Original Article
Comparative analysis of colorectal mixed adenoneuroendocrine carcinoma and adenocarcinoma with neuroendocrine differentiation: a population-based study

Ling-Jun Song, Lin Yuan

Pathology Center, Shanghai General Hospital/Faculty of Basic Medicine, Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China

Abstract: Background: Colorectal mixed adenoneuroendocrine carcinoma (MANEC) and adenocarcinoma with neuroendocrine differentiation (ANED) are recognized as different tumors pathologically and clinically. In a population-based study, the clinicopathologic characteristics and treatment strategies of the two tumors were comparatively analyzed. Methods: Patients with colorectal adenocarcinoma (ADEC), neuroendocrine carcinoma (NEC), MANEC and ANED were identified diagnosis from 2010 to 2014 using the Surveillance, Epidemiology, and End Results (SEER) database. The clinicopathologic data were analyzed by Chi-square test, univariable and multivariable Cox regression. Nomogram was performed to provide a prognostic evaluation for colorectal MANEC and ANED. Results: Totally 82121 patients were recruited in this cohort. There was no difference between MANEC and ANED in clinicopathologic characteristics and prognosis (P>0.05). The survival data showed that 1-year and 3-year survival rates were 84.70% and 67.83% for ADEC, 66.83% and 51.98% for NEC, and 54.27% and 37.68% for MANEC and ANED, respectively. Stage and surgery were independent prognostic factors of colorectal MANEC/ANED. We also found that the prognosis was significantly different without vs with chemotherapy (P=0.000) in stage III colorectal MANEC/ANED; without vs with surgery (P=0.007), and without vs with chemotherapy (P=0.000) in stage IV colorectal MANEC/ANED. Radiation did nothing for improving the prognosis of colorectal MANEC/ANED in stage III and stage IV (P=0.557, 0.677). Conclusions: MANEC and ANED should be merged into the same category pathologically and clinically, and had the poorest prognosis. Stage and surgery were independent prognostic risk factors for colorectal MANEC/ANED. The prognosis of MANEC/ANED could not benefit from radiation.

Keywords: Colorectal mixed adenoendocrine carcinoma, adenocarcinoma with neuroendocrine differentiation, multivariable cox regression, predictive nomogram, treatment strategies

Introduction

According to the World Health Organization (WHO) 2010 classification, the neuroendocrine tumors of digestive system are divided into three categories as neuroendocrine tumor (NET), neuroendocrine carcinoma (NEC), and mixed adenoendocrine carcinoma (MANEC) [1]. NEC consists of small cell NEC and large cell NEC. In the colon and rectum, large cell NEC accounted for 75% of cases, whereas in the anus most are small cell NEC [2]. NEC expresses one or more neuroendocrine markers, such as CgA, Syn and CD56, PGP9.5 and GFAP. MANEC are composed of NEC and exocrine component, each representing at least 30% of

the tumor [3, 4]. NEC component expresses neuroendocrine markers, such as chromogranin, synaptophysin and CD56, but adenocarcinoma component does not (**Figure 1**). When the tumor is mainly composed of adenocarcinoma, and the NEC component presents less than 30%, it is defined as adenocarcinoma with neuroendocrine differentiation (ANED) [5]. Pathologists need to determine whether the final diagnosis is MANEC or ANED based on the proportion of NEC in mixed tumors, which composed of morphologically recognizable adenocarcinoma and NEC components.

Several reports have found that the prognosis of NEC and MANEC is poorer than adenocarci-
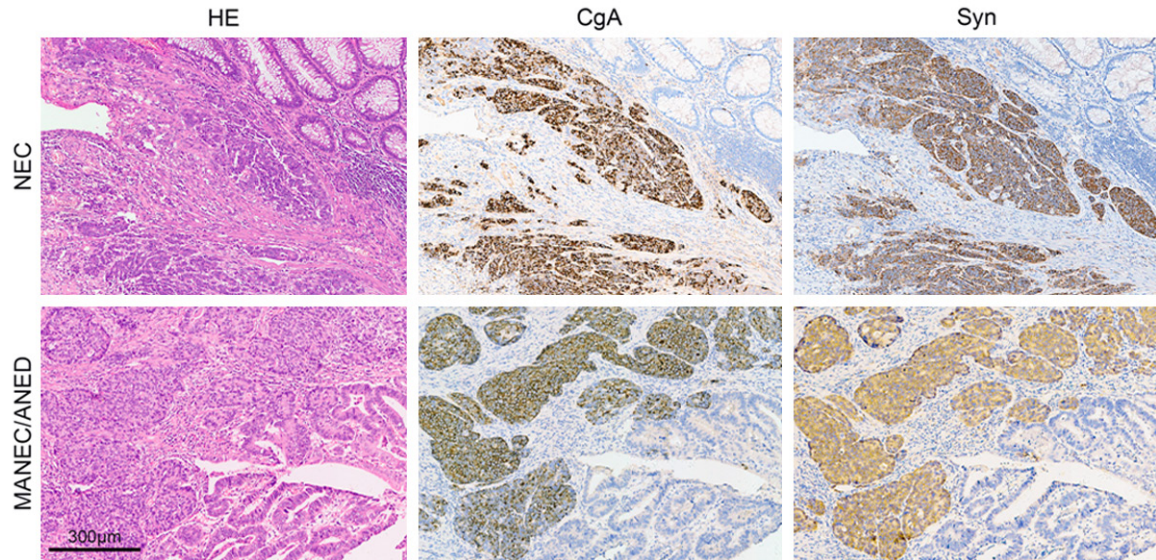
Figure 1. H&E. staining and immunohistochemical staining of CgA and Syn for NEC and MANEC/ANED.

