Relationship between Clinicopathological Profile and Tumor Budding Status in Colorectal Adenocarcinoma at Dr. Cipto Mangunkusumo General National Hospital: A Retrospective Study

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ABSTRACT

Background: Colorectal adenocarcinoma (CA) is one of the most common malignancies. Tumor budding (TB) status is associated with poor prognosis in patients. Prognosis is influenced by the clinicopathological profile. This study aims to determine the relationship between the clinicopathological profile with TB status in CA at Dr. Cipto Mangunkusumo General National Hospital.

Method: A cross-sectional retrospective analytic study using secondary data in the form of cases in large bowel malignancy resection preparations at the Department of Anatomical Pathology in 2019-2021. A total of 213 samples were taken from all cases according to the inclusion and exclusion criteria. Chi square statistical analysis was performed to see the clinicopathological relationship with TB status.

Results: Most common TB status were low grade with 92 cases. Most cases were ≥ 50 years old (64.3%), male (50.7%), located in the left colon (77.5%), histopathological degree low grade (85.9%), depth of invasion on pT3 (61.5%), lymphovascular invasion (LVI) (50.2%), lymph node metastasis (52.6%), stage 3 American Joint Committee on Cancer (AJCC) (42.3%), without perineural invasion (PNI) (79.3%) and without distant metastases (82.6%). Statistical analysis test showed that there was a significant relationship between the degree of histopathology, depth of invasion, LVI, lymph node metastasis, and AJCC stage ($p < 0.001$) and tumor location ($p = 0.036$).

Conclusion: TB status was significantly related histopathological degree, LVI, lymph node metastasis, depth of invasion, AJCC stage, and tumor location. TB status was not associated with PNI and distant organ metastases.

Keywords: colorectal adenocarcinoma, histopathological degree, tumor budding

ABSTRAK

Latar belakang: Adenokarsinoma kolorektal (AKR) termasuk salah satu jenis keganasan yang terbanyak. Status tumor budding (TB) berkaitan dengan prognosis buruk pada pasien. Prognosis dipengaruhi oleh profil klinikopatologik. Penelitian ini bertujuan mengetahui hubungan profil klinikopatologik dengan status TB pada AKR di Rumah Sakit Dr. Cipto Mangunkusumo.

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Metode: *Penelitian retrospektif analitik desain potong lintang dengan data sekunder berupa kasus pada sediaan reseksi keganasan usus besar di Departemen Patologi Anatomik tahun 2019-2021. Sampel berjumlah 213 diambil dari keseluruhan kasus menurut kriteria inklusi dan eksklusi. Dilakukan analisis statistik chi square untuk melihat hubungan klinikopatologik dengan status TB.*

Hasil: *Status TB terbanyak adalah derajat rendah sebanyak 92 kasus. Sebagian besar kasus berusia ≥ 50 tahun (64,3%), jenis kelamin laki-laki (50,7%), terletak di kolon kiri (77,5%), derajat histopatologi low grade (85,9%), kedalaman invasi pada pT3 (61,5%), ditemukan invasi limfovaskular (50,2%), terdapat metastasis kelenjar getah bening (52,6%), stadium 3 American Joint Committee on Cancer (AJCC) (42,3%), tanpa invasi perineural (PNI) (79,3%) dan tanpa metastasis jauh (82,6%). Uji analisis statistik menunjukkan bahwa terdapat hubungan bermakna antara derajat histopatologi, kedalaman invasi, LVI, metastasis kelenjar getah bening, dan stadium AJCC (p < 0,001) serta lokasi tumor (p = 0,036).*

Simpulan: *Status TB berhubungan signifikan dengan derajat histopatologi, LVI, metastasis kelenjar getah bening, kedalaman invasi, stadium AJCC dan lokasi tumor. Status TB tidak berhubungan dengan PNI dan metastasis organ jauh.*

