

## ORIGINAL ARTICLE

# The Clinicopathological Impact of Tumor Necrosis And Tumor-Infiltrating Lymphocytes in Colorectal Adenocarcinoma

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## ABSTRACT

**Introduction:** Tumor necrosis and tumor-infiltrating lymphocytes (TILs) have been correlated to the prognosis of various cancers, including colorectal adenocarcinoma. A hematoxylin-eosin (HE)-stained slide can be used to evaluate tumor necrosis and TILs. However, these factors are not consistently reported in cases of colorectal adenocarcinoma. This study aimed to investigate the correlation between tumor necrosis, TILs, and their combination with clinicopathological characteristics in patients with colorectal adenocarcinoma. **Methods:** A cross-sectional study on 36 colorectal adenocarcinoma resection samples evaluated tumor necrosis and TILs from H&E-stained slides, assessing their correlation with clinicopathological characteristics through statistical analysis. **Results:** Tumor necrosis correlated with higher grade, deeper invasion, and distant metastasis ( $p < 0.05$ ), while TILs were associated with lower depth of invasion ( $p < 0.05$ ). Multivariate analysis showed that well-differentiated tumor and without distant metastasis correlated with tumor necrosis. The combination categories of tumor necrosis and TILs were not associated with clinicopathological characteristics. **Conclusion:** Tumor necrosis and TILs may be independent prognostic factors in colorectal adenocarcinoma. Tumor necrosis was associated with unfavorable clinicopathological factors, while TILs were associated with favorable prognostic indicators.

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examination can observe tumor necrosis and tumor microenvironments (TME), such as tumor-infiltrating lymphocytes (TILs), which are associated with poor prognosis in solid tumors (5–7).

## INTRODUCTION

Colorectal adenocarcinoma is the third most common cancer in the world and has a poor prognosis (1,2). Prognostic factors in colon cancer include clinical patient conditions, CEA level, molecular testing such as KRAS or PTEN mutations, and histopathological examinations (3,4). Histopathological examinations in colorectal adenocarcinoma only focus on tumors, including type, grade, stage, and genetic mutations (4). Hematoxylin-Eosin (HE)-staining in histopathological

Interactions between cellular and non-cellular components of the tumor microenvironment play a crucial role in mediating immune responses that are closely related to the prognosis of colorectal cancer (8,9). The grade of tumor-infiltrating lymphocytes was statistically significantly associated with T, N status, and grade of colorectal adenocarcinoma. High-density TIL was associated with improved prognosis, meanwhile low-grade tumor-infiltrating lymphocytes are indicative of high-stage tumors with a poor prognosis(8–10). TIL density is routinely reported in breast cancer but not in colorectal adenocarcinoma (11). Interestingly, the colon,

as an organ, is susceptible to malignancies associated with chronic inflammation (12).

Tumor necrosis is an area of dead cells and is associated with hypoxia caused by rapid tumor growth. In many studies, tumor necrosis has been associated with poor prognosis in breast cancer, urothelial cancer, and renal cancer (5,6,13). The extent of necrosis reveals the degree of hypoxia in the tumor. In addition, increased cellular hypoxia in solid tumors also affects metastatic potential and prognosis (14).

New biomarkers are needed for colorectal adenocarcinoma, which are expected to be prognostic factors. There have been few studies that have simultaneously examined tumor necrosis and TILs in colorectal adenocarcinoma. This study aims to assess tumor necrosis, TILs, and their combination and correlate them with clinicopathological characteristics of colorectal adenocarcinoma.

## MATERIALS AND METHODS

### Patients

The study included 36 FFPE samples from colorectal adenocarcinoma patients from January to December 2020 at The Laboratory of Anatomic Pathology, Dr. Soedarso General Hospital, Pontianak, Indonesia. Patients were selected based on resection specimens, completed clinicopathological characteristics, and the histological type was adenocarcinoma. The samples were cut into 4  $\mu$ m, stained with hematoxylin-eosin, and observed using a light microscope.

### Evaluation of Tumor Necrosis, Tumor Infiltrating Lymphocytes, and Their Combination

Tumor necrosis and TILs were assessed using a light microscope. Tumor necrosis was categorized into absent (0%), focal (<10%), moderate (10-30%), and extensive (>40%) (15,16). TILs density was categorized into low (<42%) and high ( $\geq$ 42%), with an average percentage from five different fields (7). This study used a new method, combining the results of evaluating TILs and tumor necrosis. The obtained are categorized into eight groups: 1). low-absent; 2). low-focal; 3). low-moderate; 4). low-extensive; 5). high-absent; 6). high-focal; 7). high-moderate; 8). high-extensive.

