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Effect of tumor budding and tumor infiltrating lymphocytes on prognosis of colorectal mucinous adenocancers

Fatmagul Kusku Cabuk[1], Damlanur Sakiz[1], Elif Eda Ozer[2], Zehra Sibel Kahraman[1]

[1]Bakirkoy Dr Sadi Konuk Training and Research Hospital, Department of Pathology, Istanbul Turkey
[2]Bakirkoy Dr Sadi Konuk Training and Research Hospital, Department of Radiation Oncology, Istanbul Turkey

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Abstract
Mucinous adenocarcinoma (MAC) has a worse prognosis than do classical adenocarcinoma. Increased tumor budding (TB) and lower number of tumor infiltrating lymphocytes (TIL) negatively affect prognosis in classical adenocarcinoma. In this study, we investigated whether TB and TIL values influenced prognosis in patients with MAC. Forty-five patients diagnosed with MAC between 2011 and 2015 were included in the study. TB and TIL values of the patients were re-evaluated. In assessing TIL, lymphocytes that infiltrated the stroma and tumor were evaluated separately. The effects of these findings on the prognostic parameters and the disease-free survival (DFS) and overall survival (OS) were evaluated. The mean follow-up period was 26.07 months (min: 1 max: 82, SD:24.86), OS 28.04 months (min: 1 max: 82 SD:24.81). The results between TB and OS (p = 0.066) and DFS (p = 0.205) were not significant. The relationship between sTIL and OS (p = 0.367) and DFS (p = 0.949) was not significant. The relationship between iTIL and OS (p = 0.502) and DFS (p = 0.870) was not significant. The only significant difference between clinicopathological parameters and TB, sTIL, and iTIL was between grade and TB (p = 0.016). In terms of clinicopathologic parameters, stage with OS (p = 0.008) and DFS (0.016), lymph node metastasis with OS (p = 0.010) and DFS (0.008), LVI (lymph vascular invasion) with OS (p = 0.034) and DFS (p = 0.015), PNI (perineural invasion) and DFS (p = 0.007) was significant. In this study, the effect of TB and TIL on the prognosis of MAC was investigated. We found that these data did not correlate with the prognosis of the tumor and the prognostic parameters (LVI, PNI, lymph node metastasis, stage) in the staging system remained as the gold standard for predicting prognosis.

Keywords: Colorectal mucinous adenocarcinoma, tumor budding, tumor infiltrating lymphocytes, disease-free survival, overall survival, prognostic parameters

Introduction

Colorectal cancer is one of the most common cancers and the major causes of cancer-related deaths worldwide [1]. Colon cancer is the third most common cancer type in the world [2]. More than 90% of carcinomas localized in the colon are adenocarcinoma [2]. Mucinous carcinoma was first described by Parham in 1923 [3]. The separate identification of mucinous adenocarcinoma (MAC) from the classical adenocarcinoma by the world health organization (WHO) was also in 1979 [2]. Many studies have shown that MACs have a worse prognosis than classical adenocarcinoma [2,4,5].

In recent studies, it has been reported that increased tumor budding (TB) was associated with poor prognosis [6,7]. Tumor budding criteria were reported by the International Tumor Budding Consensus Conference (ITBCC) in 2016 [8]. According to the ITBCC criteria, in mucinous carcinoma, it should be done carefully and the count of the tumor cells in mucinous pools should not be considered [8].

In recent years, it has been showed that the number of lymphocytes in the tumor is an important prognostic factor [9,10]. In 1986, Jass first reported that tumor infiltrating lymphocytes (TILs) were a novel independent prognostic factor in colorectal carcinomas [11]. Tumor infiltrating lymphocytes are important for prognosis in cancers such as ovary, lung, breast cancer [9,10]. However, TIL is controversial due to limited number of studies in colorectal carcinomas [10]. The number of studies examining TIL values in MAC is very small [12].

In this study, we investigated whether tumor budding and tumor infiltrating lymphocytes influence the prognosis in MAC.

