The association of mucinous histology with clinicopathological characteristics and long-term oncological outcome in patients with colorectal cancer

Ann Ital Chir, 2020 91, 6: 639-648
pii: S0003469X20033990
Online ahead of print 28 Sept. 2020
free reading: www.annitalchir.com

Volkan Tümay*, Osman Serhat Guner**

**Department of General Surgery, Acıbadem Mehmet Ali Aydınlar University, Bursa Hospital, Bursa, Turkey*
***Department of General Surgery, Acıbadem Mehmet Ali Aydınlar University, Bodrum Hospital, Turkey*

The association of mucinous histology with clinicopathological characteristics and long-term oncological outcome in patients with colorectal cancer

BACKGROUND: *This study aimed to evaluate clinicopathological characteristics and long-term oncological outcome with respect to mucinous histology of tumor in colorectal cancer (CRC) patients.*
METHODS: *A total of 372 patients who underwent resection surgery due to CRC between March 2006 and March 2019 were included in this retrospective study. Patients were divided into two groups according to degree of mucinous component including mucinous carcinoma group (n=48, ≥50% mucinous component) and non-mucinous carcinoma (n=324, <50% mucinous component) group. Data on patient demographics, tumor characteristics, treatment characteristics, metastasis and recurrence rates, disease free survival (DFS), and overall survival (OS) times were recorded.*
RESULTS: *Mucinous vs. non-mucinous carcinoma was associated with higher rate of T4 stage (p=0.036) and high grade tumors (p=0.001) with extranodal invasion (p=0.019) Both the OS time (75.9±13.1 vs. 110.8±5.6 months, p=0.019) and DFS time (98.5±15.6 vs. 140.5±5.1 months, p=0.003) were significantly shorter in colon cancer patients with vs. without mucinous carcinoma despite their higher likelihood of receiving chemotherapy (89.6 vs. 71.9%, p=0.009). Multivariate analysis revealed presence of perineural invasion (HR 1.865, p=0.002), extranodal invasion (HR 1.869, p=0.009), T4 stage (HR 1.617, p=0.019), and M1 stage tumors (HR 3.643, p<0.001) but not mucinous carcinoma to significantly predict poor survival in CRC patients.*
CONCLUSION: *In conclusion, our findings indicate colorectal tumors with mucinous carcinoma histology to have a more aggressive tumor characteristics and advanced disease stage on admission in CRC patients as well as shorter OS time and DFS time specifically in colon cancer patients despite receiving chemotherapy.*

KEY WORDS: Chemotherapy, Colorectal cancer, Long-term follow up, Mucinous histology, Survival

Introduction

Colorectal cancer (CRC) is the most common digestive system malignancy and the fourth most common cause of cancer-associated mortalities worldwide[1-3]. In accordance with improved individualized treatments, histological and genetic subtype classification become important for identification of potential candidates of adjuvant systemic therapy along with better judgment of prognosis and survival[4,5].

The mucinous adenocarcinoma is a rare specific histological subtype of CRC defined by the World Health Organization (WHO) as mucinous components that comprise ≥50% of the tumor volume[6]. Considering mucinous adenocarcinoma in the diagnostic work-up of CRC patients seems important in terms of its association with poorer survival and lower chemotherapy response when compared to non-mucinous adenocarcinoma as reported in some studies [7-10].

Pervenuto in Redazione Giugno 2020. Accettato per la pubblicazione Luglio 2020
Correspondence to: Volkan Tümay, Department of General Surgery, Acibadem Bursa Hospital, Fatih Sultan Mehmet Bulvari, Sumer Sk. No:1, 16110 Nilufer, Bursa, Turkey (e-mail: vtumay72@gmail.com)

However, the clinical relevance of mucinous carcinoma in CRC patients remains uncertain and there is considerable debate regarding its prognostic significance due to conflicting data on its association with the oncological outcome in terms of presence and nature of such effect (independent or dependent on site, stage or genetic features) and potential differences in colon and rectal cancer patients as well as its relation to chemo-radiotheraphy (CRT) response [5,11-21].
This retrospective long-term follow up study was therefore designed to evaluate the association of mucinous histology of tumor with clinicopathological characteristics and oncological outcome in CRC patients.

Materials and methods

Study Population

A total of 372 patients (mean(SD) age: 61.6(14.0) years, 63.2% were males) who underwent resection surgery due to CRC between March 2006 and March 2019 were included in this retrospective study. Overall, 469 patients were operated due to CRC within the study period in our hospital, while the study population subjected to final analysis was composed of 372 patients with exclusion of 25 patients with synchronous disease due to non-standardized treatment, 9 rectal cancer patients with complete pathological response due to unknown histologic features after neoadjuvant chemo-radiotherapy (CRT) and 63 patients due to lost to follow up. Patients were divided into two groups according to degree of mucinous component including mucinous carcinoma group (n=48, ≥50% mucinous component) and non-mucinous carcinoma (n=324, <50% mucinous component) group.
The study protocol was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee (Date of Approval: 11/02/2020; Reference number/Protocol No: 2020-02/7). Due to the retrospective non-experimental nature of the study, informed consent was not required.

Study Parameters

Data on patient demographics (age, gender), American Society of Anesthesiologists (ASA) status, diagnosis (colon, rectum), family history for CRC, tumor characteristics including localization, TNM stage, diameter, grade, presence of micro satellite instability (MSI), signet-ring cell component, extra-nodal, perineural or lymphovascular invasion, treatment characteristics (surgery, CRT), metastasis (on admission or follow up), recurrence rate, disease free survival (DFS) time and overall survival (OS) time were recorded in each patient and evaluated in mucinous carcinoma and non-mucinous carcinoma groups. Tumor stages were classified based on clinical stage in rectal cancer patients initiated with neoadjuvant therapy, while based on pathological findings in other rectal and colon cancer patients [22]. The tumors extending up to the splenic flexure were considered as right-sided colon tumors and those extending from splenic flexure to recto-sigmoid juncture were considered as left-sided colon tumors.

