

# The Relationship of Tumor Infiltrating Lymphocytes (TIL) with The Clinicopathological Picture of Colorectal Carcinoma at Prof. Dr. I.G.N.G Ngoerah Hospital Denpasar

Grace Mary Aritonang<sup>1\*</sup>, I Wayan Sudarsa<sup>2</sup>, I Made Mulyawan<sup>3</sup>

<sup>1</sup>Department of General Surgery, Faculty of Medicine, Udayana University, Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia (80113)

<sup>2</sup>Division of Oncology, Department of Surgery, Faculty of Medicine, Udayana University, Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia (80113)

<sup>3</sup>Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Udayana University, Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia (80113)

\*Corresponding author details: Grace Mary Aritonang; [gracemaritonang@yahoo.com](mailto:gracemaritonang@yahoo.com)

## ABSTRACT

**Background:** Colorectal carcinoma is defined as a malignancy in the gastrointestinal tract originating from the colon tissue, which can be the colon and/or rectum, ranking 3rd worldwide and is the 3rd most common type of cancer in men and 2nd most common cancer in women. The innate immune system has a role in the pathogenesis of colorectal carcinoma, both in the suppression of tumor cells and the escape of tumor cells from the immune system. Tumor Infiltrating Lymphocytes (TIL) are all types of immune cells that migrate from the blood to tumor cells, which can recognize and kill tumor cells. The relationship between TIL and clinicopathologic features in colorectal carcinoma has been widely mentioned in various literature and previous studies. **Objective:** To determine the relationship between TIL density and clinicopathological features of colorectal carcinoma (age, gender, primary tumor site, clinical stage, histopathology type, and grade of differentiation) at Prof. Dr. I.G.N.G Ngoerah Hospital, Denpasar. **Methods:** This study is an analytical observational study with a cross-sectional research design. The study population was patients who had been diagnosed with colorectal carcinoma from January 2021 to January 2023, with a total of 36 samples. Data taken from the Department of Clinical Pathology and the Medical Records Department of Prof. Dr. I.G.N.G. Ngoerah Denpasar Hospital and analyzed using SPSS ver 25.0, and univariate and bivariate tests were carried out. **Results:** In the bivariate test using chi square, a significant relationship was found between TIL density and the histopathology type of colorectal carcinoma ( $p = 0.002$ ) as well as between TIL density and the degree of colorectal carcinoma differentiation ( $p < 0.001$ ). In the Spearman correlation test and obtained a significant correlation was negative with a  $p$ -value of 0.002,  $CC = -496$ . **Conclusion:** There was no relationship and correlation between TIL and age, gender, primary tumor site and clinical stage in colorectal carcinoma patients, and there was a significant relationship between histopathology type and grading differentiation with TIL in colorectal carcinoma patients but histopathology type was not found to be correlated with TIL while grading differentiation had a significant correlation with TIL in colorectal carcinoma patients.

**Keywords:** tumor Infiltrating Lymphocytes; TIL; colorectal carcinoma; age; gender; histopathological type; grade of differentiation; clinical stage.

## INTRODUCTION

Colorectal carcinoma (CRC) is defined as a malignancy in the gastrointestinal tract originating from the tissues of the colon, which can be the colon and/or rectum (Hassanpour & Dehghani, 2017; Mathur et al., 2015). Most cancers that occur in the colon or rectum are adenocarcinomas, which are cancers that originate from glandular tissue and account for 95% of all tumor cases in the colon (Ameer, 2021). According to Globocan 2020, colorectal carcinoma ranks 3rd worldwide and is the 3rd most common type of cancer

in men and the 2nd most common cancer in women (GLOBOCAN, 2020). Colorectal carcinoma also accounts for 10% of all cancer cases and is the second leading cause of cancer-related deaths worldwide (WHO, 2023).

The clinical symptoms of CRC vary widely. Some patients experience a change in bowel habits. In certain cases where there is chronic gastrointestinal bleeding, anemia may be present, and patients often present as weak and pale.

Unexplained weight loss can also be a sign of malignancy, but it is not specific to colorectal carcinoma (American Cancer Society, 2021). Patients diagnosed with stage I colorectal carcinoma have an initial five-year survival rate of 74%, where tumor cell invasion is limited to certain organs (in situ) and no distant spread (metastasis) has occurred. However, at stage IV, when tumor cells spread to distant organs, the first five-year survival rate after diagnosis decreases to only 6% (Labeda et al., 2022).