noma (ADEC) in colorectal cancer [6-9]. It was reported that colorectal NEC is a highly aggressive tumor with about 40%-50% metastases at diagnosis, and most common metastases occur in liver [10, 11]. The median survival of NEC is 10.4 months, and 3-year survival is 17.2% [1, 12]. Massimo Milione [3] reported that the median overall survival (OS) of colorectal MANEC was 12.2 months. However, because ANED cases are rare, relevant reports are almost absent. In addition, there is no relevant study on the comparative analysis of clinicopathologic data and treatment strategy for colorectal MANEC and ANED. How does MANEC differ from ANED except for the proportion of morphologic components? Should they be treated differently?

Materials and methods

Study cohort

Incidence-SEER 18 Regs Custom Data (with additional treatment fields, Nov 2016 Sub, 1973-2014 varying) from SEER*Stat 8.3.4 database was used. We selected patients diagnosed as colon cancer ("ICD-O-3 Hist/behav, malignant", including 8140/3 (Adenocarcinoma, NOS, n=612115), 8246/3 (Neuroendocrine carcinoma, NOS, n=3728), 8013/3 (Large cell neuroendocrine carcinoma, n=216, which was classified as neuroendocrine carcinoma), 8041/3 (Small cell carcinoma, NOS, n=723,

which was classified as neuroendocrine carcinoma), 8042/3 (Oat cell carcinoma, n=7, which was classified as neuroendocrine carcinoma), 8244/3 (Mixed adenoneuroendocrine carcinoma, n=557), 8574/3 (Adenocarcinoma with neuroendocrine differentiation, n=325)) from 2010 to 2014 (n=617671). Patients with exact tumor size (range, 0-989 mm), age (16-108 years), race (white, black and others), site (right colon, including cecum, ascending colon, hepatic flexure and transverse colon; left colon, including descending colon, splenic flexure and sigmoid colon; rectum, including rectosigmoid junction and rectum), tumor cell differentiation (well, moderately, poorly and undifferentiated), AJCC 7th Stage (0+I, II, III, IV), bone metastases (yes or no), brain metastases (yes or no), liver metastases (yes or no), lung metastases (yes or no), marital status (married, unmarried and single, divorced and separated, widowed), chemotherapy (yes or no/unknown), radiation (yes or no), cause-specific death classification (overall-cause survival, OS, including alive and dead) and survival time data (0-59 months) were included in the cohort. Patients with unknown surgery (including "Recommended, unknown if performed" and "Unknown; death certificate; or autopsy only") were excluded. Finally, 82121 patients were recruited in the study cohort.

X-tile, a statistical model [13], was used to develop optimal cut-off points for age and tumor size. According to the X-tile program, the

Table 1. Clinicopathologic data of different histologic types of colorectal cancer

