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Post-chemotherapy response of paclitaxel and carboplatin regimens in advanced stage cervical cancer types of histopathology squamous cell carcinoma and adenocarcinoma
Yohanes Adhitya Prakasa Sukoco, Meitria Syahadatina Noor, Ferry Armanza, Hariadi Yuseran, Renny Aditya
Online First: Feb 23, 2023

Effects of Lactobacillus plantarum IS-10506 on gastric protective mucosal factors: an ibuprofen-induced gastric mucosal injury
Andy Darma, Wibi Riawan, Khadijah Rizky Sumitro, Alpha Fardah Athiyyah, Reza Ranuh, Mohammad Fathul Qorib, Ingrid Surono, Subijanto Marto Sudarmo

Prevalence of multidrug-resistant Escherichia coli isolated from Jember hospital food handler in Indonesia
Enny Suswati, Supangat, Laksmi Indreswari, Farhatina Nur U'alifa, Faiza Nabila, Inas Gama Putri Hertian
Online First: Feb 28, 2023

Sarcopenia as an indicator of nutritional status and outcome predictor for colorectal cancer in Javanese ethnic
Damianus Hipolitus, Adeodatus Yuda Handaya, Agus Barmawi
Online First: Dec 5, 2022

Comparison of chlorhexidine 0.7% and modified Petroff's method on sputum decontamination for culture method to detect Mycobacterium tuberculosis
Rizky Hurriah Lumbessy, Ni Made Mertaniasih, Lindawati Alimsardjono, Soedarsono Soedarsono
Online First: Jan 7, 2023

Comparison of agility between the flat foot and normal foot in East Java Puslatda athletes
Bobby Kennedy, Damayanti Tinduh, Ditaruni Asrina Utami, I Putu Alit Pawana, Soenarnatalina Melaniani
Online First: Jan 24, 2023

A novel preoperative scoring system to predict postoperative mortality after pancreaticoduodenectomy in pancreatic cancer patients at Dr. Soetomo General Hospital: a retrospective cohort study
Arland Chandra, Tomy Lesmana
Online First: Feb 20, 2023

Relationship between angiopoietin-like-protein-2 levels and anti-mullerian hormone levels in polycystic ovary syndrome of reproductive age
Dyan Asih Rachmawati, Ashon Sa'adi, Budi Utomo, Arif Tunjungseto
Online First: Feb 26, 2023

The impact of COVID-19 vaccination for healthcare workers on the SARS-CoV-2 antibody titers in a tertiary hospital in Jakarta, Indonesia
Rika Bur, Febby Elvanesa Sandra Dewi, Virmandiani, Yosanti Elsa Kartikawati, Meutia Gebrina, Aditya Rifqi Fauzi, Duddy Mulyawan, Ni Sayu Dewi Budhiyani
Online First: Dec 25, 2022

Factors that determine inpatient satisfaction on nursing care: a cross-sectional study
Sulaiman , Anggriani , Maryaningsih, Ronald Erwansyah, Lagut Sutandra Siregar, Muchti Yuda Pratama, Selly Oktaria, Rahmadi Ali, Yenni Gustiani Tarigan
Online First: Jan 24, 2023

Development of nursing learning program for COVID-19 patients with e-learning method for nursing department students Poltekkes Ministry of Health Jayapura Papua
Blestina Maryorita, Jems Kifen Roget Maay
Online First: Jan 24, 2023

Tumour infiltrating lymphocyts (TILs) as an excellent predictive factor for neoadjuvant chemotherapy response on locally advanced rectal cancer patient

Florencia Adys, Iwan Kristian, Sahudi, Alphania Rahniayu
Online First: Jan 19, 2023

Correlation between age, the clinical status of patients, operator characteristics, and surgical landmarks with the incidence of tracheotomy complications
Evan Anindito, Muhtarum Yusuf, Titiek H Ahadiah
Online First: Mar 10, 2023

Fibroblast growth factor 2 expression on lymph node metastasis in early-stage breast cancer
Diani Kartini, Sonar Soni Panigoro, Islam Akbar Alam, Alban Willian Dien, Dewi Sumaryani Soemarko, Tantri Hellyanti, Jessica Dewati Wardhana, Kevin Varian Marcevianto
Online First: Feb 4, 2023

Application for COVID-19 severity diagnosis and asynchronous telehealth: development and prototype of G-COV
Budi Yanti, Adinda Zahra Ayufi Ramadhani, Zarfan Fawwaz Muhamad, Rahmad, Al Yafi
Online First: Dec 15, 2022

The effectiveness of roll-on aromatherapy of calamansi orange peel essential oil (Citrofortunella microcarpa) on reducing anxiety and pain in labor
Yetti Purnama, Kurnia Dewiani, Suci Rahmawati, Linda Yusanti, Linda Yulyani
Online First: Feb 13, 2023

Unmet need for Family Planning (FP) in 7 national development areas in Indonesia
Musafaah Musafaah, Tris Eryando, Meiwita Paulina Budiharsana, Martya Rahmaniati, Hadrianti Lasari
Online First: Mar 6, 2023

Integrated care: Health Promotion Hospital Model to Improve Family Support for Patients with Chronic Disease
Rina Kurniawati, Mahendro Prasetyo Kusumo
Online First: Feb 16, 2023

The impact of bullying on adolescent self-concept
Laili Nur Hidayati, Silfia Wahyuni
Online First: Jan 30, 2023