The immune system is thought to have an effect on suppressing the growth of a carcinoma. Macfarlane Burnet and Lewis Thomas outlined a concept of immunosurveillance in the pathogenesis of colorectal carcinoma. It is believed that the immune system has the capacity to promote an effective immunological reaction against tumor cell-specific neoantigens, and is able to eliminate and suppress cancer cell development even before clinical expression (Chen et al, 2021; Colona M, 2018). In general, both the acquired and innate immune systems play a role in the pathogenesis of TRC, both in the suppression of tumor cells and the escape of tumor cells from the immune system. In particular, the infiltration density of CD8+ and CD45+ T lymphocytes has been shown to have better prognostic value than classic tumor metastasis classification factors. Other immune subsets, such as macrophages, NK cells, or unconventional lymphocytes, are thought to play an important role in tumor cell escape. (Colonna, M., 2018).

High intra-tumor TIL density of infiltrating CD3+ and CD8+ lymphocytes is said to be associated with reduced local spread of tumor cells, lower incidence of metastasis, and better prognosis. Tumor Infiltrating Lymphocytes (TILs) are all types of immune cells that migrate from the blood to tumor cells and can recognize and kill tumor cells. TILs consist of all lymphocyte cell populations that have invaded tumor tissue (T cells and B cells). TILs have specific histological features of various cancers and are believed to reflect an individual's antitumor immune response. TILs have been described in a number of solid tumor types, including colorectal cancer, and are one of the important biomarkers in predicting the prognosis of SCI and the effectiveness and success of treatment. (Liu et al, 2015; Cagir B, 2022)

## METHODS

This study is an analytical observational study with a cross-sectional research design; data were taken from the Department of Clinical Pathology and the Medical Records Installation of Prof. Dr. I.G.N.G. Ngoerah Hospital, Denpasar. The study population was patients who had been diagnosed with colorectal carcinoma from January 2021 to January 2023, with a total of 36 samples.

The inclusion criteria in this study are 1) All patients who have confirmed colorectal carcinoma through anatomical pathology examination, 2) Recorded histopathology type, differentiation grading and TIL value on the results of anatomical pathology examination, 3) Have complete medical record data that includes age and gender, 4) Treated at Prof. Dr.

I.G.N.G Ngoerah Hospital in January 2021-January 2023. The exclusion criteria in this study represent all patients who did not meet the inclusion criteria. Data analysis was performed using SPSS for Windows version 25.0 software. The univariate and bivariate tests were carried out.

## RESULTS

**TABLE 1:** Description of the Research Subjects.

Characteristic	(n=36)
<b>Age</b>	
≤50 years	14 (38.9%)
>50 years	22 (61.1%)
<b>Gender</b>	
Female	13 (36.1%)
Male	23 (63.9%)
<b>Primary Tumor Site</b>	
Colon Ascending	3 (8.4%)
Colon Transversum	3 (8.3%)
Colon Descending	3 (8.3%)
Sigmoid	4 (11.1%)
Rectosigmoid	10 (27.8%)
Rectum	11 (30.6%)
Anus	2 (5.6%)
<b>Grading Differentiation</b>	
Undifferentiated	3 (8.3%)
Poorly Differentiated	5 (13.9%)
Moderately Differentiated	23 (63.9%)
Well Differentiated	5 (13.9%)
<b>Histopathologic Type</b>	
Adenocarcinoma	31 (86.1%)
Signet Ring Cell	1 (2.8%)
Mucinous Adenocarcinoma	2 (5.6%)
Intramucosal Carcinoma	1 (2.8%)
Squamous Cell Carcinoma	1 (2.8%)
<b>Clinical Stage Disease</b>	
St. IIA	6 (16.7%)
St. IIC	1 (2.8%)
St. IIIB	1 (2.8%)
St. IIIC	10 (27.8%)
St. IVA	16 (44.4%)
St.IVB	2 (5.6%)
<b>TIL score</b>	
Mild	14 (38.9%)
Moderate	16 (44.4%)
Severe	2 (5.6%)
Prominent	4 (11.1%)

### Analysis of the relationship between TIL frequency and Age

Bivariate analysis with chi-square test was performed to see the relationship between the Tumor Infiltrating Lymphocytes value and age. The results of the chi-square test showed that there was no significant relationship between Tumor Infiltrating Lymphocytes and age (p 0.663).