Histopathological evaluations

Tissue specimens, stained with hematoxylin and eosin, were evaluated in terms of the predominant histological type, the presence or absence of mucinous component on the largest cross sections of tumors, and the ratio of the area of mucinous component to that of the entire tumor (mucinous component ratio). Based on WHO definition, tumors with mucinous component accounting for ≥ 50 % of the cancer tissue were classified as mucinous carcinoma, while those with <50% mucinous component were considered as non-mucinous carcinoma.[6] MSI status was assessed by immunohistochemical stains for mismatch repair (MMR) proteins and MSI was considered to be evident in cases of high instability. Signet-ring cell components were evaluated and defined as signet-ring cell carcinoma by the presence of ≥50% of tumor cells. Histological grade was evaluated based on two-tier grading scheme (low, high) [23].

Follow Up Period

In accordance with National Comprehensive Cancer Network (NCCN) guidelines, patients were followed up in 3-month intervals in the first 2 years and in 6 months intervals in the following 3 years[24,25]. Biomarker levels were analyzed at each visit, while thoraco-abdominopelvic computerized tomography and colonoscopy were performed once yearly. Positron emission tomography (PET-CT) was optional. For the purpose of this study, patients or relatives were contacted to confirm survival status. Peritoneal seeding was diagnosed intraoperatively and postoperative recurrence as peritoneal carcinomatosis was diagnosed based on findings during reoperation or radiologic imaging modalities indicating the presence of abnormal intraperitoneal nodules or peritoneal thickening. Liver metastases were categorized as isolated hepatic (only liver), extrahepatic (only non-liver) and mixed (concomitant liver and non-liver) metastases.

Chemotherapy And Radiotherapy

Stage II high risk patients (high grade tumors, lymphovascular and perineural invasion, positive surgical mar-

gin, perforation, < 12 lymph node yield) and stage III patients received standard adjuvant chemotherapy (FOLFOX, XELOX regimes), while oxaliplatin- and irinotecan –based palliative chemotherapy was applied in stage IV patients. Patients with locally advanced (cT3, cT4 and /or node positive) rectal cancer with middle-distal location received CRT, while short course neoadjuvant radiotherapy was applied in cT3N0 cases depending on tumor location and magnetic resonance imaging (MRI) findings. Patients who received CRT were categorized as "chemotherapy-administered" patients. Patients were operated average 10 weeks after the neoadjuvant radiotherapy.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY). The normality of continuous variables was investigated by Shapiro-Wilk's test. For comparison of two normally distributed groups Student t test was used. Non-parametric statistical methods were used for values with skewed distribution. For comparison of two non-normally distributed groups Mann Whitney U test was used. The primary outcome was OS, defined as the interval between the date of surgical treatment and either the date of death or the censoring date for follow-up (31 December 2019), whichever was earlier. Death from any cause was regarded as an event and the subjects who were still alive at the end of follow-up were censored. Survival curves were generated according to the Kaplan–Meier method and survival distributions were compared with the use of the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) for multivariate analyses were computed using the Cox proportional hazards regression models. All tests of significance were two tailed; differences at p values of ≤0.05 were considered to be significant. Data were expressed as mean ± standard deviation (SD), median (minimum-maximum), and percent (%) where appropriate.

Results

Patient Demographics and Clinicopathological Characteristics

A total of 372 patients (mean(SD) age: 61.6(14.0) years, 63.2% were males) were included in this study. Mucinous adenocarcinoma was evident in 12.9% of CRC patients. Colon cancer was the diagnosis in 58.8% of patients. Family history for CRC was evident in 13.2% of patients. Right hemicolectomy (n=114; 30.6%) and low anterior resection (n=112; 30.1%) were the most common surgical operations. After a mean follow-up for 60.6 (SD 45.3) months, 63.7% of the patients were alive. No significant difference was noted between mucinous and non-mucinous carcinoma groups in terms of demographic and clinical characteristics (Table I).

Tumor Characteristics on Admission in Study Groups

In the mucinous carcinoma group as compared with non-mucinous carcinoma group, the percentage of patients with T4 stage tumors were significantly higher (43.8 vs. 25.6%, p=0.036), tumor diameter was larger (5.3± 2.7 vs. 4.2± 2.0 cm, p=0.002) and the presence of Signet ring cell (8.3 vs. 2.8%, p=0.050), MSI (10.4 vs. 3.7%, p=0.038), high grade tumor (45.8 vs. 23.8%, p=0.001) and extranodal invasion (50.0 vs. 32.7%, p=0.019) were significantly more common (Table II)

Table I - *Patient demographics and clinicopathological characteristics*

		Total (n=372)	Mucinous carcinoma (n=48)	Non-mucinous carcinoma (n=324)	p value
Gender (male), n(%)		235(63.2)	34(70.8)	201(62.0)	0.238[1]
Age (year), mean±SD		61.6±14.0	58.8±17.3	62.0±13.4	0.135[2]
ASA status, n(%)	1	195(52.4)	30(62.5)	165(50.9)	0.337[1]
	2	111(29.8)	9(18.75)	102(31.5)	
	3	52(14.0)	7(14.6)	45(13.9)	
	4	14(3.8)	2(4.2)	12(3.7)	
Tumor localization, n(%)	right colon	114(30.6)	20(41.7)	94(29.0)	0.202[1]
	left colon	105(28.2)	12(25.0)	93(28.7)	
	rectum	153(41.1)	16(33.3)	137(42.3)	
Familial CRC history		49(13.2)	5(10.4)	44(13.6)	0.545[1]
Mucinous component (%), mean±SD		14.3±27.5	80.2±14.1	4.5±9.8	0.000[2]
Follow-up (month), mean±SD		60.6±45.3	60.2±45.7	57.5±48.2	0.660[3]
Survivors, n(%)		237(63.7)	26(54.2)	211(65.1)	0.141[1]

ASA: American Society of Anesthesiologists; CRC: colorectal cancer
[1]Pearson Chi-Square test; [2] Mann-Whitney U test; [3]Student t-test