Distribution pattern and spatial analysis of factors for tuberculosis (TB) cases in Bandar Lampung City in 2022
Maria Tuntun, Siti Aminah, Yusrizal CH
Online First: Dec 6, 2022

The clinicopathological characteristics of borderline ovarian tumors at Dr. Soetomo Hospital, Surabaya: a single center study
Setyo Teguh Waluyo, Brahmana Askandar Tjokroprawiro
Online First: Feb 4, 2023

Efficacy of tadalafil and l-arginine combination therapy compared with tadalafil-only for the treatment of erectile dysfunction: A systematic review and meta-analysis
Donny Austine Wibisono, Furqan Hidayatullah, Fikri Rizaldi, Tarmono Djojodimedjo
Online First: Jan 20, 2023

ORIGINAL ARTICLE

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Tumour infiltrating lymphocyts (TILs) as an excellent predictive factor for neoadjuvant chemotherapy response on locally advanced rectal cancer patient

Florencia Adys[1*], Iwan Kristian[2], Sahudi[3], Alphania Rahniayu[4]

[1]Department of Surgery, Faculty of Medicine Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia;
[2]Senior Staff, Digestive Surgery Division, Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia;
[3]Senior Staff, Head and Neck Surgery Division, Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia;
[4]Senior Staff, Anatomical Pathology Departement, Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya, Indonesia;

*Corresponding author:
Florencia Adys;
Digestive Surgery Division, Department of Surgery, Faculty of Medicine Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;
flobedum@gmail.com

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ABSTRACT

Background: In Indonesia, 16,000 new cases of rectal cancer were found in 2020, with a mortality rate up to 50%. Most of the cases were locally advanced rectal cancer which requires neoadjuvant therapy. A predictive marker that can indicate how neoadjuvant therapy is responding is thus required, and TILs is a biomarker that has been extensively studied as a reliable predictive and prognostic marker in colorectal cancer. This study aims to determine if the response to neoadjuvant chemotherapy in locally advanced rectal cancer (LARC) patients correlated with the density of TILs.

Methods: This study was a retrospective cohort study with samples taken from rectal cancer patients who visited Dr. Soetomo General Hospital between 2017 and 2021. TILs density measurements were taken from stromal tumors on pre-NAC biopsy examination with H&E staining. Evaluation of chemotherapy response based on abdominal CT scan. The density of TILs using 45% cut-off.

Results: A total 38 samples were studied, 21 were male and 17 were female. Twenty-nine samples (86.2%) with weak TILs density showed negative chemotherapy response or progressive tumors. There is a significant correlation between the density of TILs and the chemotherapy response of the sample studied (p=0.002).

Conclusion: TILs are an excellent predictive marker for neoadjuvant chemotherapy response in LARC patients and can be examined using H&E staining method which is cheap and fast but still has predictive value.

Keywords: infiltrating lymphocyte, rectal cancer, neoadjuvant chemotherapy.
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INTRODUCTION

Colorectal cancer is one of the health problems faced worldwide. In terms of cancer incidence, colorectal cancer is the third most common cancer in the world, with 1.9 million new cases expected in 2020.[1] According to GLOBOCAN data 2021, colorectal cancer has the second-highest cancer mortality rate around the world with approximately 900,000 deaths in 2020. The incidence of colorectal cancer is rather high in Indonesia. There were 16,059 new cases of rectal cancer in 2020, with Locally Advanced Rectal Cancer (LARC) accounting for 49.7% of those occurrences. Of those patients, 86.3% will be over 50, and 8,342 will die from it.[1]

Chemoradiotherapy treatment is used for locally advanced rectal cancer (LARC), which is targeted to reduce size and stage of tumor in order to maximize the likelihood of sphincter preservation during surgery and tumor-free resection while reducing the risk of local recurrence.[2] Neoadjuvant therapy will cause the patient harm when it is ineffective, such as delayed surgery or immune system suppression. FOLFOX or its modification (mFOLFOX6), with a range of 2 weeks between administrations, is one of the neoadjuvant chemotherapy regimens that can be used.[3]

Neoadjuvant chemotherapy alone without radiotherapy in patients with locally advanced rectal cancer did not significantly affect PCR, resection R0, or local recurrence, according to a meta-analysis study by Lin in 2020. Neoadjuvant chemotherapy alone was stated to have a lower incidence of distant metastases and a higher rate of sphincter preservation than the chemoradiotherapy group.[4] Therefore, the subjects in this study were those who had locally advanced rectal cancer that just received neoadjuvant chemotherapy alone without radiation.

Repeating a CT or MRI scan 6–8 weeks after the initial treatment is the most objective way to evaluate the patient's response to neoadjuvant chemotherapy. Radiological exams performed before and after the treatment were compared to evaluate the effectiveness of the administered neoadjuvant chemotherapy. Pada penelitian ini, The RECIST 1.1 criteria have been followed for assessing response to neoadjuvant chemotherapy since they are easy to use, standardized and reliable criteria for both clinicians and radiologists.[5]

Currently, it is thought that tumor-infiltrating lymphocytes (TILs) can be used to predict how well the immune

system would respond to a tumor and prevent it from growing. Accordingly, numerous studies have been conducted to determine whether TILs can be used as a marker for neoadjuvant chemotherapy response in patients with locally advanced rectal cancer, with the conclusion that patients with strong TILs density have a favorable neoadjuvant chemotherapy response.[6-9] According to the International TILs Working Group (ITWG) system, this TILs examination can be performed by measuring the lymphocyte cells in the stromal tumor from a patient's biopsy sample before neoadjuvant treatment. Even though it is commonly done, reasonably quick, and inexpensive, histopathological examination with H&E staining still has the capacity to serve as a predictor and prognostic factor.[10]

This study is expected to be able to offer clinicians a new predictive marker that can be immediately, inexpensively, and easily performed in patients with locally advanced rectal cancer before starting neoadjuvant chemotherapy, assisting in determining whether the chemotherapy to be administered is beneficial or ineffective and minimizing the chance of postponing surgery.