### Statistical Analysis

The correlation between clinicopathological characteristics with tumor necrosis, TILs, and their combination categories was analyzed using Spearman's correlation test. Variables that showed significant correlation in bivariate analysis were included in the multivariate analysis using binary logistic or ordinal regression, calculating Odds Ratio (OR) and

95% Confidence Interval (CI). The p-values <0.05 were significantly correlated. Statistical analysis was performed using SPSS version 25.

### Ethical Clearance

This study was approved by the Ethical Clearance Committee of The Faculty of Medicine, Universitas Tanjungpura (Reference No:12297/UN22.9/PT.01.04/2024).

## RESULTS

### Clinicopathological Characteristics, Tumor Necrosis, and Tumor-Infiltrating Lymphocytes

The study included 36 patients aged 24-88 years, with the largest age group being 51-60 years (50%). The most common tumor location was the rectum (58.3%). The majority were in stage II (41.7%) and well-differentiated (52.8%). The most findings were focal group of tumor necrosis (36.1%), low group of TILs (52.8%), and high TILs-focal necrosis (19.4%).

### Correlation between Tumor Necrosis, Tumor-Infiltrating Lymphocytes, and Their Combination with Clinicopathological Characteristics

Table I showed that tumor necrosis was found to be positively correlated with grading, tumor depth invasion, and distant metastasis ( $p = 0.009$ ,  $r = 0.430$ ;  $p = 0.008$ ,  $r = 0.436$ ;  $p = 0.045$ ,  $r = 0.336$ ) and TILs significantly negatively correlated with tumor depth invasion ( $p = 0.030$ ,  $r = -0.361$ ). However, the combination of tumor necrosis and TILs did not show any correlation with clinicopathological characteristics. The results of the multivariate analysis test showed that well-differentiated tumors (OR = 0,000; 95%CI = -21,417 - -18,550) and the absence of distant metastasis (OR = 0,145; 95%CI = -3,767 - -0,098) significantly correlated with a reduced incidence of extensive necrosis (Table II).

## DISCUSSION

This study found that most tumor necrosis was in the focal group (36.1%), consistent with the previous study (15). Tumor necrosis showed a significant correlation with histopathological grade. Higher grades are associated with more extensive areas of tumor necrosis. Deeper tumor invasion are associated with greater necrosis area, and the extent of tumor necrosis increases the likelihood of distant metastasis. Multivariate analysis revealed that patients without distant metastasis tended to have lower necrosis rates than those with distant metastasis. This may indicate that distant metastasis may play a role in increased tumor necrosis. Nearly fifty percent of colorectal adenocarcinoma patients have tumor necrosis, and previous studies have also associated tumor necrosis with unfavorable cancer progression,

**Table I: Correlation between Tumor Necrosis, Tumor Infiltrating Lymphocytes, and Their Combination with Clinicopathological Characteristics**

Variables	Total n (%)	Tumor Necrosis (n=36)		TILs (n=36)		Combination of Tumor Necrosis & TILs (n=36)	
		p	r	p	r	p	r
Age	21-30	2	5.6				
	31-40	2	5.6				
	41-50	4	11.1	0.609	0.088	0.689	0.069
	51-60	18	50				0.558
	61-70	3	8.3				0.101
>70	7	19.4					
Gender	Male	18	50	0.561	-0.1	0.331	-0.167
	Female	18	50				0.396
Tumor Site	Right Colon	6	16.7				
	Left Colon	9	25	0.163	0.238	0.428	0.136
	Rectum	21	58.3				0.365
Grade	Well Diff	19	52.8				
	Moderately Diff	15	41.7	0.009*	0.43	0.228	-0.206
	Poorly Diff	2	5.6				0.902
Tumor Depth Invasion	pT1	2	5.6				
	pT2	4	11.1	0.008*	0.436	0.030*	-0.361
	pT3	28	77.8				0.559
	pT4	2	5.6				-0.101
Lymph Node Metastasis	Positive	19	52.8	0.268	-0.19	0.185	0.226
	Negative	17	47.2				0.344
Distant Metastasis	Positive	6	16.7	0.045*	0.336	0.47	-0.124
	Negative	30	83.3				0.883
TNM Stage	I	4	11.1				
	II	15	41.7	0.144	0.249	0.11	-0.271
	III	10	27.8				0.351
	IV	7	19.4				-0.16