Table II - *Tumor characteristics on admission in study groups*

		Total (n=372)	Mucinous carcinoma (n=48)	Non-mucinous carcinoma (n=324)	p value[1]
T Stage, n(%)	0	4(1.1)	0(0)	4(1.2)	0.036
	1	20(5.4)	0(0)	20(6.2)	
	2	35(9.4)	2(4.2)	33(10.2)	
	3	209(56.2)	25(52.1)	184(56.8)	
	4	104(28)	21(43.8)	83(25.6)	
N Stage, , n(%)	0	170(45.7)	16(33.3)	154(47.5)	0.129
	1	101(27.2)	14(29.2)	87(26.9)	
	2	101(27.2)	18(37.5)	83(25.6)	
TNM Stage, , n(%)	1	44(11.8)	1(2.1)	43(13.3)	0.109
	2	120(32.3)	17(35.4)	103(31.8)	
	3	129(34.7)	21(43.8)	108(33.3)	
	4	79(21.2)	9(18.8)	70(21.6)	
Diameter (cm), mean±SD		4.3± 2.1	5.3± 2.7	4.2± 2.0	0.002[2]
Signet ring cell, n(%)		13(3.5)	4(8.3)	9(2.8)	0.050
Micro satellite instability, n(%)		17(4.7)	5(10.4)	12(3.7)	0.038
Grade (high), n(%)		99(26.6)	22(45.8)	77(23.8)	0.001
Extranodal invasion, n(%)		130(34.9)	24(50.0)	106(32.7)	0.019
Perineural invasion, n(%)		192(51.6)	24(50.0)	168(51.9)	0.811
Lymphatic invasion, n(%)		115(30.9)	18(37.5)	97(29.9)	0.290
Venous invasion, n(%)	extramural	30(8.1)	4(8.3)	26(8)	0.942
	intramural	40(10.8)	6(12.5)	34(10.5)	0.675

[1]χ^2 test; [2]Mann-Whitney U test

Table III - *METASTASIS and recurrence in study groups*

	Total (n=372)	Mucinous carcinoma (n=48)	Non-mucinous carcinoma (n=324)	p value
Admission, n(%)				
Isolated hepatic metastasis	59(15.9)	5(10.4)	54(16.7)	0.269
Isolated extrahepatic metastasis	10(2.7)	5(10.4)	5(1.5)	<0.001
Mixed metastasis	13(3.5)	1(2.1)	12(3.7)	0.568
M1 Stage	82(22.0)	11(22.9)	71(21.9)	0.876
Follow-up, n(%)				
Isolated hepatic metastasis	8(2.8)	3(8.1)	5(2.0)	0.033
Isolated extrahepatic metastasis	24(8.3)	3(8.1)	21(8.3)	0.300
Mixed metastasis	12(4.1)	3(8.1)	9(3.6)	0.639
Systemic recurrence	44(15.2)	9(24.3)	35(13.8)	0.097
Local recurrence	6(2.1)	2(5.4)	4(1.6)	0.120

χ^2 test

Metastasis and Recurrence in Study Groups

In patients with mucinous carcinoma as compared to those with non-mucinous carcinoma, presence of isolated extrahepatic metastasis (10.4 vs. 1.5%, p<0.001) on admission and presence of isolated hepatic metastasis on follow up (8.1 vs. 2.0%, p=0.033) were significantly more common (Table III).

Systemic and local recurrence rates were 15.2% and 2.1% in the overall study population, with no significant difference between mucinous carcinoma and non-mucinous carcinoma groups (Table III).

Chemotherapy and Radiotherapy in Study Groups

Patients with mucinous carcinoma as compared to those with non-mucinous carcinoma had higher likelihood of receiving chemotherapy overall (89.6 vs. 71.9%, p=0.009), particularly for colon cancer (84.4 vs. 64.7%, p=0.028), while no significant difference was noted between study groups in terms of radiotherapy administration (Table V).

TABLE IV - *Chemotherapy and radiotherapy in study groups*

Chemotherapy		**Total**	**Mucinous carcinoma**	**Non-mucinous carcinoma**	**p value**
Overall	n	372	48	324	
	Received CT	276(74.2)	43(89.6)	233(71.9)	0.009
	No CT	96(25.8)	5(10.4)	91(28.1)	
Colon cancer patients	n	219	32	187	
	Received CT	148(67.6)	27(84.4)	121(64.7)	0.028
	No CT	71(32.4)	5(15.6)	66(35.3)	
Rectal cancer patients	n	153	16	137	
	Received CT	128(83.7)	16(100)	112(81.8)	0.062
	No CT	25(16.3)	0(0)	25(18.2)	
Radiotherapy		**Total (n=153)**	**Mucinous carcinoma (n=16)**	**Non-mucinous carcinoma (n=137)**	**p value**
Short neoadjuvant		18 (11.8)	2 (12.5)	16 (11.7)	0.974
Long neoadjuvant		45 (29.4)	4 (25)	41 (29.9)	
Adjuvant		39 (25.5)	4 (25)	35 (25.5)	
None		51 (33.3)	6 (37.5)	45 (32.8)	

CT: chemotherapy, $\chi 2$ test

TABLE V - *OS and DFS times in study groups*

Mean±SD (95% CI LB-UB)	**Total (n=372)**	**Mucinous carcinoma (n=48)**	**Non-mucinous carcinoma (n=324)**	**p value**
OS time (month)				
Total	106.6±3.9 (99-114.2)	90±10.1 (70.2-109.7)	108.7±4.2 (100.6-116.9)	0.152
Colon cancer	106.2±5.2 (96-116.5)	75.9±13.1 (50.3-101.6)	110.8±5.6 (99.9-121.6)	0.019
Rectal cancer	105±5.6 (94.1-116)	113.6±13.6 (86.8-140.3)	104±6 (92.2-115.8)	0.566
DFS time (month)				
Total	136.5±3.8(129.1-143.9)	112±11.6(89.4-134.7)	139.2±3.9 (131.6-146.9)	0.025
Colon cancer	135±5.1 (125-145)	98.5±15.6 (67.9-129.1)	140.5±5.1 (130.5-150.5)	0.003
Rectal cancer	135.1±5.3 (124.7-145.5)	133.1±14.2 (105.3-160.9)	134.2±5.8 (122.9-145.5)	0.696

CI: confidence interval; LB: lower bound; UB: upper bound; OS: Overall survival; DFS: Disease-free survival Log rank mantel test

OS AND DFS TIMES IN STUDY GROUPS

The mean±SD OS time was 106.6±3.9 months and DFS time was 136.5±3.8 months in the overall study population, with no significant difference in OS time between mucinous carcinoma and non-mucinous carcinoma groups, whereas DFS time was significantly shorter (112±11.6 vs. 139.2±3.9 months, p=0.025) in the mucinous carcinoma group (Table V).