METHODS

Study Design and Participants

This is a retrospective cohort study conducted at Digestive Surgery Division Outpatient Installation at Dr. Soetomo General Hospital. We found 38 samples that were taken using consecutive sampling based on inclusion and exclusion criteria by collecting medical record data of LARC patients who visited between January 2017 and December 2021. Every patient with LARC who underwent biopsy and received neoadjuvant chemotherapy mfolfox6 met the inclusion criteria for this study. These patients previously underwent abdominal CT scans before and after receiving neoadjuvant chemotherapy mfolfox6 to evaluate their chemotherapy response using the CT scan findings. Patients with inadequate medical record information, those who received radiotherapy or chemotherapy prior to biopsy, or those who did not receive chemotherapy as planned were excluded from the study.

Data Collection

We reviewed the medical records of patients with LARC who met this study's inclusion and exclusion criteria. We collected names, gender, age, body mass index, comorbidities, pathological examination results, and radiological features from abdominal CT Scan consisting of tumor size, lymph node, and metastasis status. All patients received neoadjuvant chemotherapy mfolfox6, as scheduled for 8 to 12 weeks. All the data were obtained from paper-based and electronic medical records.

Tumor-Infiltrating Lymphocyte Measurement

TILs density was measured from biopsy specimens stained using H&E, at the Department of Anatomical Pathology, Dr. Soetomo General Hospital, before neoadjuvant chemotherapy mfolfox6 was given. The measurement uses the criteria of the International TILs Working Group (ITWG) which have been adopted for colorectal cancer. Infiltrating lymphocytes only calculated on stromal tumors not intratumoral. To prevent bias in calculating the TILs percentage, the TILs were only evaluated by a single expert pathologist in this study.

Statistical Analysis

The data were analyzed using the SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Cross-tabulations and frequency distribution tables are used to present the data. The Chi-square test and logistic regression were used to analyze independent and dependent variables. The ideal cut-off value for the TILs to predict the chemotherapy response was determined using receiver operating characteristic (ROC) curve analysis.

RESULTS

Characteristics of Subjects

As seen in Table 1, 17 women (44.7%) and 21 men (55.3%) were among the study subjects. The subjects' ages varied from the third to the sixth decade, with the highest number in the fourth decade (18 samples, 47.4%), followed by the fifth decade (9 samples, 23.7%). In this study, comorbidities were present in 4 samples (10.5%) while 34 samples (89.5%) were comorbidity-free. As for nutritional status, most of the samples (32 samples, 84.2%) had normal nutritional status, 2 samples (5.3%) were underweight, 3 samples (7.9%) were overweight, and 1 sample (2.6%) with obesity. This study had three types of tumor differentiation: well-differentiated adenocarcinomas, moderately-differentiated adenocarcinomas, and poorly-differentiated adenocarcinomas. There were 24 samples (63.2%) with well-differentiated adenocarcinoma, 5 with moderate differentiation (13.2%), and 9 with poor differentiation (23.7%).

Subjects Tumor Staging Before and After Neoadjuvant Chemotherapy

Based on the Tumor-Nodule-Metastase (TNM) features, the tumor stage was classified into two groups: the stage before neoadjuvant chemotherapy (pre-NAC) and the stage group after neoadjuvant chemotherapy (post-NAC). Following the size of the tumor, from the pre-NAC group, 34 study samples (89.5%) had T4b, 2 samples (5.3%) with T4a and 3 samples (7.89%) with T3. As for nodal status, it was discovered that a total of 23 samples (60.5%) had N2b stage, compared to 1 sample (2.6%) had N2a, 10 samples (26.3%) and 4 samples (10.5%) for N2 stage and N1 stage, respectively. All samples (100%) in the pre-NAC group had no metastases (M0). In the post-NAC group, most of samples (34 samples, 89.5%) had T4b stage and 4 samples for T4a stage (10.5%). For nodal status, the N2b was found in 22 samples (57.9%), the N2a in 1 sample (2.6%), the N2 stage in 9 samples (23.7%), and the N1 stage in 6 samples (15.8%). Metastasis was found in 17 samples and 21 samples with M0 stage (55.3%). Tumor staging before and after neoadjuvant chemotherapy is shown in Table 2.

Correlation Between Patient's Characteristics and The Chemotherapy Response

As shown in Table 3, We analyze the correlation between patient characteristics and chemotherapy response. Chemotherapy response in this study was divided into positive responses (complete response and partial response) and negative responses (stable disease and progressive disease). Five (23.8%)

Table 1. Characteristics of Subjects.

