (1.9%)
	Liver and lung	1 (1.9%)
	Liver and bone	2 (3.8%)
	Liver, lung, and bone	1 (1.9%)
TIL CD8+	High expression	20 (37.7%)
	Normal expression	33 (62.3%)
Chemotherapy regimen	Fluorouracil and leucovorin	24 (45.3%)
	Fluorouracil, leucovorin and oxaliplatin.	21 (39.6%)
	Capecitabine	8 (15.1%)
Chemotherapy response	Complete response	3 (5.7%)
	Partial response	17 (32.1%)
	Stabile disease	15 (28.3%)
	Progressive disease	18 (34%)

3.2. Univariate analysis of variables and TILs CD8+ expression

Two variables showed a statistically significant relationship ($p < 0.05$) in univariate analysis with TILs CD8+ expression, as shown in **Table 2**. In stage III, there were 17 (32.1%) subjects with high expression of TILs CD8+ compared to stage IV. Patients with high TILs CD8+ expression showed more in the partial response group compared to the progressive disease group, which showed more patients with low TILs CD8+ expression.

Table 2. Univariate analysis for subject characteristics with TILs CD8+ expression.

	High expression	Normal expression	Chi-square (*p*-value)
Age			0.087
Early onset (<50)	2 (3.8%)	10 (18.9%)	
Late onset (≥50)	18 (34%)	23 (43.4%)	
Sex			0.337
Male	7 (13.2%)	16 (30.2%)	
Female	13 (24.5%)	17 (32.1%)	
Histologic type			0.506
Adenocarcinoma	19 (35.8%)	30 (56.6%)	
Mucinous Adenocarcinoma	0	2 (5.6%)	
Signet ring cell	1 (1.9%)	1 (1.9%)	
Grade			0.272
Well differentiated	18 (34%)	24 (45.3%)	
Moderately differentiated	0	5 (9.4%)	

Table 2. (*Continued*).

	High expression	Normal expression	Chi-square (*p*-value)
Poorly differentiated	1 (1.9%)	1 (1.9%)	
Specific	1 (1.9%)	3 (5.7%)	
Tumor location			0.862
Caecum	0	2 (3.8%)	
Ascending	1 (1.9%)	3 (5.7%)	
Transverse	2 (3.8%)	2 (3.8%)	
Descending	1 (1.9%)	1 (1.9%)	
Sigmoid	2 (3.8%)	3 (5.7%)	
Rectum	14 (26.4%)	22 (41.5%)	
Tumor location			0.801
Colon	6 (11.3%)	11 (20.8%)	
Rectum	14 (26.4%)	22 (41.5%)	
Stage			0.003
II	2 (3.8%)	4 (7.5%)	
III	17 (32.1%)	13 (24.5%)	
IV	1 (1.9%)	16 (30.2%)	
Chemotherapy response			0.001
Complete response	1 (1.9%)	2 (3.8%)	
Partial response	16 (30.2%)	1 (1.9%)	
Stabile disease	3 (5.7%)	12 (22.6%)	
Progressive disease	0	18 (34%)	

4. Discussion

4.1. Study population characteristics

Based on **Table 1**, it is known that the age of the research subjects showed an average of 57.45 ± 12.81 years and a median of 59 years. This shows that patients who come to a tertiary general hospital in West Java, Indonesia, with a diagnosis of colorectal cancer are mostly over 50 years old. Based on epidemiological studies, the results of this study are in accordance with the profile of patients with CRC, most of whom are over 50 years old[27,28]. Data showing that the risk of CRC increases in the fifth decade of life has been widely cited. In some studies, the risk increases by up to one percent (1%) for each additional 10 years of age starting at age 50[27–30].

Based on sex, there were 30 females (56.6%) and 23 males (43.4%). There were more female subjects than male subjects. The prevalence of CRC is not too different by sex, with a prevalence rate of 1:23 (4.3%) in men and 1:25 (4.0%) in women[27,29,30]. There is a protective effect of the hormones estrogen and progesterone; it is thought that it is only effective before menopause, but this effect decreases at menopause, so that the incidence of colorectal cancer in women over the age of 50 can increase[31].