| Variable	No.	MANEC (%)	ANED (%)	p value	No.	ADEC (%)	NEC (%)	MANEC/ANED (%)	Total p value	p value ADEC vs NEC	ADEC vs MANEC/ANED	NEC vs MANEC/ANED
Age (years)				0.249					0.000	0.000	0.654	0.003
≤70	79	39 (49.37)	40 (50.63)		46500	45721 (56.41)	700 (74.15)	79 (60.31)				
71-80	29	10 (34.48)	19 (65.52)		19351	19175 (23.66)	147 (15.57)	29 (22.14)				
>80	23	8 (34.78)	15 (65.22)		16270	16150 (19.93)	97 (10.28)	23 (17.56)				
Tumor size (mm)				0.544					0.000	0.000	0.009	0.000
≤34	31	16 (51.61)	15 (48.39)		24875	24369 (30.07)	475 (50.32)	31 (23.66)				
35-59	49	21 (42.86)	28 (57.14)		34967	34689 (42.8)	229 (24.26)	49 (37.40)				
>59	51	20 (39.22)	31 (60.78)		22279	21988 (27.13)	240 (25.42)	51 (38.93)				
Race				0.512					0.000	0.000	0.278	0.077
White	109	45 (41.28)	64 (58.72)		65164	64354 (79.40)	701 (74.26)	109 (83.21)				
Black	15	8 (53.33)	7 (46.67)		9223	9055 (11.17)	153 (16.21)	15 (11.45)				
Others	7	4 (57.14)	3 (42.86)		7734	7637 (9.42)	90 (9.53)	7 (5.34)				
Sex				0.871					0.196	0.192	0.211	0.475
Male	61	27 (44.26)	34 (55.74)		42700	42168 (52.03)	471 (49.89)	61 (46.56)				
Female	70	30 (42.86)	40 (57.14)		39421	38878 (47.97)	473 (50.11)	70 (53.44)				
Site				0.586					0.000	0.000	0.000	0.000
Right colon	88	41 (46.59)	47 (53.41)		37136	36606 (45.17)	442 (46.82)	88 (67.18)				
Left colon	18	7 (38.89)	11 (61.11)		23307	23217 (28.65)	72 (7.63)	18 (13.74)				
Rectum	25	9 (36.00)	16 (64.00)		21678	21223 (26.19)	430 (45.55)	25 (19.08)				
Differentiation				0.094					0.000	0.000	0.000	0.000
Well	2	2 (100)	0 (0)		5629	5258 (6.49)	369 (39.09)	2 (1.53)				
Moderately	17	9 (52.94)	8 (47.06)		60413	60290 (74.39)	106 (11.23)	17 (12.98)				
Poorly	87	32 (36.78)	55 (63.22)		13567	13184 (16.27)	296 (31.36)	87 (66.41)				
Undifferentiated	25	14 (56.00)	11 (44.00)		2512	2314 (2.86)	173 (18.33)	25 (19.08)				
Stage				0.138					0.000	0.000	0.000	0.000
0+I	8	6 (75.00)	2 (25.00)		14367	14104 (17.4)	255 (27.01)	8 (6.11)				
II	21	9 (42.86)	12 (57.14)		26691	26584 (32.8)	86 (9.11)	21 (16.03)				
III	52	25 (48.08)	27 (51.92)		27661	27345 (33.74)	264 (27.97)	52 (39.69)				
IV	50	17 (34.00)	33 (66.00)		13402	13013 (16.06)	339 (35.91)	50 (38.17)				
Marital status				0.182					0.000	0.000	0.593	0.006
Married	67	34 (50.75)	33 (49.25)		45536	44887 (55.38)	582 (61.65)	67 (51.15)				
Unmarried and single	21	10 (47.62)	11 (52.38)		13219	13025 (16.07)	173 (18.33)	21 (16.03)				
Divorced and Separated	19	5 (26.32)	14 (73.68)		9060	8943 (11.03)	98 (10.38)	19 (14.50)				
Widowed	24	8 (33.33)	16 (66.67)		14306	14191 (17.51)	91 (9.64)	24 (18.32)				
Bone metastases				0.805					0.000	0.000	0.000	0.991
Yes	4	1 (25.00)	3 (75.00)		499	466 (0.57)	29 (3.07)	4 (3.05)				
No	127	56 (44.09)	71 (55.91)		81622	80580 (99.43)	915 (96.93)	127 (96.95)				
Brain metastases				1.000					0.000	0.000	0.000	0.541
Yes	2	1 (50.00)	1 (50.00)		151	140 (0.17)	9 (0.95)	2 (1.53)				
No	129	56 (43.41)	73 (56.59)		81970	80906 (99.83)	935 (99.05)	129 (98.47)				
Liver metastases				0.411					0.000	0.000	0.000	0.597
Yes	37	14 (37.84)	23 (62.16)		10013	9688 (11.95)	288 (30.51)	37 (28.24)				
No	94	43 (45.74)	51 (54.26)		72108	71358 (88.05)	656 (69.49)	94 (71.76)				
Lung metastases				0.182					0.073	0.821	0.023	0.034
Yes	9	2 (22.22)	7 (77.78)		2722	2683 (3.31)	30 (3.18)	9 (6.87)				
No	122	55 (45.08)	67 (54.92)		79399	78363 (96.69)	914 (96.82)	122 (93.13)				
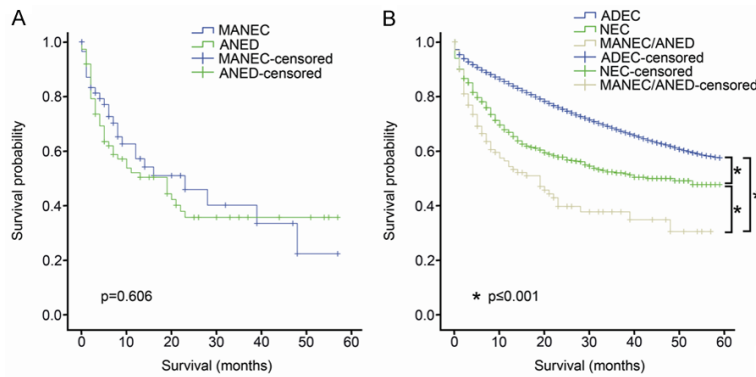
Figure 2. Kaplan-Meier (log-rank test) analysis of different histologic types of colorectal cancer. A. Kaplan-Meier (log-rank test) analysis of colorectal MANEC and ANED. B. Kaplan-Meier (log-rank test) analysis of colorectal ADEC, NEC and MANEC/ANED.