Characteristics	n	Percentage
Sex		
Male	21	55.3
Female	17	44.7
Age		
<30	1	2.6
30-39	5	13.2
40-49	18	47.4
50-59	9	23.7
<=60	5	13.2
Nutritional Status		
Underweight	2	5.3
Normal	32	84.2
Overweight	3	7.9
Obesity	1	2.6
Comorbidities		
No	34	89.5
Yes	4	10.5
Histopathology Type		
Adenocarcinoma Well Differentiated	24	63.2
Adenocarcinoma Moderately Differentiated	5	13.2
Adenocarcinoma Poorly Differentiated	9	23.7

Table 2. Subjects Tumor Staging Before and After Neoadjuvant Chemotherapy.

Pre-NAC			Post-NAC		
	n	Percentage		n	Percentage
T			**T**		
T3	3	7.89	T3	0	0
T4a	2	5.3	T4a	4	10.5
T4b	33	86.8	T4b	34	89.5
N			**N**		
N1	4	10.5	N1	6	15.8
N2	10	26.3	N2	9	23.7
N2a	1	2.6	N2a	1	2.6
N2b	23	60.5	N2b	22	57.9
M			**M**		
M0	38	100	M0	21	55.3
M1	0	0	M1	17	44.7

of the 21 male sex samples had positive chemotherapy responses, while 16 (76.2%) had negative chemotherapy responses. Meanwhile, in 17 female samples, 5 (29.4%) had a positive chemotherapy response and 12 (70.5%) had a negative chemotherapy response (p-value = 0.601).

In this study, the highest distribution of age groups was found at the age of 40-49 years with total 18 samples. There were 5 positive chemotherapy responses (27.7%) and 13 negative chemotherapy responses (72.2%) among the 18 samples. In the 50-59 year age group, 4 samples (44.4%) had a positive chemotherapy response and 5 samples (55.5%) had a negative chemotherapy response out of 9 samples. Out of five samples in the 30-39 age group, all (100%) had a negative chemotherapy response. In the over-60 age group, 1 sample (20%) had a positive chemotherapy response and 4 samples (80%) had a negative chemotherapy response. Finally, one sample (100%) in the general group under 30 years old had a negative chemotherapy response (p-value = 0.412).

Meanwhile, two samples with underweight nutritional status had a 100% negative response to chemotherapy. Of the 32 samples in the normal nutritional status group, 10 (31.2%) had a positive and 22 (68.8%) had a negative chemotherapy response. Out of three total samples in the overweight nutritional status group, all had a negative chemotherapy response (100%). A negative chemotherapy response (100%) was obtained in the obese nutritional status group (p-value = 0.0601).

Six samples (or 25%) of the 24 total samples from the well-differentiated adenocarcinoma group in this study showed positive chemotherapy responses, while 18 samples (or 75% of the samples) had negative chemotherapy responses. Out of a total of 5 samples in the moderately differentiated adenocarcinoma group, 1 sample (20%) responded favorably to chemotherapy, while the other 4 samples (80%) did not. Out of a total of 9 samples in the poorly differentiated adenocarcinoma group, 3 samples (33.3%) responded well to chemotherapy, while 6 samples (66.7%) did not (p-value = 0.83).

We found no significant correlation between sex, age, body mass index, histopathology type, and tumor stage with the chemotherapy response (P > 0.05 for all).

The Cut-off Value for TILs Density

TILs high-low-density cut-off measurements using the Receiver Operating Characteristic (ROC) curve (Figure 1). This curve analysis will enable a comparison of the cut-off values, which will be decided upon based on sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) percentages. The ROC curve analysis found that 45% was the best cutoff value, with accuracy of 81%, sensitivity of 60%, specificity of 89.3%, PPV of 66.7%, and NPV of 86.2%. It was discovered that 3 samples (33.3%) had a negative chemotherapy response and 6 (66.7%)

Table 3. Correlation between Patient's Characteristics and The Chemotherapy Response.

Characteristics	n	Chemotherapy Response		P value
		Positive (n = 10)	Negative (n = 28)	
Sex				
Male	21	5 (23.8%)	16 (76.2%)	0.601
Female	17	5 (29.4%)	12 (70.5%)	
Age				
<30	1	0	1 (100%)	
31-39	5	0	5 (100%)	
40-49	18	5 (27.7%)	13 (72.2%)	0.435
50-59	9	4 (44.4%)	5 (55.5%)	
>=60	5	1 (20%)	4 (80%)	
Nutritional Status				
Underweight	2	0	2 (100%)	
Normal	32	10 (31.2%)	22 (68.8%)	0.062
Overweight	3	0	3 (100%)	
Obesity	1	0	1 (100%)	
Comorbidities				
Yes	4	0	4 (100%)	0.289
No	34	10 (29.5%)	24 (70.5%)	
Histopathology type				
Adenocarcinoma Well Differentiated	24	6 (25%)	18 (75%)	
Adenocarcinoma Moderately Differentiated	5	1 (20%)	4 (80%)	0.83
Adenocarcinoma Poorly Differentiated	9	3 (33.3%)	6 (66.7%)	
Stage				
3b	5	1 (20.0)	4 (80.0)	0.73
3c	33	9 (27.3)	24 (72.7)	

had a positive chemotherapy response in the group with a strong TILS density, which included 9 samples. In contrast, it was discovered that out of a total of 29 samples in the group with weak TILS density, 25 samples (86.2%) had a negative chemotherapy response and 4 samples (13.8%) had a positive chemotherapy response (p-value = 0.002). Comparison for each cut-off value of TILs Density was shown in Table 4.