**TABLE 2:** Chi-Square Test of the Relationship Between TIL Frequency and Age.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	1.586 <sup>a</sup>	3	.663
Likelihood Ratio	2.277	3	.517
Linear-by-Linear Association	.026	1	.873
N of Valid Cases	36		

**Analysis of the relationship between TIL frequency and Gender**

Bivariate analysis with chi-square test was performed to see the relationship between the Tumor Infiltrating Lymphocytes value and gender. The results of the chi-square test showed that there was no significant relationship between Tumor Infiltrating Lymphocytes and gender (p 0.941).

**TABLE 3:** Chi-Square Test of the Relationship Between TIL Frequency and Gender.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.396 <sup>a</sup>	3	.941
Likelihood Ratio	.401	3	.940
Linear-by-Linear Association	.041	1	.839
N of Valid Cases	36		

**Analysis of the relationship between TIL frequency and Primary Tumor Site**

Bivariate analysis with chi-square test was performed to see the relationship between the Tumor Infiltrating Lymphocytes value and primary tumor site. The results of the chi-square test showed that there was no significant relationship between Tumor Infiltrating Lymphocytes and primary tumor site (p 0.422).

**TABLE 4:** Chi-Square Test of the Relationship Between TIL Frequency and Primary Tumor Site.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	21.611 <sup>a</sup>	21	.422
Likelihood Ratio	23.097	21	.339
Linear-by-Linear Association	.705	1	.401
N of Valid Cases	36		

**Analysis of the relationship between TIL frequency and Histopathology Type**

Bivariate analysis with chi-square test was performed to see the relationship between Tumor Infiltrating Lymphocytes value and histopathology type of colorectal carcinoma. The results of the chi-square test showed that there was a significant relationship between Tumor Infiltrating Lymphocytes and the histopathology type of colorectal carcinoma (p 0.002).

**TABLE 5:** Chi-Square Test of the Relationship Between TIL Frequency and Histopathology Type.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	31.624 <sup>a</sup>	12	.002
Likelihood Ratio	16.987	12	.150
Linear-by-Linear Association	6.573	1	.010
N of Valid Cases	36		

**Analysis of the relationship between TIL frequency and differentiation grading**

Bivariate analysis with the chi-square test was performed to see the relationship between the value of Tumor Infiltrating Lymphocytes and grading differentiation of colorectal carcinoma. The results of the chi-square test showed that there was a significant relationship between Tumor Infiltrating Lymphocytes and grading differentiation (p<0.001).

**TABLE 6:** Chi-Square Test of the Relationship Between TIL Frequency and Differentiation Grading.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	41.707 <sup>a</sup>	9	<.001
Likelihood Ratio	36.962	9	<.001
Linear-by-Linear Association	6.009	1	.014
N of Valid Cases	36		

**Analysis of the relationship between TIL frequency and Clinical Stage**

Bivariate analysis with chi-square test was performed to see the relationship between the Tumor Infiltrating Lymphocytes value and clinical stage. The results of the chi-square test showed that there was no significant relationship between Tumor Infiltrating Lymphocytes and clinical stage (p 0.967).

**TABLE 7:** Chi-Square Test of the Relationship Between TIL Frequency and Clinical Stage.

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.633 <sup>a</sup>	15	.967
Likelihood Ratio	7.949	15	.926
Linear-by-Linear Association	.013	1	.909
N of Valid Cases	36		

**Analysis of TIL Correlation Test with Age**

In the Spearman correlation test used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and age, there was no significant correlation with a p value of 0.731.

**TABLE 8:** Spearman Correlation Between TIL Frequency and Age.

Variable TIL	Grading differentiation
Correlation coefficient	059
P	0.731
n	36

**Analysis of TIL Correlation Test with Gender**

In the Spearman correlation test used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and gender, there was no significant correlation with a p value of 0.944.

**TABLE 9:** Spearman Correlation Between TIL Frequency and Gender.

Variable TIL	Grading differentiation
Correlation coefficient	012
P	0.944
n	36

**Analysis of TIL Correlation Test with Primary Tumor Site**

In the Spearman correlation test used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and primary tumor site, there was no significant correlation with a p value of 0.361.

**TABLE 10:** Spearman Correlation Between TIL Frequency and Primary Tumor Site.

Variable TIL	Grading differentiation
Correlation coefficient	-157
P	0.361
n	36

**Analysis of TIL Correlation Test with Histopathology Type**

In the Spearman correlation test used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and histopathology type, there was no significant correlation with a p value of 0.193.