optimal cut-off points were 70 and 80 (years) for age, 34 and 59 (mm) for tumor size. Then patients were divided into three groups for age (≤70, 71-80, >80 years) and tumor size (≤34, 35-59, >59 mm), respectively.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA), and R software version 3.03. Chi-square test was used to evaluate the clinicopathologic data of the four different histological types of colorectal cancer. In this study, Kaplan-Meier (log-rank test) and Cox Regression (univariable and multivariable) analyses assessed risk factors for OS prognosis. Hazard ratio (HRs) were presented with there 95% CIs [14]. Selected variables were incorporated in the nomogram created by R software using "rms" package to predict the probability of 1-year and 3-year OS. The Concordance index (C-index) was used to quantify the predictive accuracy [15]. Calibration plots were generated to examine the performance characteristic of the predictive nomogram. A two-tailed P<0.05 was considered significant.

Results

Clinicopathologic characteristics of four different histologic types of colorectal cancer

This study was comprised of 42700 males and 39421 females, with a male-female ratio of 1.08:1. The ages of the patients ranged from 16 to 108 years (median age, 68 years). The mortality rate for OS was 26.21%

(21526/82121). The study cohort was composed of ADEC (n=81046), NEC (n=944), MANEC (n=57) and ANED (n=74). The result showed that the median age of ADEC, NEC, MANEC and ANED was 68 years (16-108 years), 60 years (18-95 years), 66 years (30-89 years) and 69 years (28-94 years), respectively. The tumor size of ADEC, NEC, MANEC and ANED was 45 mm (0-989 mm), 33.5 mm (1-989 mm), 50 mm (3-989 mm) and 50 mm (11-350 mm), respectively. Chi-square test (**Table 1**) revealed that there was no difference between MANEC and ANED in age (P=0.249), tumor size (P=0.544), race (P=0.512), sex (P=0.871), site (P=0.586), differentiation (P=0.094), stage (P=0.138), marital status (P=0.182), bone metastases (P=0.805), brain metastases (P=1.000), liver metastases (P=0.411) and lung metastases (P=0.182). The survival data showed that there was no statistically significant difference between MANEC and ANED in the prognosis (P=0.606, **Figure 2A**). So, we merged MANEC and ANED into one category in the subsequent study. Comparative analysis within every two groups among the three histological types of colorectal cancer (ADEC, NEC and MANEC/ANED) showed that the proportion of the patients with age >80 years in MANEC/ANED was 17.56% (23/131), higher than NEC (10.28%, 97/944, P=0.003), and there was no difference between MANEC/ANED and ADEC (P=0.654) in age. The proportion of MANEC/ANED in tumor size >59 mm was 38.93% (51/131), higher than that of ADEC (27.13%, 21988/81046, P=0.009) and NEC (25.42%, 240/944, P=0.000). The proportion of MANEC/ANED in right colon was 67.18% (88/131), also higher than that of ADEC (45.17%, 36606/81046, P=0.000) and NEC (46.82%, 442/944, P=0.000). The proportion of MANEC/ANED with poorly differentiation was 66.41% (87/131), significantly higher than that of ADEC (16.27%, 13184/81046, P=0.000) and NEC (31.36, 296/944, P=0.000). The proportion of MANEC/ANED in stage III and IV were 39.69% (52/131) and 38.17% (50/131), both higher than that of ADEC (stage III, 33.74%, 27345/81046; and stage IV, 16.06%,

Table 2. Univariable and Multivariable Cox Regression for all-cause mortality among patients with colorectal MANEC/ANED

Variables	Total	Univariable		Multivariable	
		HR (95% CI)	p value	HR (95% CI)	p value
Age (years)		NA	0.658		
≤70	79				
71-80	29				
>80	23				
Size (mm)		1.420 (1.015-1.986)	0.041		
≤34	31			1 [Reference]	NA
35-59	49			NA	0.863
>59	51			NA	0.390
Race		NA	0.588		
White	109				
Black	15				
Others	7				
Sex		NA	0.592		
Male	61				
Female	70				
Site		NA	0.095		
Right colon	88				
Left colon	18				
Rectum	25				
Differentiation		1.535 (1.031-2.287)	0.035		
Well	2			1 [Reference]	NA
Moderately	17			NA	0.150
Poorly	87			NA	0.873
Undifferentiated	25			NA	0.111
Stage		3.192 (2.162-4.710)	0.000		
0+I	8			1 [Reference]	NA
II	21			1.526 (0.153-15.229)	0.719
III	52			7.842 (1.015-60.616)	0.048
IV	50			18.688 (2.493-140.110)	0.004
Marital status		NA	0.534		
Married	67				
Unmarried and single	21				
Divorced and Separated	19				
Widowed	24				
Bone metastases		6.132 (2.141-17.565)	0.001		
No	127			1 [Reference]	NA
Yes	4			NA	0.088
Brain metastases		7.066 (1.654-30.176)	0.008		
No	129			1 [Reference]	NA
Yes	2			NA	0.086
Liver metastases		3.609 (2.169-6.003)	0.000		
No	94			1 [Reference]	NA
Yes	37			NA	0.928
Lung metastases		4.154 (1.922-8.981)	0.000		
No	122			1 [Reference]	NA
Yes	9			NA	0.334