Based on the results of anatomical pathology examination, as many as 49 subjects (92.5%) showed adenocarcinoma, followed by signet ring cells in two subjects (5.6%), and mucin adenocarcinoma in two subjects (5.6%). Meanwhile, based on grade, 42 subjects (79.2%) showed good differentiation, while five and two subjects, respectively (9.4% and 3.8%), had moderate and poor differentiation. Based on literature studies, the type of adenocarcinoma in colorectal cancer is found in up to 90% of the total cases. The differentiation of the adenocarcinoma itself can be in the form of comedo, medullary, micropapillary, mucinous, or signet ring

cells. moderate grade or differentiation, which is as much as 70%, followed by good differentiation of around 10%–20%[32].

Based on the location of the tumor found, as many as 17 subjects (32.1%) showed the tumor was in the colon; the location of this tumor included the cecum, ascending colon, transverse, descending, and sigmoid. In 36 subjects (67.9%), the tumor was in the rectum; this location covered the proximal to distal rectum. The results of this study are in accordance with the existing literature, which states that about 70% of total colorectal cancer occurs in the descending colon (10% descending colon, 10% sigmoid, and 50% rectum), and then about 29.5% occurs in the ascending and transverse colons[33]. Most of the study subjects, that is, 30 subjects (56.6%), had stage III CRC, and 6 subjects (11.3%) had stage II CRC. Patients with stage IV CRC were found in 17 subjects (32.1%) with the most metastases in the liver, that is, 12/17 (70.5%).

4.2. Expression of tumor infiltrating lymphocytes CD8+ in colorectal cancer patients

In this study, the proportion of TILs with CD8+ expression appeared to be higher when compared to several other studies. Univariate analysis of TILs CD8+ expression showed no significant relationship between TILs CD8+ expression and age, sex, type of anatomic pathology, grade, or tumor location, but showed a significant relationship with stage and chemotherapy response ($P < 0.05$). Colorectal patients with high TILs CD8+ expression were less likely to have stage IV (distant metastases) when compared to those with low TILs CD8+ expression, that is, 1 versus 16 subjects. Then, CRC patients with high TILs CD8+ expression responded better to chemotherapy than those with low TILs CD8+ expression. Out of 33 subjects with low TILs CD8+ expression, 12 (22.6%) had a stable disease response and 18 (34%) had a disease response that got worse.

In the study by Sideras et al. in the Netherlands, in patients with stage IV CRC with liver metastases, 47 subjects underwent immunohistochemistry of TILs CD8+. The majority of TILs CD8+ are in the peri-tumoral region when compared to the intra-tumoral region. The median TILs CD8+ density in the intra-tumoral region was 58.6/mm^2, and the peri-tumoral median was 883/mm^2. Calculation of the ratio of TILs CD8+ in the intra-tumoral region showed this ratio as a predictor of survival (hazard ratio 0.45, 95% confidence interval 0.20–0.99, $P = 0.044$)[34]. In the study by Trabelsi et al. in Tunisia, 106 CRC patients underwent immunohistochemistry of TILs CD8+. The examination was carried out in the CT (central tumor) and IM (invasive margin) areas. The expression of TILs CD8+ in both areas showed high results, one area showed heterogeneous results, and a negative result in both areas indicates a low result. Examination showed that there were 95 subjects with high TILs CD8+ expression at 20/95 (21.05%), heterogeneous at 49/95 (51.58%), and low at 26/95 (27.37%). The results of the analysis showed that tumors with high TILs CD8+ expression showed better overall survival (OS) compared to tumors with low TILs CD8+ expression ($P = 0.005$)[35].

In the study of Guan et al. in USA using a mouse model, high TILs CD8+ levels were associated with a good therapeutic response and a good prognosis in rats receiving Folfox chemotherapy (fluorouracil, leucovorin and oxaliplatin). Folfox therapy modulates TILs CD8+, which has an impact on reducing the tumor burden of colorectal cancer[36]. In the study of Alsalman et al. in Oman, 34 CRC patients with high levels of TILs CD8+ showed a significant association with better disease-free survival (DFS)[37]. A study by Xin et al. in China showed that in 111 patients with stage IV CRC, preoperative chemotherapy would increase the antigen presented by T lymphocytes and activate TILs CD8+ infiltration. High expression of TILs CD8+ in the microtumor environment was related to Programmed Cell Death Ligand 1 (PD-L1), which affected patient survival, and TILs CD8+ in primary tumors was an independent factor, which affects prognosis, so that CD8+ infiltration in primary tumors can predict better OS in patients with stage IV CRC. Based on the results of this study, TILs CD8+ expression and clinicopathological factors in CRC patients did not show an interrelated relationship. However, based on other studies linking TILs CD8+ expression with survival in CRC patients, it showed a positive relationship between TILs CD8+ expression and increased survival in 85 patients[38]. Chemotherapeutic agents are intended to trigger particular immune responses that lead to the immunogenic