Kata kunci: *adenokarsinoma kolorektal, derajat histopatologi, tumor budding*

INTRODUCTION

Colorectal carcinoma (CRC) is a malignancy whose incidence rate ranks third in the world.[1] In Indonesia, CRC ranks fourth in terms of incidence and the fifth cause of death from cancer in 2020.[2] Dr. Cipto Mangunkusumo General National Hospital is a national referral hospital that receives quite a lot of cases. The number of CRC case visits at the Department of Anatomical Pathology in the period January 2019 to December 2021 was 715 cases, both biopsies and surgeries. The most histopathological type of CRC is colorectal adenocarcinoma (CA), which accounts for approximately 90% of the total CRC.[3] In CRC, the classification of the clinical stage of cancer and the prognostic value of patients has been using the system issued by the American Joint Committee on Cancer (AJCC). The AJCC classification uses pathological criteria in the form of primary tumor extension (pT), regional lymph nodes (N), and distant metastases (M) which is abbreviated as TNM.[3,4] In the World Health Organization (WHO) Classification of Tumours 5th Edition Digestive System Tumors in 2019, it is stated that tumor budding (TB) is a determinant of prognostic value and survival.[3]

The definition of TB is a malignant tumor cell in the form of single cells or small groups of not more than 4 cells located at the edge of an invasive tumor.[3,5,6] TB cells are morphological manifestation of the epithelial-mesenchymal transition which is a biological process that allows cells to have a mesenchymal phenotype characterized by increased migration, invasion, resistance to apoptosis, and increased production of extracellular matrix components.[3,4,7,8] It is said that

TB has the ability to separate from the main tumor, evade the body's defense response and can invade into the lymphatic and vascular vessels, and eventually underwent distant metastasize.[4] The frequency of TB was found to be increased in CRC with more advanced stages of AJCC.[9] High-grade TB (greater than or equal to 10 TB) was consistently found to be associated with depth of tumor invasion beyond the muscularis propria layer, lymph node metastases, distant organ metastases, and incidence of lymphovascular invasion (LVI).[10]

In patients diagnosed with stage 1, TB is a predictor factor for the incidence of lymph node metastasis which later will play a role in determining segmental resection procedure followed by lymphadenectomy.[11] In patients diagnosed with stage 2 CRC, high-grade TB status shows aggressive behavior.[11] In general, postoperative adjuvant chemotherapy is not performed at stage 2, but if a high-grade TB status is found, an adjuvant chemotherapy plan can be considered to be added.[4,11] Graham et al, Zlobec et al, and Chinelli et al found that high-grade TB status was associated with a poorer prognosis.[12–14] At stage 3, TB status is important predictor of local recurrence, survival, tumor regression rate, and chemosensitivity of therapy.[11,15] In patients with stage 4, TB status can be used to evaluate disease progression as a prognostic factor but not as a predictor of survival.[15]

This study aims to determine the relationship between the clinicopathological profile and TB status in CA at the Department of Anatomical Pathology, Dr. Cipto Mangunkusumo General National Hospital in 2019-2021.

METHOD

This study is a descriptive analytical retrospective study with a cross-sectional design. The sample used was secondary data obtained from the archives of the Department of Anatomical Pathology, Dr. Cipto Mangunkusumo General National Hospital within January 2019 and December 2021 period. Cases searching were carried out using the international classification of disease for oncology topographic and morphological code, 3rd edition (ICD-0-3). The topographic codes taken were C18.-, C19.-, and C20.- which were diagnosed as colorectal malignancy cases with the morphological code /3. Inclusion criteria were all resection preparations originating from the large intestine with a final diagnosis of CA. Exclusion criteria were biopsy preparations and the final diagnosis was not CA and if blocks and slides were not found. Samples were taken from all cases that met the inclusion criteria and did not meet the exclusion criteria.