	n	HR (95% CI)	P	HR (95% CI)	P
Surgery		3.319 (1.847-5.961)	0.000		
Yes	114			1 [Reference]	NA
No	17			2.592 (1.327-5.061)	0.005
Chemotherapy		NA	0.216		
Yes	76				
No	55				
Radiation		NA	0.218		
Yes	10				
No	121				

Abbreviations: HR, hazard ratio; NA, not applicable.

13013/81046, P=0.000) and NEC (stage III, 27.97%, 264/944; and stage IV, 35.91%, 339/944, P=0.000). The rate of patients in widowed with MANEC/ANED was 18.32% (24/131), higher than that of NEC (9.64%, 91/944, P=0.006). The rate of MANEC/ANED with bone metastases, brain metastases, liver metastases and lung metastases were 3.05% (4/131), 1.53% (2/131), 28.24% (37/131) and 6.87% (9/131), all significantly higher than that of ADEC (0.57%, 466/81046, P=0.000; 0.17%, 140/81046, P=0.000; 11.95%, 9688/81046, P=0.000; 3.31%, 2683/81046, P=0.023, respectively). The proportion of MANEC/ANED with lung metastases was also higher than that of NEC (3.18%, 30/944, P=0.034). In addition, there were no significant differences between MANEC/ANED and ADEC in race (P=0.278), sex (P=0.211) and marital status (P=0.593), and there were no statistically significant differences between MANEC/ANED and NEC in race (P=0.077), sex (P=0.475), bone metastases (P=0.991), brain metastases (P=0.541), and liver metastases (P=0.597). The survival curve showed that there was significant difference between ADEC, NEC and MANEC/ANED in prognosis (ADEC vs NEC, P=0.000; ADEC vs MANEC/ANED, P=0.000; NEC vs MANEC/ANED, P=0.001, Figure 2B). The 1-year and 3-year survival rates were 84.70% and 67.83% for ADEC, 66.83% and 51.98% for NEC, and 54.27% and 37.68% for MANEC/ANED, respectively.

Univariable and multivariable cox regression and predictive nomogram for OS in patients with colorectal MANEC/ANED

The univariable analysis showed that tumor size, cell differentiation, stage, bone metastases, brain metastases, liver metastases, lung metastases and surgery were related with prognosis of colorectal MANEC/ANED. The multivariate analysis demonstrated that TNM stage and surgery were independent prognostic factors of colorectal MANEC/ANED. By multivariable analysis, it displayed that stage III (vs stage 0+I; HR, 7.842; 95% CI, 1.015-60.616; P=0.048, Table 2), stage IV (vs stage 0+I; HR, 18.688; 95% CI, 2.493-140.110; P=0.004), without surgery (vs surgery; HR, 2.592; 95% CI, 1.327-5.061; P=0.005) were associated with significantly poorer prognosis. The nomogram (Figure 3A) to predict OS was created based on 2 independent prognostic factors, stage (0+I, II, III or IV) and surgery (yes or no). Higher total points based on the sum of the assigned number of points for each factor in the nomogram was associated with a worse prognosis. For example, a patient with Stage (IV) and Surgery (no) would have a total of 13 points (10 points for stage, 3 points for surgery), for a predicted 1-year and 3-year OS of 50% and 15%, respectively. For internal validation, calibration plots of the nomogram predicting 1- and 3-year survival performed well with the ideal model (Figure 3B). The C-index of the multivariate prognostic model based on 2 independent prognostic factors was 0.742.