death of cancer cells. The chemotherapeutic agents may encourage the activation of IFN-γ-producing CD8+ T cells. In addition to TILs CD8+, which are essential for tumor-specific cellular adaptive immunity, activation of innate and particular immune effectors may boost the body's ability to fight against tumors. As a result, the expression of high TIL CD8+ may be a predictor of a stronger host immune response against a tumor[39]. The statistically significant result helped guide and serve as a reference for which therapy should be given to patients with high or low expression of TILs CD8+. It also showed that the expression of TILs CD8+ helped predict a better prognosis since the expression correlates with a better chemotherapy response and a more suitable therapy for each patient. The limitations of this study were that the short period of time might not have resulted in the actual findings, as TILs CD8+, according to several studies, can undergo exhaustion and dysfunction due to chronic expression of immune checkpoints. Larger data were also needed as the tertiary hospital where this study was held only covered one part of the area in which the subjects might have similar characteristics; thus, larger areas are needed to include subjects with different characteristics to consider other factors that might influence this research. As the trend of colorectal cancer patients is changing, the examination of risk factors, genetic mutations, and immunological status of colorectal cancer patients has become important to implement personalized cancer treatment, especially in advanced colorectal cancer[22,40–44].

The results of this study are expected to provide direction for subsequent studies with similar topics and different samples. Further studies are needed regarding the comparison between the effectiveness of individualized therapy and non-individualized therapy, whether patients with low TILs CD8+ can utilize immunotherapy to increase the activity of these cells, whether combination therapy can further improve the prognosis because it can affect TILs CD8+, and lastly, for subjects with high TILs CD8+ expression, when is the best time to start immunotherapy as TILs CD8+ can experience dysfunction and exhaustion, and whether immunotherapy will be the right choice.

5. Conclusion

High expression of TILs CD8+ in colorectal cancer patients results in a better chemotherapy response. The known side effects of chemotherapy, such as toxicity, are one of the reasons why many studies have developed therapies that specifically work in certain cells; thus, alternatives such as immunotherapy are now emerging. Immune checkpoint inhibitors prevent TILs CD8+ from experiencing exhaustion and dysfunction, so patients with high expression of TILs CD8+ can benefit from this mechanism, while patients with low expression of TILs CD8+ might as well benefit from immunotherapy. Combination therapy has shown great success for patients that do not respond to checkpoint inhibitor monotherapy, as the majority of chemotherapy was found to have immunostimulatory effects rather than immunosuppressive properties. Other options of therapy, such as targeted therapy, might be considered for patients who are unresponsive to immunotherapy.

Author contributions

Conceptualization, DB, KL, RR, BAASS, YS, PN, LYH and BMD; methodology, DB, KL, RR, BAASS, YS, PN, LYH and BMD; formal analysis, DB, KL, RR, BAASS, YS, PN, LYH and BMD; investigation, DB, KL, RR, BAASS, YS, PN, LYH and BMD; resources, DB, KL, RR, BAASS, YS, PN, LYH and BMD; data curation, DB, KL, RR, BAASS, YS, PN, LYH and BMD; writing—original draft preparation, DB, KL, RR, BAASS, YS, PN, LYH and BMD; writing—review and editing, DB, KL, RR, BAASS, YS, PN, LYH and BMD; supervision, DB, KL, RR, BAASS, YS, PN, LYH and BMD; project administration, DB, KL, RR, BAASS, YS, PN, LYH and BMD. All authors have read and agreed to the published version of the manuscript.

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Statements and declarations

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Ethical declaration

The Hospital ethics committee approved the study with No. Ethical Approval LB.02.01/X.6.5/08/2022. Every research participant signed an informed consent form before participating in the study.

Data availability

All data and tables used to support the findings of this study are included within the article and available upon request to the corresponding author.

Provenance and peer review

Not commissioned, externally peer reviewed.

Conflict of interest

The authors declare no conflicts of interest.

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