When the diagnosis was considered, both the OS time (75.9±13.1 vs. 110.8±5.6 months, p=0.019) and DFS time (98.5±15.6 vs. 140.5±5.1 months, p=0.003) were significantly shorter in colon cancer patients with mucinous carcinoma as compared to those with non-mucinous carcinoma, whereas in rectal cancer patients the amount of mucinous component had no significant impact on survival times (Table V).

UNIVARIATE ANALYSIS FOR RISK FACTORS FOR POOR SURVIVAL

Univariate analysis revealed larger tumor diameter (>4 cm, HR 1.471, p=0.025), high grade tumors (HR 1.911, p<0.001), presence of lymphatic invasion (HR 2.361, p<0.001), perineural invasion (HR 2.992, p<0.001), extranodal invasion (HR 3.416, p<0.001), intramural venous invasion (HR 2.671, p<0.001), signet-ring cell carcinoma (HR 3.489, p<0.001), T4 stage (HR 2.691, p<0.001), N 1-2 stage (HR 3.516, p<0.001) and M1 stage on admission (HR 5.196, p<0.001) to be significantly associated with poor survival in overall study population (Table VI).

Presence of mucinous carcinoma was associated with poor survival (HR 1.919, p=0.021) only in the colon cancer patients, while higher tumor grade (HR 2.140,

TABLE VI - *Univariate analysis for risk factors for poor survival*

Variables	Total (n=372) HR	95% CI(LB-UB)	p	Colon cancer (n=219) HR	95% CI(LB-UB)	p	Rectal cancer (n=153) HR	95% CI(LB-UB)	p
Age (>60 years vs. 60 years)	1.040	0.740-1.461	0.823	0.744	0.470-1.178	0.208	1.548	0.924-2.594	0.097
Gender (male vs. female)	1.425	0.992-2.048	0.055	1.408	0.885-2.241	0.149	1.498	0.831-2.698	0.179
Diagnosis (colon vs. rectum)	1.041	0.740-1.464	0.818	N/A	N/A	N/A	N/A	N/A	N/A
Diameter (>4 cm vs. 4 cm)	1.471	1.049-2.064	0.025	1.366	0.868-2.150	0.177	1.616	0.960-2.720	0.071
MSI (present vs. absent)	0.578	0.142-2.355	0.445	0.562	0.136-2.315	0.425	0.049	0.136-2.315	0.818
Grade (high vs. low)	1.911	1.347-2.711	<0.001	2.140	1.347-3.400	0.001	1.660	0.971-2.836	0.064
Lymphatic invasion (present vs. absent)	2.361	1.658-3.364	<0.001	3.040	1.923-4.805	<0.001	1.578	0.854-2.915	0.145
Perineural invasion (present vs. absent)	2.992	2.084-4.296	<0.001	3.520	2.185-5.672	<0.001	2.440	1.397-4.263	0.002
Extranodal invasion (present vs. absent)	3.416	2.425-4.814	<0.001	4.190	2.647-6.634	<0.001	2.652	1.578-4.458	<0.001
Venous invasion (present vs. absent)	2.671	1.816-3.929	<0.001	3.370	2.052-5.537	<0.001	1.915	1.024-3.581	0.042
Histology (mucinous vs. non-mucinous)	1.394	0.883-2.202	0.154	1.919	1.103-3.337	0.021	0.781	0.334-1.822	0.567
Signet-ring cell (present vs. absent)	3.489	1.761-6.915	<0.001	3.631	1.308-10.083	0.013	3.646	1.431-9.293	0.007
T Stage (T4 vs. other T stages)	2.691	1.904-3.803	<0.001	2.858	1.814-4.504	<0.001	3.061	1.644-5.699	<0.001
N Stage (N(+) vs. N0)	3.516	2.379-5.197	<0.001	4.237	2.462-7.290	<0.001	2.809	1.592-4.956	<0.001
M Stage admission (M1 vs. M0)	5.196	3.656-7.385	<0.001	5.583	3.511-8.876	<0.001	4.569	2.652-7.870	<0.001

Cox proportional hazards model. Univariate analysis; HR: hazard ratio; CI: Confidence interval; LB: Lower bound; UB: Upper bound; MSI: Micro satellite instability; N/A: Not applicable

TABLE VII - *Cox regression multivariate analysis for determinants of poor survival*