Material and Methods

Forty-five patients who were diagnosed with colorectal MAC between the years 2011 and 2015 and who had follow-up and

***Coresponding Author:** Fatmagul Kusku Cabuk, Bakirkoy Dr Sadi Konuk Training and Research Hospital, Department of Pathology, Istanbul Turkey
E-mail: fatmagulkusku@hotmail.com

access to the slides from the pathology archive were included in the study. None of the patients included in the study had received neoadjuvant chemotherapy and/or radiotherapy preoperatively. Haematoxylin-eosin preparations of the patients were re-evaluated for tumor budding (TT), stromal tumor infiltrating lymphocytes (sTIL), intratumoral tumor infiltrating lymphocytes (iTIL) and the presence of metastatic lymph nodes in each case. The recurrence of MAC, the date of the patients' last hospital visit time, and final status (dead, alive) was determined.

Tumor budding was evaluated according to ITBCC criteria [8]. Tumor buds were evaluated in areas other than mucinous pools in each tumor (Figure 1). It was divided into low (0-4), medium (5-9) and high (≥10) according to the number of buds.

International TILs Working Group criteria were used to asses tumor infiltrating lymphocytes [9]. The sTIL in the tumor was evaluated as percentage (Figure 2). The percentages of the lymphocytes in the stroma were divided into three groups as below 10%, 10-50% and above 50%, respectively, as suggested by Swisher et al. [13]. Intratumoral lymphocytes (iTIL) were counted in the single high magnification area where lymphocytes were in contact with tumor (Figure 3).

The effects of tumor infiltrated lymphocytes and tumor bud groups on disease-free survival (DFS) and overall survival (OS) were evaluated in the Kaplan-Meier survival curve and long rank values. Kruskal Wallis was used for other statistical data. SPSS (SPSS version 17.0, SPSS Inc. Chicago, IL, USA) was used. P values less than 0.05 were considered as significant. ROC analysis were made to identify a cut-off value for iTIL values.

Results

Out of forty-five patients, 27 were male (60%) and 18 were female (40%). The mean age was 61.62 (min: 37, max: 85; SD: 12.65) years. The most common tumor localization was the rectosigmoid region (48.9%). There were 26 (57.8%) patients with the highest grade of tumor and 28 (62.2%) patients with T3 in the tumor stage. Perineural invasion (PNI) was present in 22 patients (48.9%), lymphovascular invasion (LVI) in 25 (55.6%), and lymph node metastasis in 33 (73.3%) patients. Twenty-five (55.6%) patients died and 11 (24.4%) had recurrence. Other clinicopathological features were summarized in Table 1.

The mean follow-up was 26.07 (min: 1, max: 82 SD: 24.86) months, OS was 28.04 (min: 1, max: 82 SD: 24.81) months.

The mean tumor budding was 4.44 (min: 0, max: 15 SD: 4.12). In the Kaplan Meier survival analysis, when tumor budding was divided into the three groups, the result between TB and OS (p = 0.066) and DFS (p = 0.205) was not significant.

The mean sTIL percent was 18.76 (min: 0, max: 80 SD: 21.67). There were no significant relationships between sTIL and OS (p = 0.367) or DFS (p = 0.949). The mean iTIL was 2.24 (min: 1, max: 5 SD: 2.24). When we wanted to divide iTIL into the groups for statistical analysis, 1.5 was accepted as a threshold value with 54.5% sensitivity and 52% specificity in ROC curve. Accordingly, iTIL were grouped as ≤1 and >1 and statistical analyses were performed. There were no significant relationships between iTIL and OS (p = 0.502) or DFS (p = 0.870).

Table 1. Sociodemographic, clinical and laboratory data of patients

	n (%)
Sex	
Female	18 (40)
Male	27 (60)
Age	
≤50	9 (20)
> 50	36 (80)
Localization	
Cecum	7 (15.6)
Ascending colon	13 (28.9)
Transvers colon	1 (2.2)
Descending colon	2 (4.4)
Rectosigmoid	22 (48.9)
Grade	
Low	5 (11.1)
Moderate	26 (57.8)
High	14 (31.1)
Perineural Invasion	
Yes	22 (48.9)
No	23 (51.1)
Lymphovascular Invasion	
Yes	25 (55.6)
No	20 (44.4)
T stage	
T2	4 (8.9)
T3	28 (62.2)
T4a	9 (20)
T4b	4 (8.9)
Stage	
Low Stage (I-II)	11 (24.4)
High Stage (III-IV)	34 (75.6)
TNM Stage	
Stage I	4 (8.9)
Stage II	7 (15.6)
Stage III	25 (55.6)
Stage IV	9 (20)
Lymph node metastasis	
Yes	33 (73.3)
No	12 (26.7)
Recurrence	
Yes	11 (24.4)
No	34 (75.6)
Exitus	
Yes	25 (55.6)
No	20 (44.4)
Stromal tumor infiltrating lymphocytes	
≤%10	24 (53.3)
%10-50	16 (16)
>%50	5 (5)
Tumor budding	
Low (0-4)	24 (53.3)
Moderate(5-9)	13 (28.9)
High (>10)	8 (17.8)

When the relationship between tumor budding, sTIL, iTIL and clinicopathological parameters were examined, only the relationship between grade and TB was found to be significant (p = 0.016). The TB was higher in high grade MACs. There was no significant relationship between the other parameters (Table 2).