Analysis of treatment strategies for colorectal MANEC/ANED in different stages

Predict OS based on surgery, chemotherapy and radiation (Table 3) showed that among patients with colorectal MANEC/ANED in stage III, all the patients implemented surgery (n=52), 33 patients among them taken chemotherapy, and only 6 patients adopted radiation. By multivariable analysis, it displayed that patients without chemotherapy (vs with chemotherapy) in stage III colorectal MANEC/ANED have worse prognosis (HR, 4.377; 95% CI, 1.958-9.786;
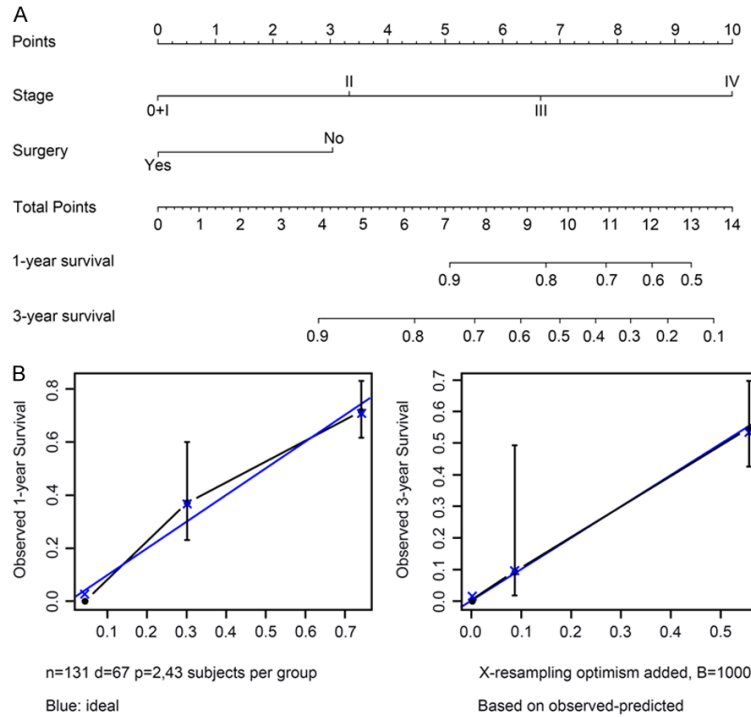
Figure 3. Nomogram Predicting Survival in Patients with colorectal MANEC/ANED. A. The nomogram to predict OS was created based on 2 independent prognostic factors. Notes: AJCC 7th Stage (0+I, II, III and IV) and Surgery (Yes and No). B. Calibration Plot Comparing Predicted and Actual Survival Probabilities at 1-year and 3-year survival. The blue line represents the performance of an ideal nomogram. The black line indicates the performance of proposed nomogram. Black circles are sub-cohorts of the data set; X is the bootstrapped corrected estimate of nomogram with 1000 resamples. Vertical bars represent 95% CI. It seemed that the nomogram predicted accurately 1- and 3-year OS.

P=0.000). The mortality of the patients with chemotherapy in stage III colorectal MANEC/ANED was 33.33% (11/33), and that of patients without chemotherapy was 73.68% (14/19, P=0.000). In stage IV colorectal MANEC/ANED (n=50), 35 patients underwent surgery (vs without surgery, n=15), 33 patients took chemotherapy (vs without chemotherapy, n=17), and 1 patient had radiation (vs without radiation, n=49). By multivariable analysis, among patients with stage IV colorectal MANEC/ANED, going without surgery (vs with surgery; HR, 2.627; 95% CI, 1.297-5.322; P=0.007), or without chemotherapy (vs with chemotherapy; HR, 4.396; 95% CI, 2.059-9.384; P=0.000) were associated with significantly poorer prognosis. The mortality of the patients with surgery in stage IV colorectal MANEC/ANED was 68.57% (24/35), and that of patients without surgery was 93.33% (14/15, P=0.007). The mortality of the patients with chemotherapy in stage IV

colorectal MANEC/ANED was 72.73% (24/33), and that of patients without chemotherapy was 82.35% (14/17, P= 0.000). It seemed that radiation was of no use to improve the prognosis of colorectal MANEC/ANED in stage III (P=0.557) and stage IV (P=0.677).

Discussion

According to the World Health Organization (WHO) 2010 classification [1], the diagnosis of MANEC or ANED is determined by higher than or less than 30% NEC component in mixed tumors, that are composed of adenocarcinoma and NEC components. Is ANED totally different from MANEC? What is the difference between the two types of colorectal cancer besides morphologic proportions? No prior study reported on comparing MANEC and ANED. After thorough comparative analysis, our data showed that MANEC and ANED should be merged into the same category, because there was no difference between MANEC and ANEC in clinicopathologic characteristics and survival (**Table 1** and **Figure 2A**).

The study demonstrated that the tumor size in patients with MANEC/ANED was comparatively larger than ADEC and NEC, and the tumor cell differentiation in patients with MANEC/ANED was relatively poorer than ADEC and NEC. The TNM stage of MANEC/ANED patients at diagnosis was comparatively later than with ADEC and NEC patients. Several reports suggested that the prognosis of NEC and MANEC is poorer than ADEC in colorectal cancer [6-9, 12]. Our survival data indicated that the prognosis of MANEC/ANED was the poorest, compared with ADEC and NEC, and the prognosis of NEC patients was poorer than ADEC patients (**Figure 2B**), which was consistent with the references. Our study also found that there was no difference between the prognosis of MANEC patients

Table 3. Multivariable Cox Regression for all-cause mortality among colorectal MANEC/ANED patients with different treatment in stage III and stage IV