The clinical and pathological data collected were age, gender, tumor location, histopathological degree, pT, LVI, perineural invasion (PNI), lymph node metastasis, distant organ metastases, incision margins, and AJCC stage. The age recorded was the patient's age during first diagnosis in years. Tumor location is location where the tumor grows in the large intestine; divided into the right colon (cecum, ascending colon, hepatic flexure, and 2/3 proximal of the transverse colon) and left colon (1/3 distal of the transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum).[16] The degree of tumor histopathology was defined as the arrangement of tumors forming a glandular pattern. If the glandular pattern is 50%, it is called low grade, whereas if the gland pattern is < 50%, it is called high grade. The parameter pT is the deepest invasion of the tumor based on the lining of the large intestine which is classified according to WHO: (1) pT1 if the tumor penetrates the muscularis mucosal layer and reaches the submucosa; (2) pT2 if the tumor invades the muscularis propria layer; (3) pT3 if the tumor invades the subserosa or pericolic layer or perirectal tissue; (4) pT4 if the tumor directly invades other organs or structures and/ or cause perforation of the visceral peritoneum. Lymph node metastases was considered when tumor cells are found in the lymph nodes and distant organ metastases when tumor cells are found in other organs. The incision margin or the surgical incision margin is free when no tumor cells are found and not free when tumor cells are found. Based on TNM, CRC is classified into four stages of AJCC, namely stage I (T1-2N0M0), stage II (T3-4N0M0), or stage III (T1-4N1-2M0), or stage IV (T1-4N1-4M1).[17] LVI is defined as tumor cells found in blood vessels (usually veins) or lymph vessels, while PNI is defined as tumor cells found along or around nerves and nerve sheaths.[18]

The number of research samples that met the inclusion criteria and did not fall into the exclusion criteria were 213 cases. The clinicopathological data were found to be complete on the patient's status. A re-evaluation of all histopathological preparations from the hematoxylin and eosin (HE) smeared slides was taken from the archives of the Department of Anatomical Pathology Dr. Cipto Mangunkusumo General National Hospital by the researcher and supervisor who is an anatomical pathologist. Reassessment of the degree of tumor histopathology was performed using the WHO classification of tumours of the digestive system, 2019. If there was a difference between the previous results and the time of reassessment, the results that would be used in this study were the results during reassessment.

The calculation of the number of TB and determination of TB status was performed using the reference from the international tumor budding consensus conference (ITBCC) 2016. The method of calculating TB began with selecting the HE slide with the most invasive tumor area. Observations were made using an olympus CX23 microscope (field diameter 20 mm, field area 0.785 mm^2) with medium magnification (10x objective) to determine 10 areas where the number of TB will be counted. The assessed area was the invasive front, which was located peritumoral or between the main tumor. Of the 10 areas, one area was determined as a hot-spot calculation. TB counts were performed using an objective magnification of 20x to count TB detached from the main tumor by 1 to 4 cells. The result of TB calculation is divided by the normalization factor (1.00). It is considered as low-grade TB if the number was 0-4 TB, medium-grade TB if the number was 5-9 TB, and high-grade TB if the number was more than or equal to 10 TB.[5,11]

The research data were statistically analyzed using the statistical package for social science (SPSS) version 25.0 application. The analysis was carried out using the chi square test or Fisher's test if the chi square conditions were not met. The results were declared statistically significant if the p value < 0.05.

RESULTS

Research was conducted using archive data from the Department of Anatomical Pathology, Dr. Cipto Mangunkusumo General National Hospital in 2019-2021 and 213 CA cases were found. The most CA

subtypes were NOS adenocarcinoma as many as 168 (78.9%) and non-NOS adenocarcinoma as many as 45 (21.1%).