Variables	Total (n=372) HR	95,0% CI	p	Colon cancer (n=219) HR	95,0% CI	p	Rectal cancer (n=153) HR	95,0% CI	p
Diameter (>4 cm vs. 4 cm)	1.116	(0.767-1.623)	0.567	0.922	(0.541-1.569)	0.764	1.544	(0.855-2.791)	0.150
Grade (high vs. low)	1.330	(0.882-2.007)	0.174	1.528	(0.848-2.755)	0.158	1.035	(0.544-1.970)	0.916
Lymphatic invasion (present vs. absent)	0.983	(0.642-1.507)	0.938	1.051	(0.570-1.936)	0.874	0.705	(0.343-1.448)	0.341
Perineural invasion (present vs. absent)	1.865	(1.248-2.786)	0.002	2.191	(1.263-3.802)	0.005	1.273	(0.654-2.477)	0.477
Extranodal invasion (present vs. absent)	1.869	(1.172-2.981)	0.009	2.254	(1.355-3.747)	<0.001	2.622	(1.190-5.780)	0.017
Venous invasion (present vs. absent)	1.031	(0.635-1.674)	0.902	1.880	(0.987-3.581)	0.055	1.393	(0.660-2.940)	0.384
Histology (mucinous vs. non-mucinous)	1.020	(0.627-1.659)	0.936	1.552	(0.830-2.902)	0.168	0.474	(0.181-1.241)	0.128
Signet-ring cell carcinoma (present vs. absent)	2.041	(0.968-4.303)	0.061	1.306	(0.433-3.941)	0.636	3.229	(1.028-10.144)	0.045
T Stage (T4 vs. other T stages)	1.617	(1.084-2.413)	0.019	1.276	(0.709-2.295)	0.416	2.922	(1.405-6.078)	0.004
N Stage (N(+) vs. N0)	1.083	(0.610-1.922)	0.786	1.169	(0.526-2.601)	0.701	0.930	(0.389-2.219)	0.869
M Stage admission (M1 vs. M0)	3.643	(2.476-5.361)	<0.001	3.191	(1.858-5.479)	<0.001	5.295	(2.841-9.868)	<0.001

Cox regression multivariate analysis. HR: hazard ratio; CI: Confidence interval; LB: Lower bound; UB: Upper bound

p=0.001) and presence of lymphatic invasion (HR 3.040, p<0.001) were also associated with poor survival only in colon cancer patients (Table VI).

Cox-Regression Multivariate Analysis for Risk Factors for Poor Survival

Multivariate analysis revealed presence of perineural invasion (HR 1.865, 95% CI 1.248 to 2.786, p=0.002), extranodal invasion (HR 1.869, 95% CI 1.172 to 2.981, p=0.009), T4 stage (HR 1.617, 95% CI 1.084 to 2.413, p=0.019), and M1 stage tumors (HR 3.643, 95% CI 2.476 to 5.631, p<0.001) on admission to significantly predict poorer survival in the overall study population (Table VII).

Presence of perineural invasion (HR 2.191, 95% CI 1.263 to 3.802, p=0.005) significantly predicted the poor survival only in colon cancer patients, while presence of signet-ring cell carcinoma (HR3.229, 95% CI 1.028 to 10.144, p=0.045) and T4 stage tumors (HR 2.922, 95% CI 1.405 to 6.078, p=0.004) significantly predicted the poor survival only in rectal cancer patients. In colon cancer and rectal cancer patients, extranodal invasion (HR 2.254, p<0.001 and HR 2.622, p=0.017, respectively) and M1 stage tumor on admission (HR 2.254, p<0.001 and HR 2.622, p=0.017, respectively) significantly predicted the poor survival (Table VII).

No significant impact of mucinous histology was noted on survival in the multivariate analysis, regardless of the diagnosis (Table VII).

Discussion

Our findings revealed significant association of mucinous carcinoma with aggressive tumor characteristics (large tumor diameter, T4 stage, high grade tumor, extranodal invasion and signet ring cell component) in CRC patients along with higher likelihood of MSI and isolated extrahepatic (on admission) or hepatic (on follow up) metastases. Presence of mucinous carcinoma was also associated with lower OS and DFS times in colon cancer patients, despite higher likelihood of receiving chemotherapy. However, while mucinous carcinoma was associated with poor survival in colon cancer patients in the univariate analysis, multivariate analysis did not reveal a significant impact of mucinous histology on survival in CRC patients. The predictors of poor survival in multivariate analysis were perineural invasion in colon cancer patients, signet-ring cell carcinoma and T4 stage tumors in rectal cancer patients, while extranodal invasion and M1 stage tumor in both colon cancer and rectal cancer patients.

Our findings revealed the mucinous carcinoma in 12.9% of patients with CRC, supporting the consideration of 1.6–25.4% of all CRC cases to have mucinous carcinoma [13,26]. Albeit not significant, there was a tendency for higher likelihood of right-sided vs. left-sided colon tumors to have mucinous component >50% in our cohort. Being consistent with past reports indicated mucinous carcinoma to develop more often in the right side of the colon [13,27], this finding seems also important given the likelihood of right-sided colon cancer not only to have a more mucinous type cancer but also a high grade tumor and advanced TNM stage [28].

In the current study, presence vs. absence of mucinous carcinoma was associated with larger tumor diameter, more aggressive tumor pathology (signet ring cell component, extranodal invasion and high grade tumors) and advanced disease stage (T4 stage and extrahepatic metastasis) and higher incidence of MSI in CRC patients. This supports the published data on association of colorectal tumors with vs. without mucinous component (over 50%) with a larger tumor size [13,27], higher tumor grade and advanced T-stage [16], higher likelihood of MSI[29] and higher rate of perineural infiltration and more advanced cancer[30] in recent studies with CRC patients. Notably, absence of signet ring cells and mucinous component but the presence of MSI is considered to be favorable prognostic factors in CRC patients with peritoneal carcinomatosis [31], while even a minor signet ring cell component in CRCs was associated with higher cancer-specific mortality [32]. In our cohort, while presence of signet ring cell component was associated with poor survival in CRC patients regardless of the diagnosis, multivariate analysis revealed its predictive role only in rectal cancer patients.

Albeit not confirmed in multivariate analysis, OS time and DFS time were significantly shorter in colon cancer patients with vs. without mucinous carcinoma in our study, despite their higher likelihood of receiving chemotherapy. Similar to our findings, in a past study among CRC patients, authors reported poorer OS in CRC patients with mucinous vs. non-mucinous carcinoma in the combined analysis but no impact of mucinous histology on survival in the multivariable Cox regression analysis [33]. Indeed, our findings indicated that presence of mucinous carcinoma was significantly associated with all factors determined to be significant predictors of poor survival in the multivariate analysis, including perineural invasion (in colon cancer patients), signet-ring cell carcinoma and T4 stage tumors (in rectal cancer patients) and extranodal invasion and M1 stage tumor on admission (in both colon cancer and rectal cancer patients). Accordingly, our findings seem to support the role of mucinous carcinoma in survival outcome among CRC patients due to its association with an advanced disease stage and high grade tumor, subsequently resulting in poorer survival, rather than as an independent poor prognostic factor per se [33]. Higher likelihood of having T4 stage disease on admission by our CRC patients with mucinous carcinoma seems also notable in this regard, given that the poor prognostic impact of mucinous histology is considered to emerge with increasing stage of disease and to turn into significantly poorer survival in stage IV colon cancer [33].