TILs = Tumor-infiltrating lymphocytes; \* = p-value < 0.05 (statistically significant); r = coefficient correlation

**Table II: Multivariate Analysis Correlation between Tumor Necrosis and Tumor Infiltrating Lymphocytes, with Grade, Tumor Depth Invasion and Distant Metastasis.**

Variables	OR	Tumor Necrosis (n=36)		TILs (n=36)	
		95% CI	p	OR	95% CI
Grade	Well Diff	0,000	0,000*		
	Moderately Diff	-	-21,417 - -18,550		
	Poorly Diff	Reference	-18,863 - -18,863		
Tumor Depth Invasion	pT1-pT2	0,316	0,243	0,133	0,014 - 1,288
	pT3-pT4	Reference	-3,081 - -0,781		
Distant Metastasis	Negative	0,145	0,039*		
	Positive	Reference	-3,767 - -0,098		

TILs = Tumor-infiltrating lymphocytes; OR = Odds Ratio; \* = p-value < 0.05 (statistically significant)

including 5-year overall survival and progression-free survival (14,17).

Necrosis, a process linked to hypoxic conditions, vascular insufficiency, and incomplete neovascularization in the TME, is influenced by inflammation and local inflammatory response. It releases damage-associated molecular patterns (DAMPs) and pro-inflammatory cytokines, thereby increasing tumor aggressiveness. Necrosis also activates cell proliferation and anti-apoptotic pathways, hallmarks of cancer progression (16,17). The interplay between immune cells and the tumor microenvironment is crucial to understanding cancer progression. The local inflammatory response tends to be notably subdued in tumors with significant necrosis. Colorectal cancers often exhibit varying levels of necrosis, which is considered an adverse prognostic factor. Conversely, vigorous local anti-tumor immune response is linked to more favorable outcomes in colorectal cancer (16,18).

In this study, TILs showed more in a low group (52,8%) and correlated with tumor depth invasion. Previous studies have also shown a significant correlation between TILs and tumor invasion depth (7,8). TILs are pivotal in the immune response against cancer. Key cellular components include CD8+ T cells, which are known for their cytotoxic effects, and CD4+ T helper cells (CD4+Th). CD4+Th cells assist in the clonal expansion of antigen-specific CD8+ T cells and promote interferon-gamma production (IFN- $\gamma$ ). This cytokine, in turn, enhances the proliferation and functionality of effector molecules in both CD8+ T cells and natural killer (NK) cells, helping to suppress cancer progression, particularly in colorectal adenocarcinoma. TILs negatively correlate with tumor invasion depth, and high-density TILs are associated with better outcomes. They also serve as a prognostic indicator for overall survival in colon and rectal cancer (8,9).

This study showed a combination of tumor necrosis and TILs did not correlate with clinicopathological parameters. The previous studies also showed no correlation between tumor necrosis and TILs in DMBA-induced rat breasts (19). The direction of correlation between tumor necrosis and TILs and clinicopathological parameters was the opposite.

Histopathological examination of colorectal adenocarcinoma enables observation of tumor cells and their surrounding components, such as tumor necrosis and TILs. These features can be easily evaluated using H&E stained slides through semiquantitative methods. Tumor necrosis and TILs have been found to correlate with various clinicopathological factors and may help determine patient survival (18,20). As a result, the observation of tumor necrosis and TILs on H&E-stained slides can be routinely included in pathological reports for colorectal adenocarcinoma.