Table 1 shows the distribution of demographic data in 213 CA cases consisting of 50.7% males and 49.3% females. The age of most patients (64.3%) was in the category of 50 years. The location of the tumor was generally (77.5%) in the left intestine. The limit of the incision that was considered tumor-free was 84.98%. Most TB status were low grade 92 (43.2%) cases, followed by moderate grade 71 (33.3%) cases, and high grade 50 (23.5%) cases. The histopathological degree was dominated by the low-grade group with 183 cases (85.9%), while the high-grade group consisted of 30 cases (14.1%). The depth of pT3 invasion was the most common, with 131 cases (61.5%). Tumors with LVI were more common (50.2%) than those without LVI (49.8%). Tumors with PNI were found in 44 cases (20.7%). Lymph node involvement (the presence of lymph node metastases) were negative in 101 cases (47.4%) and positive in 112 cases (53.6%). Generally, the patients were in the absence of distant organ metastases with a total of 176 cases (82.6%). There were 37 cases (17.4%) of patients who had distant organ metastases. Based on the AJCC stage, the most common tumors were in stage 3 with 90 cases (42.3%), followed by stage 2 in 67 cases (31.5%), stage 4 in 31 cases (14.5%) and the least was stage 1 with 25 cases (11.7%).

The characteristics of the relationship between TB status and clinicopathology profile in CA were listed in Table 2. In low grade histopathology, the highest TB status was low grade in 89 cases (48.63%). In high grade histopathology, the most TB status was high grade TB in 16 cases (53.33%). Clinicopathological characteristics in histopathological degree was associated with TB status (p value < 0.001).

At a depth of invasion equivalent to pT3, the status of low-grade TB was 45 cases (34.35%), moderate TB was 52 cases (39.69%), and high-grade TB was 34 cases (25.95%). In the statistical analysis test, group simplification was carried out so that pT1 and pT2 became group 1 and pT3 and pT4 became group 2. The number of group 1 was 35 cases (16.4%) and group 2 was 178 cases (83.6%). Among group 1, the most common TB status was low grade. Likewise in group 2, the highest TB status was also low grade. A significant relationship was found between the depth of invasion and TB status (p = 0.001).

Tumors without LVI were 106 cases (49.8%) with 60 cases (65.2%) of low-grade TB, 31 cases of moderate-grade TB (43.7%), and 15 cases of

high-grade TB (30%). In further analysis, LVI was significantly associated with TB status (p value < 0.001). Tumors without PNI was 169 (79.3%) cases with the most TB status being low grade in 79 cases (46.7%). There was no significant relationship between PNI and TB status (p = 0.105).

In cases with tumor metastases, 36 cases (39.1%) had low-grade TB status, 38 cases had medium-grade TB (53.5%), and 38 cases had high-grade TB (76.0%). The presence of lymph node metastases was associated with TB status (p < 0.001). In patients with distant organ metastases, the most TB status was low-grade in 14 cases (37.8%). Distant organ metastases were not associated with TB status (p value = 0.344).

Table 1. Clinicopathological characteristics of colorectal adenocarcinoma (n = 213)

Parameter	n (%)
Age	
< 50 years old	76 (35.7)
≥ 50 years old	137 (64.3)
Sex	
Male	108 (50.7)
Female	105 (49.3)
Tumour location	
Right colon	48 (22.5)
Left colon	165(77.5)
Incision margin	
Free	181 (85)
Not free	32 (15)
Histopathological degree	
Low grade	183 (85.9)
High grade	30 (14.1)
Depth of invasion (pT)	
pT1	3 (1.4)
pT2	32 (15)
pT3	131 (61.5)
pT4	47 (22.1)
Lymphovascular infiltration (LVI)	
No	106 (49.8)
Yes	107 (50.2)
Perineural invasion (PNI)	
No	169 (79.3)
Yes	44 (20.7)
Lymph node metastases	
No	101 (47.4)
Yes	112 (52.6)
Distant metastases	
No	176 (82.6)
Yes	35 (17.4)
Stadium AJCC	
1	25 (11.7)
2	67 (31.5)
3	90 (42.3)
4	31 (14.6)
Adenocarcinoma subtype	
NOS	168 (78.9)
Non-NOS	45 (21.1)