Nonetheless, it should be noted that mucinous vs. non-mucinous histology was also reported to be associated with a shorter long-term OS, regardless of the chemotherapy regimen, metastasis or tumor site subgroups [7-10, 34-38]. However, the published data remain inconsistent regarding the consideration of the histological subtype of the tumor as a negative prognostic factor [39], as some studies reported no association of mucinous carcinoma with an increased risk of advanced stage disease[13, 27] or high grade tumor [40] as well as similar oncological outcomes in colon cancer patients with mucinous vs. other histological tumor types [11-13,15].

Although the likelihood of mucinous carcinoma to exhibit a different outcome depending on tumor position was reported in a past study with CRC patients [41], authors noted the association of mucinous carcinoma with no survival impact, improved survival and poor survival in patients with left-sided colon tumors, right-sided colon tumors and rectal tumors, respectively [41]. In contrast, our findings revealed association of mucinous carcinoma with poor survival only in colon cancer patients. In another study, mucinous histology was reported to be associated with poor survival in left-sided CRCs and with improved survival in right-sided tumors[42]. Likewise, mucinous component was reported to be a strong independent poor prognostic factor for survival in a recent study among 301 patients with stage I-III colorectal cancer.[43] In a recent meta-analysis of eight studies in 1724 rectal cancer patients, mucinous rectal carcinoma was concluded to be a biomarker for poor response to pre-

operative CRT and an adverse prognostic indicator,[21] while rectal mucinous carcinoma was also reported to be not an independent prognostic factor in another study following the adjustment by pT category [44].

Notably, the difference in molecular subtypes between proximal and distal mucinous carcinomas has been suggested to variably affect the oncological outcome with better outcome in hyper-mutated tumors with MSI prevail in the right colon mucinous carcinomas than microsatellite-stable, BRAF-mutated left-sided mucinous carcinomas [20,45].

Patients with mucinous adenocarcinoma continue to receive both neo-adjuvant and adjuvant therapy, despite the likelihood of poor response to neoadjuvant chemoradiotherapy (CRT) in terms of down-staging and tumor regression grade [34] and a limited response to adjuvant treatment and a reduced survival benefit as compared with non-mucinous tumors [8,34,35,46]. This resistance to treatment is suggested to be related to differences in molecular signature and physical properties of mucin-containing tumors resulting in unique patterns of spread, and substantially different patterns of vascularity and tumor cellularity [47]. Indeed, adjuvant trials on the impact of mucinous histology as a negative prognostic variable revealed different results [39]. Two studies reported no statistically significant 5-year overall survival differences for patients with mucinous and non-mucinous colon cancer when treated with adjuvant chemotherapy [45,48], while association of mucinous adenocarcinoma with a more unfavorable prognosis was confirmed by two large studies in colon cancer patients receiving post-resection adjuvant chemotherapy [9,10]. Moreover, some authors recommended administration of adjuvant chemotherapy in mucinous rectal carcinoma patients even in the absence of other high-risk features for poor outcomes, given its independent association with improved survival [46].

Moreover, mucinous subtype is considered to have a particular prognostic impact in the subpopulation of patients with stage II poorly differentiated colon cancer by causing a higher prevalence of peritoneal and local recurrence and poorer long-term survival[30]. Hence, authors concluded mucinous adenocarcinoma to be a high-risk factor for stage II CRC and considered adjuvant chemotherapy to be routinely recommended for these patients [30]. In addition, improved survival rates were also reported in patients with stage II or stage III mucinous adenocarcinoma of the colon who are given adjuvant chemotherapy compared to patients not given chemotherapy [49]. In a recent study with high risk stage II grade 3 CRC patients, no associations were detected between mucinous histology and tumor characteristics, except for a higher proportion of patients with right-sided tumor in the subgroup of subjects with mucinous carcinoma, while authors suggested that patients with mucinous carcinoma histology to need adjuvant chemotherapy for a duration of 6 months rather than 3 months [39]. Our findings emphasize a change towards an advanced disease pattern in relation to mucinous component in CRC patients along with lower OS and DFS times in colon cancer patients with mucinous carcinoma despite their higher likelihood of receiving chemotherapy. Hence, the role of considering mucinous histology as a relevant factor for identifying candidate patients to be treated with chemotherapy should be further justified in larger scale prospective studies [39].

Notably, our findings revealed no significant impact of mucinous cancer histology on local or systemic recurrence rates. In fact, in a past study among rectal cancer patients, authors also reported the association of mucinous rectal cancer with shorter survival time despite no significant differences in local recurrence between mucinous and non-mucinous cancers [44]. The authors suggested the potential role of bias due to the pattern of metastatic disease in mucinous rectal cancer recurrence (predominantly peritoneal disease and distant lymph node spread) which is often not treated as aggressively with as metastases in the liver or lung [44]. Patterns of metastasis in mucinous CRC in our study seem notable in this regard, with higher rate of isolated extrahepatic metastasis on admission and that of isolated hepatic metastasis on follow up.

Certain limitations to this study should be considered. First, due to retrospective single center design, establishing the temporality between cause and effect as well as generalizing our findings to overall CRC population seem not possible. Second, the imbalance in the number of patients between the two groups of mucinous histology as well as the low sample size may have resulted in a limited statistical power and possible random conclusion. Nevertheless, despite these certain limitations, given the inconsistency of data available on prognostic role of mucinous histology in CRC patients, our findings represent a valuable contribution to the literature.