	Stage III				Stage IV			
	Total	Dead (%)	HR (95% CI)	p value	Total	Dead (%)	HR (95% CI)	p value
Surgery			NA	NA				
Yes	52	25 (48.08)			35	24 (68.57)	1 [Reference]	NA
No	0	0 (0)			15	14 (93.33)	2.627 (1.297-5.322)	0.007
Chemotherapy								
Yes	33	11 (33.33)	1 [Reference]	NA	33	24 (72.73)	1 [Reference]	NA
No	19	14 (73.68)	4.377 (1.958-9.786)	0.000	17	14 (82.35)	4.396 (2.059-9.384)	0.000
Radiation			NA	0.557			NA	0.677
Yes	6	3 (50)			1	1 (100)		
No	46	22 (47.83)			49	37 (75.51)		

Abbreviations: HR, hazard ratio; NA, not applicable.

and ANED patients. It suggested MANEC/ANED was a malignant tumor with higher malignancy and poorer prognosis, compared with ADEC and NEC. It seemed that the NEC component in MANEC was related to poor prognosis, no matter how much proportion it counted. So, we suggest that the pathologic diagnosis for this kind of tumor should be united to one category. We recommend MANEC other than ADEC with neuroendocrine differentiation. And objective description about the proportion of each component is also recommended for a complete diagnosis.

In addition, the study indicated that stage and surgery were important independent prognostic factors of colorectal MANEC/ANED patients, as showed in **Table 2**. The nomogram in this study (**Figure 3**) to predict OS was created based on 2 independent prognostic factors (stage and surgery), which could be used to guide the prognosis of patients with colorectal MANEC/ANED. It showed the most significant impact on the prognosis of colorectal MANEC/ANED was stage. Furthermore, the C-index of the multivariate prognostic model was 0.742, which performed well on internal validation.

Surgery is recommended for the treatment of Stage 0-II colorectal cancer. Treatment of Stage III colorectal cancer includes surgery and chemotherapy, and that of Stage IV contains surgery, chemotherapy, radiation and targeted therapy [16]. Excluding other factors, we only consider the effect of treatment on the prognosis of MANEC/ANED patients. Predicting OS based on surgery, chemotherapy and radiation

respectively (Table S1) showed that patients could benefit from surgery and chemotherapy, no matter which stage of colorectal ADEC. Radiation could improve the prognosis of the patients with stage III and IV of colorectal ADEC, but radiation should not be recommended to the stage 0+I patients (without surgery vs with surgery; HR, 0.720; 95% CI, 0.547-0.948; P=0.019), but the data showed that totally about 13172 stage 0+I colorectal ADEC patients were treated with radiation. Surgery is the preferred treatment for colorectal NEC as reported before [12, 17]. NEC patients can be treated with chemotherapy and radiation [18-27], particularly when surgical resection is difficult. However, radiotherapy is still controversial for improving the prognosis of patients with NEC [28, 29]. Our study showed that surgery and chemotherapy could improve NEC patient prognosis in stage IV (without surgery vs with surgery; HR, 1.566; 95% CI, 1.186-2.068; P=0.002; without chemotherapy vs with chemotherapy; HR, 1.350; 95% CI, 1.025-1.778; P=0.033). Prognosis of colorectal NEC would be made poorer by chemotherapy in stage 0+I/II/III (without chemotherapy vs with chemotherapy in stage 0+I; HR, 0.043; 95% CI, 0.009-0.200; P=0.000; without chemotherapy vs with chemotherapy in stage II; HR, 0.285; 95% CI, 0.098-0.829; P=0.021; without chemotherapy vs with chemotherapy in stage III; HR, 0.441; 95% CI, 0.298-0.652; P=0.000). Our data also demonstrated that radiation was of no use to improve the prognosis of colorectal NEC in all stages. It remains unclear to medical oncologists if a tumor with both exocrine and endocrine differentiation (MANEC) should be treated

based on protocols for conventional ADEC or NEC [30]. The treatment of MANEC is mostly adopted from therapeutic strategies of ADEC and NEC [31, 32]. It was reported that chemoradiotherapy can benefit MANEC patients [7]. Colorectal ANED is rare and an effective chemotherapy has not yet been established, according to previous report [33]. To date, researchers have no idea about the therapeutic effect of ANED. For MANEC/ANED patients, surgery was helpful to improve the prognosis of stage IV patients, and chemotherapy was useful to improve the prognosis of stage III and stage IV patients (**Table 3**), according to our analysis. It showed that surgery and chemotherapy were not helpful to improve the prognosis of MANEC/ANED patients in stage 0+I and stage II, which might due to the early progress of stage 0+I/II patients itself. In addition, radiation was of no use to improve the prognosis of MANEC/ANED patients in all stages (Table S1).