Table 2. Characteristics of the relationship between tumor budding status and the clinicopathology profile in colorectal adenocarcinoma

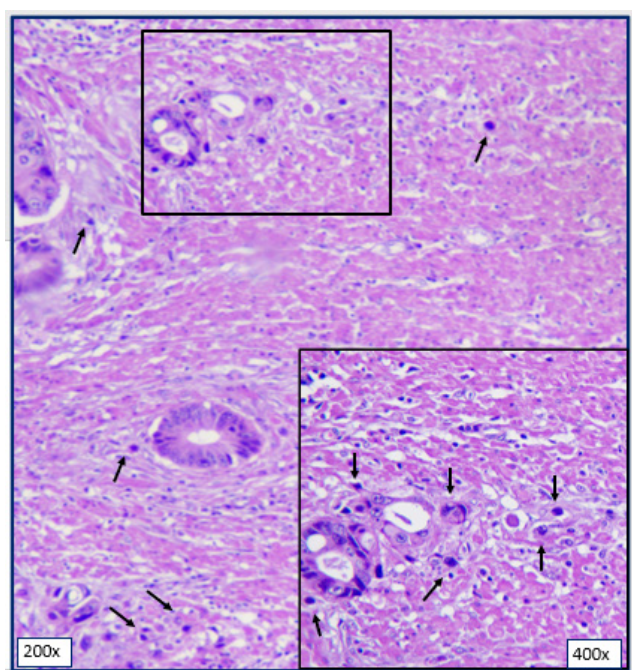| Clinicopathological characteristics	Tumor Budding Status			p value
	Low grade	Medium grade	High grade	
Age (Years)				
< 50 years	26 (34.21)	27 (35.53)	23 (30.26)	0.095
≥ 50 years	66 (48.18)	44 (32.12)	27 (19.71)	
Sex				
Male	46 (42.59)	41 (37.96)	21 (19.44)	0.230
Female	46 (43.81)	30 (28.57)	29 (27.62)	
Tumour location				
Right colon	28 (58.33)	14 (29.17)	6 (12.5)	0.034
Left colon	64 (38.79)	57 (34.55)	44 (26.67)	
Histopathological degree				
Low grade	89 (48.63)	60 (32.79)	34 (18.58)	< 0.001
High grade	3 (10)	11 (36.67)	16 (53.33)	
Depth of invasion				
pT1 and pT2	26 (74.29)	7 (20)	2 (5.71)	< 0.001
pT3 and pT4	66 (37.08)	64 (35.96)	48 (26.9)	
Lymphovascular invasion (LVI)				
No	60 (55.6)	31 (29.25)	15 (14.15)	< 0.001
Yes	32 (29.91)	40 (37.38)	35 (32.71)	
Perineural invasion (PNI)				
No	79 (46.75)	54 (31.95)	36 (21.3)	0.105
Yes	13 (29.55)	17 (38.64)	14 (31.82)	
Lymph node metastases				
No	54 (55.45)	33 (32.67)	12 (11.88)	< 0.001
Yes	36 (32.14)	38 (33.93)	38 (33.93)	
Organ metastases				
No	78 (44.32)	59 (33.52)	39 (22.16)	0.590
Yes	14 (37.84)	12 (32.43)	11 (29.73)	
Stadium AJCC				
1	21 (84)	3 (12)	1 (4)	< 0.001
2	31 (46.27)	26 (38.81)	10 (14.93)	
3	28 (31.11)	32 (35.56)	30 (33.3)	
4	12 (38.71)	10 (32.26)	9 (29.03)	

Figure 1. Tumor budding pattern (marked with arrows) in invasive area, HE 200x and HE 400x

In stages 1 and 2 of AJCC, the most common TB status were low grade as many as 21 cases (84%) and 31 cases (46.3%), respectively. Stage 3 AJCC, the most common TB status was moderate grade as many as 32 cases (36%). In stage 4 AJCC cases, the most common TB status was low grade as many as 12 cases (38.7%). AJCC stage correlated with TB status (p value = 0.001).