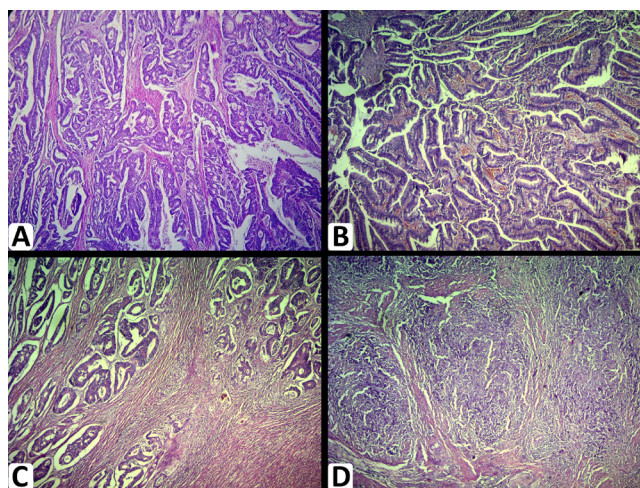


Figure. 1: Histological type and grading of the tumor. (A) The histological type is adenocarcinoma with gland and tubular formation. (B) A well-differentiated tumor is a uniform gland or simple tubule formation. (C) A moderately differentiated tumor with a more complex tubular formation. (D) Poorly differentiated tumors show less than <50 % gland formation with sheet structures. (HE stain, 40x).

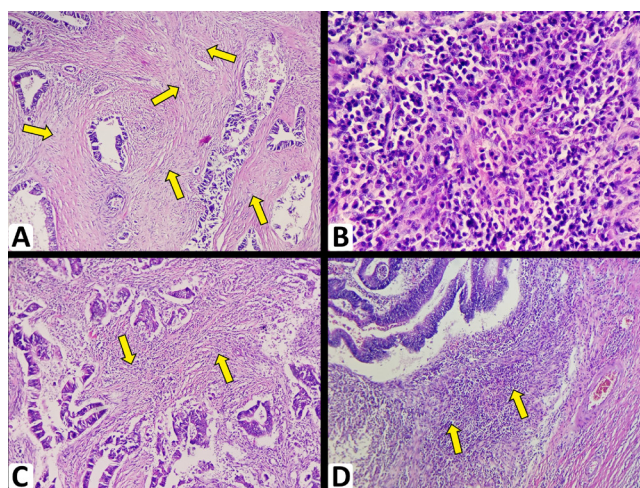


Figure. 2: Tumor-infiltrating evaluation. (A) TILs were evaluated in the stromal area surrounding tumor cells (Yellow Arrows, HE stain, 40x). (B) TILs have contained lymphocytes and plasma cells (HE stain, 400x). (C) Low-TILs (HE stain, 200x). (D) High-TILs (HE stain, 200x).

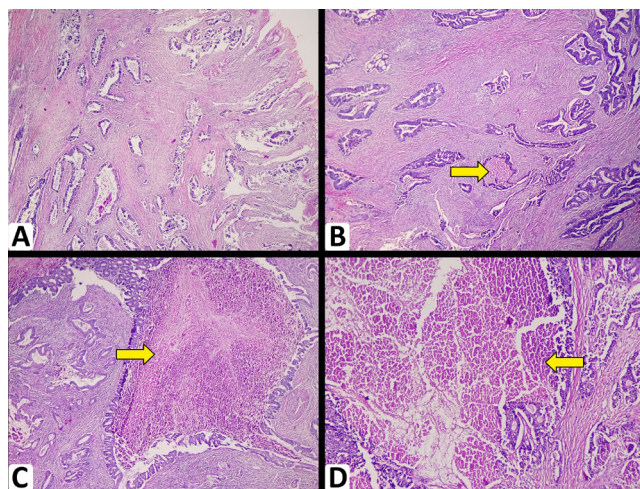


Figure. 3: Tumor necrosis evaluation. (A) Absent. No necrosis was found in areas surrounding tumor. (B) Focal. Tumor necrosis refers to necrotic areas surrounding the tumor with <10%. (C) Moderate. Tumor necrosis was 30%. (D) Extensive. Tumor necrosis was 60% of all areas. (Yellow Arrows, HE Stain, 40x).

This study has limitations regarding sample size. Samples were collected from only single location based on existing cases following specific inclusion and exclusion criteria, which may have influenced the findings. Multicenter studies are necessary to obtain larger samples to enhance the correlation between TILs and tumor necrosis with clinicopathological variables.

## CONCLUSION

Tumor necrosis and TILs can be observed in H&E-stained slides of colorectal adenocarcinoma and correlated with clinicopathological variables. Tumor necrosis was associated with unfavorable variables such as higher grade, deeper invasion, and distant metastasis, while TILs were associated with favorable indicators like lower depth invasion, potentially acting as independent prognostic factors.

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