**TABLE 11:** Spearman Correlation Between TIL Frequency and Histopathology Type.

Variable TIL	Grading differentiation
Correlation coefficient	222
P	0.193
n	36

**Analysis of TIL Correlation Test with Differentiation Grading**

The Spearman correlation test was used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and grading differentiation of colorectal carcinoma, and obtained a significant negative correlation with a p value of 0.002 (p <0.005). This shows that the higher the density of TILs, the better the grading differentiation (well differentiated).

**TABLE 12:** Spearman Correlation Between TIL Frequency and Differentiation Grading.

Variable TIL	Grading differentiation
Correlation coefficient	059
P	0.002
N	36

*P signifikan < 0.005.*

**Analysis of TIL Correlation Test with Clinical Stage**

In the Spearman correlation test used to assess the relationship between Tumor Infiltrating Lymphocytes (TIL) values and clinical stage, there was no significant correlation with a p value of 0.493.

**TABLE 13:** Spearman Correlation Between TIL Frequency and Clinical Stage.

Variable TIL	Grading differentiation
Correlation coefficient	118
P	0.493
n	36

## DISCUSSION

### Characteristics of the Research Subjects

In this study, the majority of colorectal carcinoma patients were >50 years old, with a sample size of 22 (61.1%). The American Cancer Society states that there is an increased risk of a person developing colorectal carcinoma according to age. Gender Colorectal carcinoma is the third most common cancer in men after lung and prostate, and the second most common in women after lung cancer, but the incidence of colorectal carcinoma is generally more common in men (ACS). Various literature, including the American Cancer Society, states that 70% of primary tumor sites are in the colon and 30% are in the rectum. In this study, it was found that the most common primary tumor locations were in the colon, with a total of 23 samples (64.4%), with rectosigmoid being the most common location as the primary tumor site. The most common type of histopathology found in this study was adenocarcinoma, namely 31 samples (86.1%). This is in accordance with previous literature, where 98% of colon cancer is adenocarcinoma. (WHO). Stage IVA is considered an advanced stage of colorectal carcinoma where direct infiltration to surrounding organs or distant metastases to other organs (liver, brain, bones, patu) has been obtained, this is in accordance with what is stated in Globocan and the American Cancer Society where when a person is first diagnosed with colorectal carcinoma, the patient is already at an advanced stage. The determination of TIL density in colorectal carcinoma is not the same as in breast cancer, where the cutoff used has a certain standardization. The determination of TIL density in colorectal carcinoma is influenced by the patient's immune system. (WHO)

### Relationship of TIL frequency with age, gender, primary tumor site, histopathology type, differentiation grading, and clinical stage

The aging process is believed to be associated with a decline in the body's immune system. TILs, as part of the immune system, are thought to be affected by this process. Higher TIL density was found in younger age groups than in older age groups. (Hamzah et al, 2022). In many previous studies, there is no evidence or results that can explain the cause of the difference in TIL density in women and men. However, males are found to be more predominant in colorectal carcinoma. This is often associated with lifestyle such as smoking and/or alcohol consumption (Wang et al, 2022). TIL density is usually increased in tumors with DNA mismatch repair (dMMR) deficiency that are more often located in the right colon (Galon et al, 2006; Ogino et al, 2009). Mentioning that there are differences in immune cell composition, molecular subtypes, and

microbial composition based on anatomical tumor location, researchers found significantly higher TIL density in right versus left-sided tumors (Saberzadeh et al, 2022).

A study conducted in China in 2019 evaluated the effect of histopathology type on the survival rate of colorectal carcinoma patients in China between 2004 and 2015. This study found that non-adenocarcinoma histopathology types, such as mucinous (50.7%) and signet ring adenocarcinoma (26.8%), had worse 5-year overall survival than patients with adenocarcinoma histopathology types (58%) ( $p < 0.001$ ). It was found that colorectal carcinoma patients with adenocarcinoma histopathology types had a better prognosis at advanced stages (stage III and IV) than colorectal carcinoma patients with non-adenocarcinoma types (Wu et al, 2019).