DISCUSSION

The most histopathological degrees (85.92%) were found to be low grade. This result was similar to the study conducted by Ozer SP et al in which CA was dominated by low grade histopathology.[12] In high grade histopathology, the most common TB status was in high grade (53.33%) while in low grade histopathology the most common was low grade (48.63%). This is in accordance with a study conducted by Mehta A et al and Zlobec I et al which stated that high-grade TB status was more common in tumors with high-grade histopathology.[6,13] TB increased the expression of

matrix metalloprotein (MMP) 2 and MMP9 proteins. This protein is associated with cell proliferation.[19]

The depth of invasion found in this study appeared to be dominated by pT3 (61.5%) and pT4 (22%). This showed that most patients presented at an advanced stage. Research conducted by Ozer SP et al and Chinelli J et al found that pT3 and pT4 were more dominant than pT1 and pT2.[12,14] Research by Zlobec I et al stated that there was a significant relationship between the depth of invasion and TB status (p < 0.0001).[20] Van Wyk et al stated that in tumors with pT1 and pT2, only TB status can determine significant prognostic value. This proves that TB occurs earlier in tumor invasion.[21]

The highest status of lymph node involvement in this study was the presence of lymph node metastases, with 112 cases (52.58%) with high-grade TB status were found in 38 cases (33.93%). This study was in line with the research conducted by Mehta A et al which stated that high grade TB status was a predictor of lymph node metastasis.[6] A study on the calculation of TB in preoperative CA biopsies conducted by Zlobec I et al concluded that TB status can predict the presence of lymph node metastases. Lymph node involvement was related to the nature of TB which represents EMT characteistics, which cause it to become more invasive and aggressive.[22]

Sligtly higher lymphovascular invasion cases than without LVI. Research conducted by Zlobec I et al and Ozer SP et al also gave the same result with p value < 0.001.[12,20] Lymphovascular invasion occurs because TB loses expression of e-cadherin so that TB has the ability to break away and moves into lymphovascular vessels.[4]

Reporting the presence or absence of PNI is related to recurrence and survival rates.[23] The study of Mehta et al concluded that there was no relationship between recurrence and TB status.[6] Meanwhile, in other studies stated that there was a relationship between PNI and TB status.[12,13]

There was no statistically significant relationship between TB status and distant organ metastases in this study (p = 0.344). The results of this study were in line with research conducted by Mehta A et al in which there was no significant relationship between TB status and organ metastases (p = 0.332).[6] In contrast, the study conducted by Zlobec I et al stated that there was a relationship between distant metastases and TB status (p < 0.001).[13] The difference in the results of this study is difficult to explain but may be due to differences in the distribution of stages in the study and the biological characteristics of CA in Indonesia.

AJCC staging with TB status in this study showed a significant relationship which was in line with the study conducted by Mehta et al.[6] The AJCC stage is commonly used as a prognostic factor. However, other studies have shown TB as a better prognostic factor. TB can predict the incidence of local invasion related to the depth of invasion (pT), the risk of lymph node metastases associated with nodes (N), and lymphovascular invasion associated with the incidence of distant organ metastases (M).[19] TB shows signs of an aggressive malignancy such as loss of cell adhesion properties which allows local invasion to occur.[21]

CONCLUSION

In this study, most of the TB status was low-grade TB status. It was found that there was a statistically significant relationship between the degree of tumor histopathology, LVI, lymph node metastasis, depth of invasion, AJCC stage, and tumor location with TB status. There was no significant relationship between PNI and distant organ metastases with TB status.

Unfortunately, this study only involved one center (Dr. Cipto Mangunkusumo General National Hospital) which is a national referral hospital, so the results of the study did not represent the overall results in Indonesia. It would be better if the samples also came from other centers so that the cases are more varied and can represent the total population in Indonesia.

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