No significant relationship was found between the right colon and the left colon in terms of OS (p = 0.785) or DFS (p = 0.114). There were statistically significant relationships between stage and OS (p = 0.008) or DFS (0.016). The relationship between lymph node metastasis and OS (p = 0.010) and DFS (0.008), LVI and OS (p = 0.034) and DFS (p=0.008) were significant. The relationship between PNI and DFS was significant (p = 0.007), and that between PNI and OS was non-significant (p = 0.866).

Table 2. Statistical correlation of clinopathologic parameters with tumor budding, stromal and intratumoral tumor infiltrating lymphocytes

	Tumor budding	sTIL	iTIL
Age	0.056	0.448	0.179
Sex	0.970	0.155	0.807
Localization	0.911	0.568	0.142
Grade	0.016**	0.354	0.540
PNI	0.074	0.146	0.863
LVI	0.072	0.970	0.350
T stage	0.072	0.867	0.507
LN metastasis	0.610	0.482	0.529
TNM stage	0.172	0.867	0.741
Recurrence	0.346	0.971	0.654
Exitus	0.320	0.343	0.386

** p<0.05

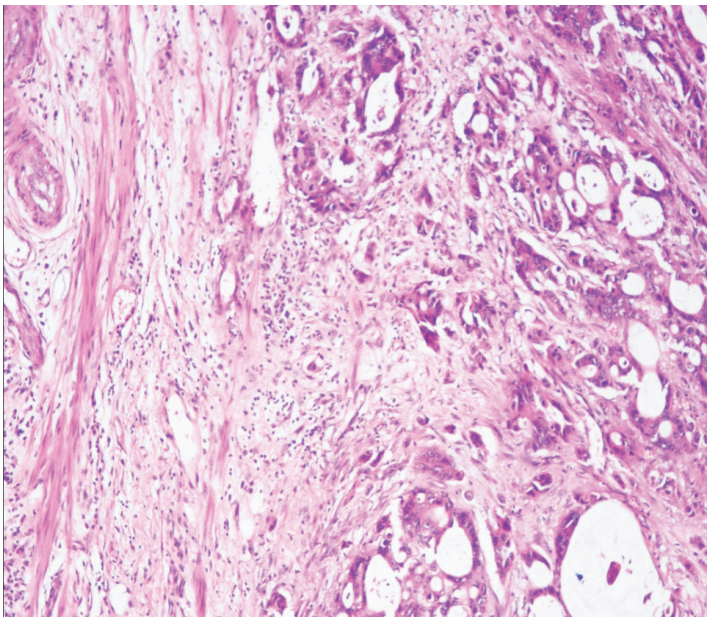

Figure 1. High numbers of tumor budding outside the mucinous pools in the tumor infiltrating areas x200 (H&E)

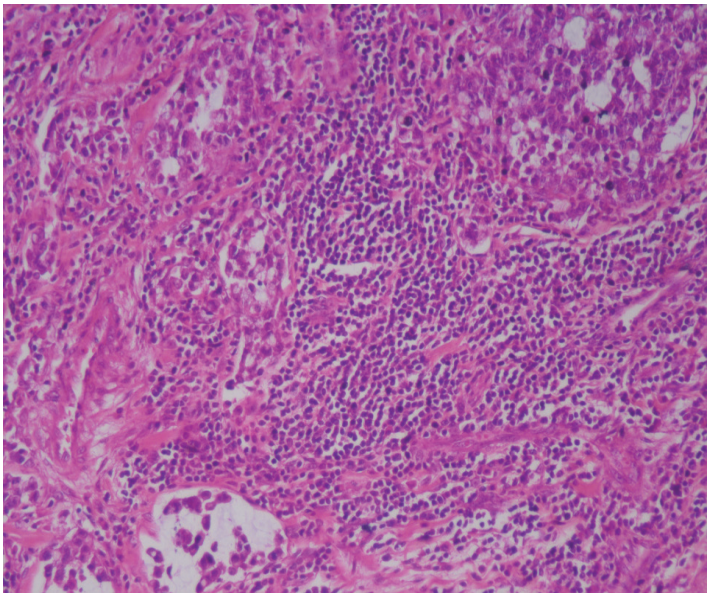

Figure 2. Tumor infiltrating lymphocytes in tumor stroma x200 (H&E)