Cell differentiation grading is one of the prognostic factors in colorectal carcinoma, where the better the differentiation grading (well differentiated), the better the prognosis of a colorectal carcinoma. Histologic grade poorly differentiated is one of the important prognostic factors in colorectal cancer cases (Barri et al., 2015). As many as 72.5% of colorectal cancers classified as poorly differentiated have mismatch repair (MMR) deficiencies that interfere with clonality and molecular aspects of the tissue. In addition, these MMR-deficient tumors also have high levels of BRAF gene mutations and fusions, TP53 and KRAS gene amplifications that cause tumor instability (Perna et al., 2021). Research conducted by Huh et al showed that colorectal cancer with a high degree of malignancy tends to have a mild degree of TIL, where mild TIL density in colorectal carcinoma has a worse prognosis value than heavy TIL density. (Huh et al, 2012). A study conducted at Dr. Soedarso Hospital, Pontianak, in 2023 showed that there was a relationship between the number of TILs with Dukes stage and TNM stage. The results of this study are said to be in line with Ko and Pyo's research that there is a relationship between immunoscore, which is a calculation of TIL with TNM stage. Another study conducted by Fuchs et al also showed that there was a significant relationship between TIL expression and TNM stage (Trianto et al, 2023).

### Correlation of TIL with age, gender, primary tumor site, histopathology type, differentiation grading, and clinical stage

This increase in age is also associated with a decrease in the immune system, which has an impact on reducing the number of TILs. This is in line with research conducted by Hamzah et al, where it was found that TIL density was higher in younger age groups than in older age groups in colorectal carcinoma. (Hamzah et al, 2022). In the Spearman correlation test conducted in this study, there was no correlation between TIL density and patient age. (P 0.731, CC 059). In a study conducted by Hamzah et al found that the number of colorectal carcinoma cases in men and women was almost the same number, although men were said to be less than women (52 vs 46 samples).

In the research of Valentini et al also stated that the frequency of colorectal carcinoma patients from study subjects between men and women was almost the same (Hamzah et al, 2023). In particular, no source has succeeded in proving that certain genders have a tendency for certain TIL values or densities. The correlation test conducted in this study showed no correlation between the number of TILs and gender (P 0.944; CC 012). It has been mentioned above that TIL density usually increases in tumors with deficiency of DNA mismatch repair (dMMR), which are more often located in the right colon (Galon et al, 2006; Ogino et al, 2009). Another study conducted by Trianto et al stated the opposite result, where there was no relationship between the location of the primary tumor and the number of TILs. In line with these results are studies conducted by Fuchs et al, Ko and Pyo, and Matsusani et al, which showed that there was no significant relationship between tumor location and TIL expression. In the Spearman correlation test conducted in this study, there was no correlation between TIL density and primary tumor location, which is in line with several studies that have been conducted previously (P 0.361; CC -157).

90% of all colorectal carcinoma cases are adenocarcinoma. Research in China in 2019 found that non-adenocarcinoma histopathology types, such as mucinous (50.7%) and signet ring adenocarcinoma (26.8%), had worse 5-year overall survival than patients with adenocarcinoma histopathology types (58%) (p<0.001). In this study, Spearman correlation test showed no significant correlation between TIL density and histopathology type of colorectal carcinoma (P 0.193; CC 222). In this study, a correlation test with Spearman was conducted to assess the relationship between the density of Tumor Infiltrating Lymphocytes (TIL) with grading differentiation of colorectal carcinoma. A significant negative correlation was obtained with a p value of 0.002 with a correlation coefficient of (-0.496). This shows that the lower the TIL density, the higher the grading of cancer cell differentiation (poorly to undifferentiated). This finding is in line with various literature that states the differentiation grading of a tumor cell is related to TIL density, where the denser the TIL density, the lower the degree of tumor cell division (Well Differentiated). In this study, a p-value of 0.002 was obtained, so that there is a significant relationship between TIL density and grading of cancer cell differentiation. While the correlation coefficient value was obtained (-0.496), which states that there is a moderate correlation between the two variables assessed. So it can be interpreted that TIL density is one of the factors that affect cell differentiation grading, but is not the main or only factor that can affect cancer cell differentiation grading. A study conducted at Dr. Soedarso Hospital, Pontianak, in 2023 showed that there was a relationship between the number of TILs with Dukes stage and TNM stage. The results of this study are said to be in line with Ko and Pyo's research that there is a relationship between immunoscore, which is a calculation of TIL with TNM stage (Trianto et al, 2023). In this study, the

Spearman correlation test was conducted, which stated that there was no significant correlation between TIL density and the clinical stage of colorectal carcinoma (P 0.493; CC 118).

## CONCLUSIONS

There was no relationship and correlation between TIL and age, gender, primary tumor location and clinical stage in colorectal carcinoma patients, and there was a significant relationship between histopathology type and grading differentiation with TIL in colorectal carcinoma patients but histopathology type was not found to be correlated with TIL while grading differentiation had a significant correlation with TIL in colorectal carcinoma patients.

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