Conclusions

In conclusion, our findings indicate colorectal tumors with mucinous carcinoma histology to have a more aggressive tumor characteristics and advanced disease stage on admission and to be associated with shorter OS and DFS times specifically in colon cancer patients despite chemotherapy. However, multivariate analysis revealed that perineural invasion, extranodal invasion, signet-ring cell component, T4 stage and M1 stage tumors rather than mucinous carcinoma histology as the factors that significant predict poor survival in CRC patients. However, given the association of all these factors also with presence of mucinous carcinoma, our findings seem to indicate the association of mucinous carcinoma with poor oncological outcome in CRC patients primarily due to its relation with aggressive tumor pathology and advance disease rather than as an independent poor prognostic factor. Nonetheless, the clini-

cal relevance of mucinous histology in prognosis of CRC should be further addressed by larger scale prospective studies in the context of both early and advanced stage disease, specific patterns of metastasis, colon cancer laterality and provision of postoperative adjuvant chemotherapy.

Acknowledgments

The authors would like to thank Prof. Dr. Abdullah Zorluoglu (Acibadem University, Istanbul, Turkey) for his most valuable contributions

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: *Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries.* CA Cancer J Clin, 2018; 68:394-424.

2. Sinicrope FA, Okamoto K, Kasi PM, Kawakami H: *Molecular biomarkers in the personalized treatment of colorectal cancer.* Clin Gastroenterol Hepatol, 2016; 14:651-658.

3. Luo C, Cen S, Ding G, Wu W: *Mucinous colorectal adenocarcinoma: clinical pathology and treatment options.* Cancer Commun (Lond), 2019; 39:13.

4. Siegel RL, Miller KD, Jemal A: *Cancer statistics 2016.* CA Cancer J Clin, 2016; 66:7-30.

5. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: *Global cancer statistics, 2012.* CA Cancer J Clin, 2015; 65:87-108.

6. Bosman FT, Carneiro F, Hruban RH, Theise ND: *WHO classification of tumours of the digestive system.* 2010; No.Ed. 4

7. Dai D, Zhou B, Zhong Y, Jin H, Wang X: *Survival of patients with resected primary colorectal mucinous adenocarcinoma: A competing risk nomogram analysis.* Oncol Lett, 2019; 18:6594-6604.

8. Catalano V, Loupakis F, Graziano F, et al: *Mucinous histology predicts for poor response rate and overall survival of patients with colorectal cancer and treated with first-line oxaliplatin- and/or irinotecan-based chemotherapy.* Br J Cancer, 2009; 100:881-887.

9. Bagante F, Spolverato G, Beal E, et al.: *Impact of histological subtype on the prognosis of patients undergoing surgery for colon cancer.* J Surg Oncol, 2018; 117:1355-363.

10. Kanda M, Oba K, Aoyama T, et al: *Japanese Foundation for Multidisciplinary Treatment of production on survival in colorectal cancer: A case-control study.* World J Gastroenterol. 2008; 14:6981-6985.

11. Consorti F, Lorenzotti A, Midiri G, Di Paola M: *Prognostic significance of mucinous carcinoma of colon and rectum: A prospective case-control study.* J Surg Oncol, 2000; 73:70-74. *Cancer. Clinical signatures of mucinous and poorly differentiated subtypes of colorectal adenocarcinomas by a propensity score analysis of an independent patient database from three phase III trials.* Dis Colon Rectum, 2018; 61:461-471.

12. Farhat MH, Barada KA, Tawil AN, Itani DM, Hatoum HA, Shamseddine AI: *Effect of mucin production on survival in colorectal cancer: A case-control study.* World J Gastroenterol, 2008; 14(45): 6981-985.

13. Maeda Y, Sadahiro S, Suzuki T, Haruki Y, Nakamura N: *Significance of the mucinous component in the histopathological classification of colon cancer.* Surg Today, 2016; 46:303-308.

14. Hogan J, Burke JP, Samaha G, et al: *Overall survival is improved in mucinous adenocarcinoma of the colon.* Int J Colorectal Dis, 2014; 29:563-69.

15. Kang H, O'Connell JB, Maggard MA, Sack J, Ko CY: *A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum.* Dis Colon Rectum, 2005; 48:1161-168.

16. Warschkow R, Tarantino I, Huttner FJ, et al: *Predictive value of mucinous histology in colon cancer: A population-based, propensity score matched analysis.* Br J Cancer, 2016; 114:1027-1032.

17. Hyngstrom JR, Hu CY, Xing Y, et al: *Clinicopathology and outcomes for mucinous and signet ring colorectal adenocarcinoma: Analysis from the National Cancer Data Base.* Ann Surg Oncol, 2012; 19:2814-2821.

18. Kim SH, Shin SJ, Lee KY, et al: *Prognostic value of mucinous histology depends on microsatellite instability status in patients with stage III colon cancer treated with adjuvant FOLFOX chemotherapy: a retrospective cohort study.* Ann Surg Oncol, 2013; 20:3407-3413.

19. Sarli L, Bottarelli L, Azzoni C, et al: *Two subtypes of mucinous adenocarcinoma of the colorectum: clinicopathological and genetic features.* Ann Surg Oncol, 2008; 15:1429-439.

20. Negri F, De Giorgi A, Gilli A, et al: *Impact of laterality and mucinous histology on relapse-free and overall survival in a registry-based colon cancer series.* Sci Rep, 2019; 9:3668.

21. McCawley N, Clancy C, O'Neill BD, Deasy J, McNamara DA, Burke JP: *Mucinous rectal adenocarcinoma is associated with a poor response to neoadjuvant chemoradiotherapy: a systematic review and meta-analysis.* Dis Colon Rectum, 2016; 59:1200-1208.

22. Amin M, Greene FL, Edge SB, et al: *The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population? based to a more "personalized" approach to cancer staging.* CA Cancer J Clin, 2017; 67:93-99.