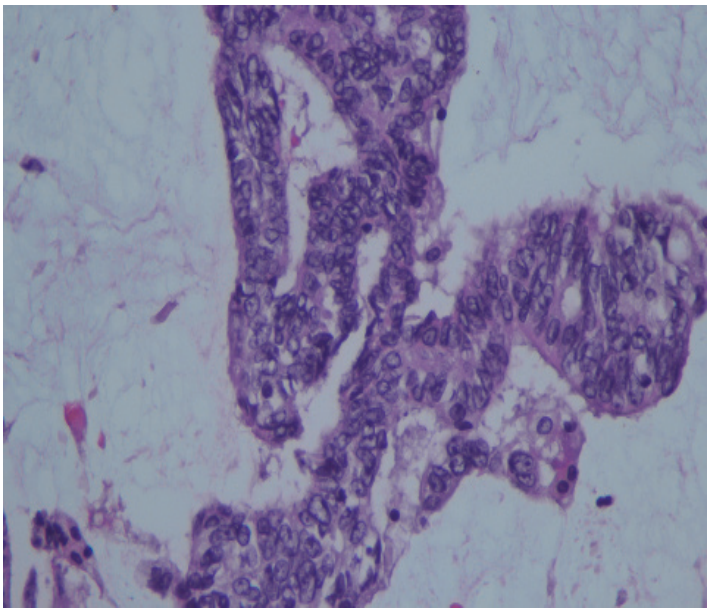

Figure 3. Lymphocytes that are in close touch with tumor cells in a high magnification area x400 (H&E)

Discussion

There are only a few articles investigating the effect of TB, sTIL and iTIL on the prognosis of the colorectal MACs [12,14].

The tumor budding has entered the final review report Collage of American Pathologists (CAP) and is now included in routine reports [15]. In a study, the relationship between TB and lymphatic invasion, blood vessel invasion, perineural invasion, lymph node metastasis, relapse, OS and DFS was found to be significant in the colorectal adenocarcinoma, but the relationship with tumor stage was not significant [16]. However, MAC was not considered in their study [16]. In the evaluation of tumor budding in colorectal carcinomas, it is difficult to evaluate two rare but specific histological types [17]. One of them is signet ring cell carcinoma. Diffuse infiltrative and discrete tumor cells increase the TB score in signet ring cell carcinoma. The other is MAC. It is also emphasized

that signet ring cells inside the mucinous ponds and small tumor cell islands should not be considered during evaluation of TB in MAC [17]. In our study, we also examined the TB values of the tumor located around the invasive area without considering the mucinous ponds. In another study, TB of colorectal carcinomas was found to be associated with lymphovascular invasion, microsatellite instability, presence of KRAS mutation, and cancer specific death [6]. It was stated that the limit value in budding is 10 [6]. TB with stage, venous invasion, histological grade, localization, chemotherapy treatment and BRAF mutation were found to be insignificant [6]. However, in that study, MACs have not been studied [6]. Similar to that in our study, Okuyama et al. had also studied MAC [14]. In their clinicopathological data, the male/female ratio indicated male predominance, the mean age was 63, and the rectum was the most common localization [14]. In our study there were more males with a mean age of 61. They found no significant relationship of TB to the age, tumor localization, tumor diameter, lymphatic invasion and significant relationship to venous invasion, lymph node metastasis, stage, and distant metastasis [14]. However, in their studies, TB was evaluated either present or absent. Within 5-year life disease free survival, TB was associated with poor prognosis and recurrence in these patients [14]. In our study, we found no significant correlation when we compared tumor budding with OS and DFS. Similarly, when we evaluate TB with age, localization, grade, LVI, PNI, recurrence and death, only the relationship between grade and TB was significant. We found higher TB in higher tumor grades.