Correlation Between TILs Density and The Chemotherapy Response

The Chi-Square Test was used to determine the correlation between the density of TILs and the chemotherapy response (shown in Table 5), and it was discovered that the stronger the density of TILs, the more favorable the response to chemotherapy was. On the other hand, the response to chemotherapy will be more adverse with a low TILs density (RR 12.5, p-value = 0.002). Three samples (33.3%) had a poor chemotherapy response, while six samples (66.7%) had a good chemotherapy response in the group with a high TILS density, which included a total of nine samples. Comparatively, out of a total of 29 samples in the group with weak TILS density, 25 samples (86.2%) had a negative chemotherapy response, whereas 4 samples (13.8%) had a positive chemotherapy response.

DISCUSSION

Males and women both develop colon cancer at the same rate, but men are more likely than women to develop rectal cancer, and more than 90% of cases are discovered in people between the ages of 40 and 50. According to this study, most of the sample was male (55.3%), and 84% were older than 40. Meltzer in 2020 investigated at how gender affected the likelihood that patients with locally advanced rectal cancer would get distant metastases following neoadjuvant treatment. According to the study, men experienced a higher incidence of liver metastases due to progression following neoadjuvant chemotherapy than women. This is because male patient's inferior mesenteric vein anatomic structures are wider, which allows larger tumor dissemination.[11] This is consistent with this study, in which up to 16 male patients (76.2%) had a poor response to treatment. However, this study was unable to demonstrate statistical significance for the association between gender and chemotherapy response. Rectal cancer is said to progress most rapidly in those over the age of 50, and start to increase in those who are 40 years old and older.[12] In this study, it was discovered that 22 patients over the age of 40 (68.75%) had a poor response to chemotherapy, despite knowing that this correlation was not statistically significant.

Additionally, obesity has been associated with a higher risk of colorectal cancer. The amount of body fat can lead to a variety of metabolic and systemic changes that set off the development of cancer. According to a study, obesity is linked to high levels of the hormone leptin in the body, which increases colon tissue cell differentiation and inhibits cell apoptosis, making elevated leptin levels one of the risk factors for tumor development.[13] Rectal cancer patients who are obese class 2 or more (BMI ≥ 35 kg/m^2) and underweight (BMI ≤ 18.5 kg/m^2) have a lower overall survival rate (HR = 1.6; 95% confidence interval (CI) 0.9-2.7) and a higher risk of developing distant metastases (HR = 1.7; 95% CI 0.9-3.3) than patients who are normal weight (BMI 18.5 - 25 kg/m^2). There was no noticeable difference in the local recurrence rate between the groups.[13] According to a study by Gan et al. published in 2021, obesity is a risk factor for a low survival rate for colorectal cancer patients since it is an independent predictor of their responsiveness to neoadjuvant chemotherapy and is linked to an elevated risk of tumor formation and progression.[14] This is consistent with this study, which found that chemotherapy had a negative effect on all patients with nutritional status of obesity and underweight (100%, p-value 0.06). However, statistical proof of the association between nutritional status and chemotherapy response in this study was not preferable.

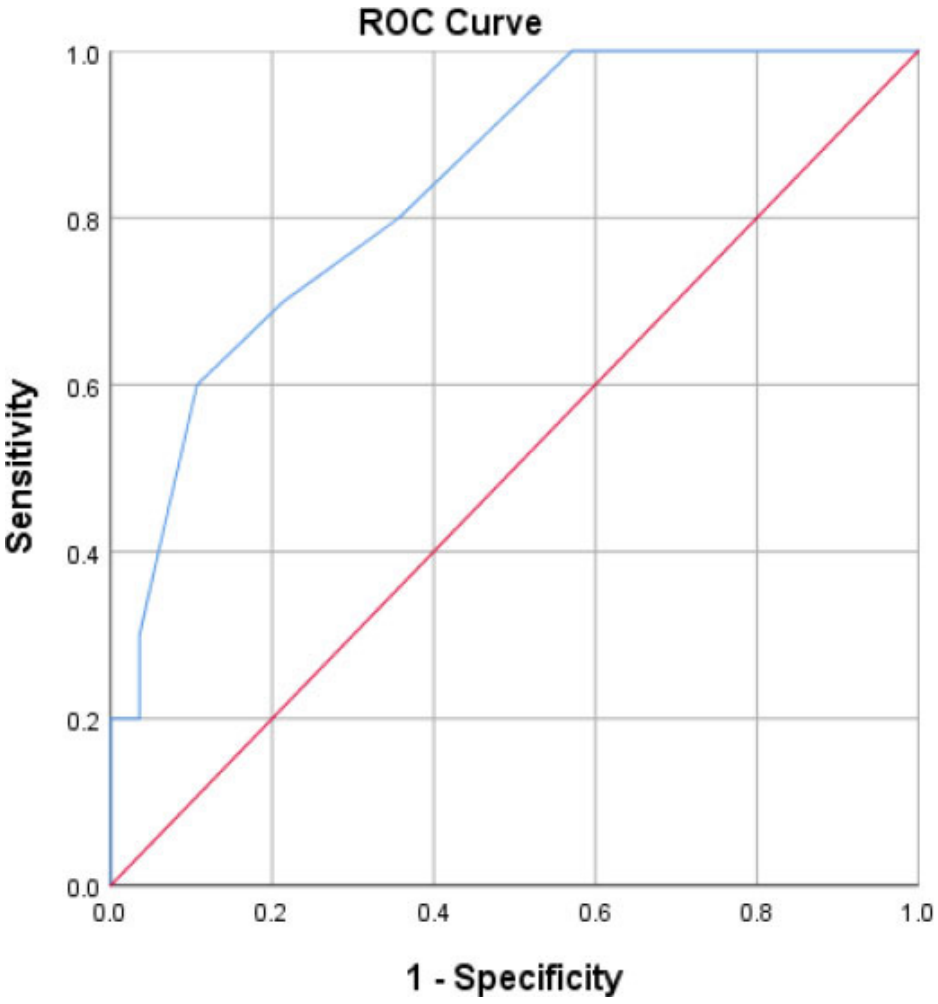

Figure 1. ROC Analysis of TILs Density.