23. Compton CC: *Updated protocol for the examination of specimens from patients with carcinomas of the colon and rectum, excluding carcinoid tumors, lymphomas, sarcomas, and tumors of the vermiform appendix: a basis for checklists. Cancer Committee.* Arch Pathol Lab Med, 2000; 124:1016-1025.

24. N*CCN Clinical Practice Guidelines in Oncology*. Colon Cancer version 2.2020. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf. Accessed 3 March 2020

25. *NCCN Clinical Practice Guidelines in Oncology*. Rectal Cancer version 2.2020. https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf. Accessed 3 March 2020

26. Hugen N, van Beek JJ, de Wilt JH, Nagtegaal ID: *Insight into mucinous colorectal carcinoma: Clues from etiology.* Ann Surg Oncol, 2014; 21:2963-2970.

27. Nozoe T, Anai H, Nasu S, Sugimachi K: *Clinicopathological characteristics of mucinous carcinoma of the colon and rectum.* J Surg Oncol, 2000; 75:103-107.

28. Hsu YL, Lin CC, Jiang JK, et al: *Clinicopathological and molecular differences in colorectal cancer according to location.* Int J Biol Markers, 2019; 34:47-53.

29. Song GA, Deng G, Bell I, Kakar S, Sleisenger MH, Kim YS: *Mucinous carcinomas of the colorectum have distinct molecular genetic characteristics.* Int J Oncol, 2005; 26:745-50.

30. Wang L, Hirano Y, Heng G, et al: *Mucinous Adenocarcinoma as a High-risk Factor in Stage II Colorectal Cancer: A Propensity Score-matched Study from Japan.* Anticancer Res, 2020; 40:1651-1659.

31. Massalou D, Benizri E, Chevallier A, et al: *Peritoneal carcinomatosis of colorectal cancer: Novel clinical and molecular outcomes.* Am J Surg, 2017; 213:377-87.

32. Pozos-Ochoa LI, Lino-Silva LS, León-Takahashi AM, Salcedo-Hernández RA: *Prognosis of signet ring cell carcinoma of the colon and rectum and their distinction of mucinous adenocarcinoma with signet ring cells. a comparative study.* Pathol Oncol Res, 2018; 24:609-616.

33. Ott C, Gerken M, Hirsch D, et al: *Advanced mucinous colorectal cancer: epidemiology, prognosis and efficacy of chemotherapeutic treatment.* Digestion, 2018; 98:143-152.

34. Negri FV, Wotherspoon A, Cunningham D, Norman AR, Chong G, Ross PJ: *Mucinous histology predicts for reduced fluorouracil responsiveness and survival in advanced colorectal cancer.* Ann Oncol, 2005; 16:1305-310.

35. Verhulst J, Ferdinande L, Demetter P, Ceelen W: *Mucinous subtype as prognostic factor in colorectal cancer: A systematic review and metaanalysis.* J Clin Pathol, 2012; 65:381-88.

36. Mekenkamp LJ, Heesterbeek KJ, Koopman M, et al: *Mucinous adenocarcinomas: Poor prognosis in metastatic colorectal cancer.* Eur J Cancer, 2012; 48:501-509.

37. Maisano R, Azzarello D, Maisano M, et al: *Mucinous histology of colon cancer predicts poor outcomes with FOLFOX regimen in metastatic colon cancer.* J Chemother, 2012; 24:212-16.

38. Umpleby HC, Ranson DL, Williamson RCN: *Peculiarities of mucinous colorectal carcinoma.* Br J Surg, 1981; 72:715-18.

39. Rosati G, Galli F, Cantore M, et al: *TOSCA (Three or Six Colon Adjuvant) Investigators. Predictive impact of mucinous tumors on the clinical outcome in patients with poorly differentiated, stage II colon cancer: A TOSCA subgroup analysis.* Oncologist, 2020; 25:e928-e935.

40. Williams DS, Mouradov D, Newman MR, et al: *Tumour infiltrating lymphocyte status is superior to histological grade, DNA mismatch repair and BRAF mutation for prognosis of colorectal adenocarcinomas with mucinous differentiation.* Mod Pathol, 2020: https://doi.org/10.1038/s41379-020-0496-1. [Epub ahead of print]

41. Gao P, Song YX, Xu YY, et al: *Does the prognosis of colorectal mucinous carcinoma depend upon the primary tumour site? Results from two independent databases.* Histopathology, 2013; 63:603-615.

42. Yang J, Du XL, Li ST, et al: *Characteristics of differently located colorectal cancers support proximal and distal classification: A population-based study of 57,847 patients.* PLoS One, 2016; 11:e0167540.

43. Micu BV, Vesa ŞC, Pop TR, Micu CM: *Evaluation of prognostic factors for 5 year-survival after surgery for colorectal cancer*. Ann Ital Chir, 2020; 91:41-48.

44. Shin US, Yu CS, Kim JH, et al: *Mucinous rectal cancer: Effectiveness of preoperative chemoradiotherapy and prognosis.* Ann Surg Oncol, 2011; 18:2232-2239.

45. Catalano V, Loupakis F, Graziano F, et al: *Prognosis of mucinous histology for patients with radically resected stage II and III colon cancer.* Ann Oncol, 2012; 23:135-141.

46. Chand M, Rasheed S, Bhangu A, et al: *Adjuvant chemotherapy improves overall survival after TME surgery in mucinous carcinoma of the rectum.* Eur J Surg Oncol, 2014; 40:240-245.

47. Hugen N, Brown G, Glynne-Jones R, de Wilt JH, Nagtegaal ID: *Advances in the care of patients with mucinous colorectal cancer.* Nat Rev Clin Oncol, 2016; 13:361-369.

48. Hugen N, Verhoeven RH, Radema SA, et al: *Prognosis and value of adjuvant chemotherapy in stage III mucinous colorectal carcinoma.* Ann Oncol, 2013; 24: 2819-2824.

49. Fields AC, Lu P, Goldberg J, Irani J, Bleday R, Melnitchouk N: *The role of adjuvant chemotherapy in stage II and III mucinous colon cancer.* J Surg Oncol, 2019; 120: 1190-1200.