In routine histopathologic studies, the density of lymphocytes on the margin of the lesion in malignant melanoma and triple negative breast cancer are reported [18-20]. In colorectal carcinomas, this is not yet included in routine reporting [15]. In this study, we investigated the effect of the percent of lymphocyte infiltration on the prognosis of MAC which is less common than colorectal carcinomas and has a worse prognosis than classical colorectal adenocarcinomas. Jukob et al. found negative relationships between sTIL and blood and lymphatic vessel infiltration, PNI, lymph node involvement or diffuse metastasis in colorectal carcinoma [21]. DFS was also found to be shorter in patients without iTIL at the tumor center [21]. In their study, approximately one third of the patients had received RT and / or CT before the operation. In our study, no patient had received CT and / or RT before the operation. In their study, there was no significant difference between sTIL and iTIL in terms of prognostic and predictive values [21]. The sTIL and iTIL values were examined separately at around and at the center of the tumor, and according to the statistical analyses, both sTIL and iTIL were found to be more significant around the tumor [21]. MAC were not included in the study and TIL levels was not correlated in adenocarcinomas with mucinous component [21]. In our study, the sTIL values around the tumor were evaluated, but the results were thought to be different from Jukob et al. study because all the tumors were mucinous. A limited number of studies exists in the literature about TIL on MAC. Millar et al. examined the TIL ratio in mucinous and classic colorectal carcinomas and found that the mucinous component decreased the TIL ratio [12]. They stated that extracellular mucin pools formed a barrier. This change in immune response was not only seen in the mucin regions of the tumor, but also in the non-mucin regions [12]. It was emphasized that these tumors exhibited more aggressive behavior as a result of TIL reduction [12]. In our study, we examined the TIL ratio in stromal and intratumoral areas. However, we couldn't achieve statistically significant results between clinicopathological data and survival. In general, TIL values were low. This supports the literature in the view that the mucin pools functions as a barrier and decrease the TIL ratios.

When the right or left colon localization of the tumor was analyzed with survival rates, we did not find any significant results in our study. The data about this topic in the literature are controversial. While some authors reported no difference between the two columns [22], some reported a better prognosis in the right colon [24,25] contrary to those reporting a worse prognosis in the right colon [23,26-28]. However, the type of tumor is also important. Qui et al reported that MACs were more localized to the right colon and had a better prognosis [28]. Hossein et al. stated that MACs were mostly localized in the left colon and rectal placement was indicative of poor prognosis, assessed by DFS and OS [25]. In our study, most of the tumors were localized to the left colon, but there was no significant difference in prognosis between the two localizations.

Prognostic parameters show poor outcomes in MAC as in other carcinomas in the presence of LVI, PNI, lymph node metastasis and advance stage [15]. LVI showed a poor prognosis and should be included in the report of TNM cancer staging system [15,29]. Stage and lymph node metastasis are an independent risk factor for poor prognosis [4,15,30]. In our study, both DFS and OS were significantly correlated with stage and lymph node metastasis. Although PNI has also been reported as an independent indicator of poor prognosis in TNM cancer staging system, some studies reported PNI not to be effective in predicting the prognosis [31]. In our study, a significant result was found between PNI and DFS; and progression was more accelerated in patients with PNI. However, there was no significant difference in overall survival in patients with or without PNI.

Our study has some limitations. First of all, our sample size was small due to rarer occurrence of MAC than that of classical adenocarcinomas. However, we had sufficient number of patients to make statistical analysis. The second limitation was evaluation of tumors from different stage, but since we aimed to evaluate the number of TILs on prognosis, we have to used different stages.

Conclusion

In this study, we showed that tumor budding and tumor infiltrating lymphocytes had no effect on the prognosis of mucinous adenocarcinomas. When the effect of histopathological data on prognosis was evaluated, similar results have been obtained with classical literature. Accordingly, LVI, PNI, lymph node metastasis, stage with DFS and OS were evaluated and all these correlations were statistically significant except for DFS with PNI. In conclusion, the reliability of the parameters included in the staging system were found to be still as the gold standard in determining the prognosis of the patients with MAC.

Conflict of interest
The authors declare that there are no conflicts of interest.

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Ethical approval
Because it is retrospective study, no consent of ethics needed

Fatmagul Kusku Cabuk ORCID:0000-0002-7833-9341
Damlanur Sakiz ORCID:0000-0002-2051-1049
Elif Eda Ozer ORCID:0000-0003-4050-0942
Zehra Sibel Kahraman ORCID:0000-0003-3902-8265

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