Table 4. Comparison of Cut-off Value for TILs Density.

Cut-off Value	Sensitivity	Specificity	PPV	NPV	Accuracy
Cut-off TIL 15	100%	42.90%	38.50%	100%	31.60%
Cut-off TIL 25	80.00%	64.30%	44.40%	90.00%	68.40%
Cut-off TIL 35	70.00%	78.60%	53.80%	88.00%	76.30%
Cut-off TIL 45	60.00%	89.30%	66.70%	86.20%	81.58%

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Following a research by Kristoffer, patients with LARC with poorly differentiated who got NCRT had a worse prognosis than those in the groups with moderately and well-differentiated adenocarcinomas due to their poorer chemotherapy response.[15] There is evidence that additional histological characteristics such lymphovascular invasion, lymph node metastases, and tumor differentiation can affect a patient's prognosis for rectal cancer. Poor differentiation, lymphovascular involvement, and lymph node metastases are all indicators of more aggressive tumors that carry a poorer prognosis.[16]

Patients who receive NCRT and have poorly differentiated rectal cancer have a poorer prognosis than those who have moderately and highly differentiated rectal cancer. First, poorly differentiated rectal tumors are more likely to be located at the anal verge than moderately or well-differentiated tumors, which increases NCRT complications and surgical challenges. Second, cancers

Table 5. Correlation Between TILs Density and The Chemotherapy Response.

TILs Density	n	Chemotherapy Response		P value	RR	95% CI
		Negative	Positive			
Strong ≥ 45%	9	3 (33.3%)	6 (66.7%)	0.002*	12.5	0.02 – 0.71
Weak < 45%	29	25 (86.2%)	4 (13.8%)			

with poor histological differentiation are more likely to infiltrate vascular and nerve tissues as well as to cross the borders. Because undifferentiated tumors are typically more aggressive than differentiated tumors, NCRT may not be able to improve the patient's chance of survival in cases of rectal cancer with this histological type.[17-20]

In this study, 6 out of 9 samples (66.7%) showed poor tumor differentiation, were found to be progressing, and did not respond well to the chemotherapy mFolfox6. Tumor differentiation can affect chemotherapy response and tumor progression, even though this study did not find a statistically significant association between tumor differentiation and chemotherapy response (p-value = 0.83). This is possible because, regardless that tumor differentiation is a potent prognostic factor for survival rates in colorectal cancer patients, the calculation presents its own challenges because it is subjective and relies on the anatomical pathologist who performs the calculations. The evaluation of tumor differentiation is subjective and imprecise due to the very heterogeneous nature of tumors and their wide variety. [21-23]

TILs density and treatment response were shown to be significantly correlated in this study (p=0.002). This is shown by the fact that out of 29 samples with low TIL density, 25 samples (86%) had a poor response to chemotherapy. This is in line with studies done by Orhan in 2022 that found that in patients with LARC undergoing NCRT, the density of TILs is a predictive marker of therapeutic response and long-term prognosis. Patients with high pre-therapy CD8 + TILs density had a better chance of achieving PCR and pTR and also showed a better OS and DFS than those with lower levels of CD8+ TIL infiltration.[9]

All stages of tumor development and progression are affected by the body's immunological response, which influences tumor behavior.[24-25] In CRC histopathological evaluation, tumor-infiltrating lymphocytes (TILs) are frequently viewed as the body's defense mechanism against tumor growth. TILs aim to gain, develop, and activate immune cells that inhibit the growth of tumors. Along with other more traditional prognostic factors, T cell tumor infiltration is a very useful prognostic factor for CRC.[24] Numerous studies have demonstrated that CRC patients' overall survival and disease-free survival rely on kind, density, and location of tumor-infiltrating lymphocytes in the primary tumor.[18-20]

In a number of recent studies, the density of CD3+ and CD8+ TIL subsets was found to be the best predictor of overall survival (OS) and disease-free survival (DFS) in patients with LARC.[6,18,19,20] The higher the density, the better the OS and DFS rates. This may not be the issue for one of the other TILs subtypes, such as Treg or FOXP3+. Poorer OS and DFS rates are correlated with high Treg or FOXP3+ density.[21] This remains open to discussion. Since this study only generally analyzes the density of TILs, further precise study based on the subgroup, is required.

The strength of this study is the first to be conducted in Indonesia, which offers advantages in examining the correlation between the density of TILs in stromal tumors and the response to neoadjuvant chemotherapy in LARC patients.

The study's weakness is that the density of TILs is not calculated by subset, where each TILs subset is thought to play a different role in tumor immunity. As a result, more research is required to calculate the density of TILs based on subsets and then evaluate the correlation of each subset to the response to neoadjuvant chemotherapy.

CONCLUSION

TILs are an excellent predictive marker for neoadjuvant chemotherapy response in LARC patients and can be examined using H&E staining method which is cheap and fast but still has predictive value.

CONFLICTS OF INTEREST

No competing interests declared.

AUTHOR CONTRIBUTION

Conceived the study: FA. Designed the study: FA, IK, and S. Analyzed the data: FA, IK, and AR. Wrote the manuscript: FA and IK. Review the manuscript: IK, S, and AR

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ETHICAL STATEMENT

This study has been approved by Ethical Committee Faculty of Medicine, Universitas Airlangga-Dr. Soetomo Hospital with ethical clearance reference number 0957/LOE/301.4.2/VII/2022.

REFERENCES

1. Merabishvili V. Review Cancer incidence in Five Continents Vol. XI / Ed. by F. Bray, M. Colombet, L. Mery, M. Piñeros, A. Znaor, R. Zanetti and J. Ferlay. IARC Sci. publ. № 166, Lyon, France, 1545 p. Probl Oncol. 2021;67(6):855. Available from: http://dx.doi.org/10.37469/0507-3758-2021-67-6-855
2. Bosset J-F, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, et al. Chemotherapy with Preoperative Radiotherapy in Rectal Cancer. N Engl J Med. 2006;355(11):1114–23. Available from: http://dx.doi.org/10.1056/nejmoa060829
3. Xu Y, Liu H, Chen J, Zhou Q. Comparisons between the National Comprehensive Cancer Network (NCCN) non-small-cell lung cancer (NSCLC) Clinical Practice Guidelines (Chinese version), the NCCN original edition, and the European Society for Medical Oncology NSCLC Guidelines in 2009. Thorac Cancer. 2010;1(2):83–6. Available from: http://dx.doi.org/10.1111/j.1759-7714.2010.00016.x
4. Lin H, Wang L, Zhong X, Zhang X, Shao L, Wu J. Meta-analysis of neoadjuvant chemotherapy versus neoadjuvant chemoradiotherapy for locally advanced rectal cancer. World J Surg Oncol. 2021;19(1):141. Available from: https://pubmed.ncbi.nlm.nih.gov/33952287
5. Schwartz LH, Litière S, de Vries E, Ford R, Gwyther S, Mandrekar S, et al. RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer. 2016/05/14. 2016;62:132–7. Available from: https://pubmed.ncbi.nlm.nih.gov/27189322
6. Mei Z, Liu Y, Liu C, Cui A, Liang Z, Wang G, et al. Tumour-infiltrating inflammation and prognosis in colorectal cancer: systematic review and meta-analysis. Br J Cancer. 2014/02/06. 2014;110(6):1595–605. Available from: https://pubmed.ncbi.nlm.nih.gov/24504370
7. Miyakita H, Sadahiro S, Suzuki T, Chan LF, Ogimi T, Okada K, et al. Tumor-Infiltrating Lymphocytes in Biopsy Specimens Obtained 7 Days after Starting Chemoradiotherapy for Rectal Cancer Are Predictors of the Response to Chemoradiotherapy. Oncology. 2020/08/14. 2020;98(12):869–75. Available from: https://pubmed.ncbi.nlm.nih.gov/32799200
8. Morris M, Platell C, Iacopetta B. Tumor-Infiltrating Lymphocytes and Perforation in Colon Cancer Predict Positive Response to

5-Fluorouracil Chemotherapy. Clin Cancer Res. 2008;14(5):1413–7. Available from: http://dx.doi.org/10.1158/1078-0432.ccr-07-1994
9. Orhan A, Khesrawi F, Tvilling Madsen M, Peuliche Vogelsang R, Dohrn N, Kanstrup Fiehn A-M, et al. Tumor-Infiltrating Lymphocytes as Biomarkers of Treatment Response and Long-Term Survival in Patients with Rectal Cancer: A Systematic Review and Meta-Analysis. Cancers (Basel). 2022;14(3):636. Available from: https://pubmed.ncbi.nlm.nih.gov/35158905
10. Fuchs TL, Sioson L, Sheen A, Jafari-Nejad K, Renaud CJ, Andrici J, et al. Assessment of Tumor-infiltrating Lymphocytes Using International TILs Working Group (ITWG) System Is a Strong Predictor of Overall Survival in Colorectal Carcinoma. Am J Surg Pathol. 2019;44(4):536–44. Available from: http://dx.doi.org/10.1097/pas.0000000000001409
11. Meltzer S, Bakke KM, Rød KL, Negård A, Flatmark K, Solbakken AM, et al. Sex-related differences in primary metastatic site in rectal cancer; associated with hemodynamic factors? Clin Transl Radiat Oncol. 2019;21:5–10. Available from: https://pubmed.ncbi.nlm.nih.gov/31872084
12. Khan S, Haider G, Abid Z, Bukhari N, Khan SZ, Abid M. Adequacy of Surgical Pathology Reports of Colorectal Carcinoma and Its Significance. Cureus. 2021;13(8):e16965–e16965. Available from: https://pubmed.ncbi.nlm.nih.gov/34540379
13. Kalb M, Langheinrich MC, Merkel S, Krautz C, Brunner M, Bénard A, et al. Influence of Body Mass Index on Long-Term Outcome in Patients with Rectal Cancer-A Single Centre Experience. Cancers (Basel). 2019;11(5):609. Available from: https://pubmed.ncbi.nlm.nih.gov/31052303
14. Gan T, Schaberg KB, He D, Mansour A, Kapoor H, Wang C, et al. Association Between Obesity and Histological Tumor Budding in Patients With Nonmetastatic Colon Cancer. JAMA Netw open. 2021;4(4):e213897–e213897. Available from: https://pubmed.ncbi.nlm.nih.gov/33792733
15. Derwinger K, Kodeda K, Bexe-Lindskog E, Taflin H. Tumour differentiation grade is associated with TNM staging and the risk of node metastasis in colorectal cancer. Acta Oncol (Madr). 2009;49(1):57–62. Available from: http://dx.doi.org/10.3109/02841860903334411
16. Chen K, Collins G, Wang H, Toh JWT. Pathological Features and Prognostication in Colorectal Cancer. Curr Oncol. 2021;28(6):5356–83. Available from: https://pubmed.ncbi.nlm.nih.gov/34940086
17. Barresi V, Bonetti LR, Ieni A, Branca G, Baron L, Tuccari G. Histologic grading based on counting poorly differentiated clusters in preoperative biopsy predicts nodal involvement and pTNM stage in colorectal cancer patients. Hum Pathol. 2014;45(2):268–75. Available from: http://dx.doi.org/10.1016/j.humpath.2013.07.046
18. Idos GE, Kwok J, Bonthala N, Kysh L, Gruber SB, Qu C. The Prognostic Implications of Tumor Infiltrating Lymphocytes in Colorectal Cancer: A Systematic Review and Meta-Analysis. Sci Rep. 2020;10(1):3360. Available from: https://pubmed.ncbi.nlm.nih.gov/32099066
19. Bai Z, Zhou Y, Ye Z, Xiong J, Lan H, Wang F. Tumor-Infiltrating Lymphocytes in Colorectal Cancer: The Fundamental Indication and Application on Immunotherapy. Front Immunol. 2022;12:808964. Available from: doi: 10.3389/fimmu.2021.808964.
20. Kong JCH, Guerra GR, Millen RM, Roth S, Xu H, Neeson PJ, et al. Tumor-Infiltrating Lymphocyte Function Predicts Response to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Cancer. JCO Precis Oncol. 2018;(2):1–15. Available from: http://dx.doi.org/10.1200/po.18.00075
21. Sherwood AM, Emerson RO, Scherer D, Habermann N, Buck K, Staffa J, et al. Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother. 2013/06/16. 2013;62(9):1453–61. Available from: https://pubmed.ncbi.nlm.nih.gov/23771160
22. Sulistyo H, Budipramana VS. Examination of Micro Vascular Density on Metastatic Colorectal Cancer of RAS Mutant-Type as Anti Vegf Therapy Predictor. Folia Medica Indones. 2021;57(2):111. Available from: http://dx.doi.org/10.20473/fmi.v57i2.18249
23. Rumapar L, Mustokoweni S, Rahniayu A. The Role of HIF-1alpha in Regional Lymph Nodes Metastasis in Colorectal Adenocarcinoma. Folia Medica Indones. 2018;54(1):1. Available from: http://dx.doi.org/10.20473/fmi.v54i1.8042
24. Saraswati PA, Budipramana VS, Rahniayu A. Grading of lymphocyte infiltrating tumor can be used as a prognosis factor compared to T Pathology and N Pathology on recurrency of colon cancer. Indones J Biomed Sci. 2021;15(1):39–42. Available from: http://dx.doi.org/10.15562/ijbs.v15i1.280
25. Wardhana AA, Lesmana T. Neutrophil Lymphocyte Ratio (NLR) as a predictive factor radiological response of Neoadjuvant Chemotherapy (NAC) in Locally Advanced Rectal Cancer (LARC). Bali Med J. 2021;10(2):717–23. Available from: http://dx.doi.org/10.15562/bmj.v10i2.2483

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PEMERINTAH PROPINSI JAWA TIMUR
RUMAH SAKIT UMUM DAERAH Dr. SOETOMO
KOMITE ETIK PENELITIAN KESEHATAN
Jl. Mayjen Prof. Dr. Moestopo No. 6-8, Telp. 031-5501071-5501073, Fax. 031-5501164
SURABAYA 60286

SURAT EXEMPTION

(" LETTER OF EXEMPTION ")

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Judul Protokol Penelitian	:	HUBUNGAN DENSITAS TUMOR INFILTRATING LYMPHOCYTE (TILS) TERHADAP RESPON KEMOTERAPI NEOADJUVAN MFOLFOX6 PADA PASIEN DENGAN LOCALLY ADVANCED RECTAL CANCER DI RSUD DR. SOETOMO SURABAYA
Dokumen yang disetujui	:	1481/106/4/VI/2022 (versi: 1)
Tanggal terbit	:	8 Juli 2022
Berlaku sampai	:	8 Juli 2023
Peneliti Utama	:	Dr. Sahudi, Sp.B (K) KL
Peneliti Lain	:	1. Iwan Kristian, dr., Sp.B-KBD 2. Florencia Adys, dr
Instalasi/Tempat Penelitian	:	RSUD Dr. Soetomo

Komite Etik Penelitian Kesehatan RSUD Dr Soetomo menyatakan bahwa dokumen diatas sesuai dengan The Office for Human Research Protections (OHRP) dibawah persyaratan the U.S. Department of Health and Human Services (HHS) *Regulasi 45 CFR bagian 46 untuk* ***exempt review***.

Dr. Elizeus Hanindito dr., Sp.An., KIC
Ketua Panel 2

Dr. Damayanti Tinduh dr., SpKFR(K)
Sekretaris Panel 2