With the development of molecular biology techniques, the treatment of colorectal cancer is no longer confined to surgery, chemotherapy and radiation. More targeted therapy is used to treat patients with colorectal cancer; for example, VEGF inhibitor, EGFR inhibitor, PD-1 inhibitor, Ziv-aflibercept and so on [16]. However, unfortunately there were no relevant data about targeted therapy for MANEC/ANED in the SEER database,and we had no exact information about chemotherapy for MANEC/ANED either.

Conclusions

Colorectal MANEC and ANED could be merged into the same category, because of their similar clinicopathologic and prognosis data. Pathologic diagnosis for this tumor should put emphasis on NEC components, with objective description of the exact proportion of both components. The prognosis of colorectal MANEC/ANED was the poorest, compared with ADEC and NEC. The nomogram of this paper could provide a prognostic evaluation of colorectal MANEC/ANED. Stage and surgery were independent prognostic risk factors for colorectal MANEC/ANED. For example, a patient with Stage (IV) and Surgery (no) would have a total of 13 points (10 points for stage, 3 points for surgery), for a predicted 1-year and 3-year OS

of 50% and 15%, respectively. Radiation was unhelpful to MANEC/ANED.

Disclosure of conflict of interest

None.

Address correspondence to: Lin Yuan, Pathology Center, Shanghai General Hospital/Faculty of Basic Medicine, Shanghai Jiao Tong University School of Medicine, No. 100, Hai-Ning Road, Shanghai 200080, P. R. China. Tel: 86-21-63240090 Ext. 4501; E-mail: yuanlin_li@126.com

References

[1] Bosman F, Carneiro F, Hruban R, Theise N. WHO classification of tumors of the digestive system. Zhonghua Bing Li Xue Za Zhi 2011; 40: 351-4.

[2] Shia J, Tang LH, Weiser MR, Brenner B, Adsay NV, Stelow EB, Saltz LB, Qin J, Landmann R, Leonard GD, Dhall D, Temple L, Guillem JG, Paty PB, Kelsen D, Wong WD, Klimstra DS. Is nonsmall cell type high-grade neuroendocrine carcinoma of the tubular gastrointestinal tract a distinct disease entity? Am J Surg Pathol 2008; 32: 719-31.

[3] Milione M, Maisonneuve P, Pellegrinelli A, Grillo F, Albarello L, Spaggiari P, Vanoli A, Tagliabue G, Pisa E, Messerini L, Centonze G, Inzani F, Scarpa A, Papotti M, Volante M, Sessa F, Fazio N, Pruneri G, Rindi G, Solcia E, La Rosa S, Capella C. Ki67 proliferative index of the neuroendocrine component drives MANEC prognosis. Endocr Relat Cancer 2018; 25: 583-93.

[4] Marando A, Dainese E, La Rosa S, Capella C. Images in endocrine pathology : oncocytic differentiation in a mixed adenoneuroendocrine carcinoma of the colon. Endocr Pathol 2013; 24: 54-6.

[5] Sorbye H, Strosberg J, Baudin E, Klimstra DS, Yao JC. Gastroenteropancreatic high-grade neuroendocrine carcinoma. Cancer 2014; 120: 2814-23.

[6] Watanabe J, Suwa Y, Ota M, Ishibe A, Masui H, Nagahori K, Tsuura Y, Endo I. Clinicopathological and prognostic evaluations of mixed adenoneuroendocrine carcinoma of the colon and rectum: a case-matched study. Dis Colon Rectum 2016; 59: 1160-7.

[7] Tanaka T, Kaneko M, Nozawa H, Emoto S, Murono K, Otani K, Sasaki K, Nishikawa T, Kiyomatsu T, Hata K, Morikawa T, Kawai K, Watanabe T. Diagnosis, assessment, and therapeutic strategy for colorectal mixed adenoneuroendocrine carcinoma. Neuroendocrinology 2017; 105: 426-34.

[8] Bernick PE, Klimstra DS, Shia J, Minsky B, Saltz L, Shi W, Thaler H, Guillem J, Paty P, Cohen AM, Wong WD. Neuroendocrine carcinomas of the colon and rectum. Dis Colon Rectum 2004; 47: 163-9.

[9] Komatsubara T, Koinuma K, Miyakura Y, Horie H, Morimoto M, Ito H, Lefor AK, Sata N, Fukushima N. Endocrine cell carcinomas of the colon and rectum: a clinicopathological evaluation. Clin J Gastroenterol 2016; 9: 1-6.

[10] Chen L, Chen J, Zhou Z. Interpretation of the latest guidelines in the treatment of gastrointestinal neuroendocrine neoplasms. Zhonghua Wei Chang Wai Ke Za Zhi 2016; 19: 1201-4.

[11] Cheung TT, Chok KS, Chan AC, Tsang S, Dai JW, Lang BH, Yau T, Chan SC, Poon RT, Fan ST, Lo CM. Long term survival analysis of hepatectomy for neuroendocrine tumour liver metastases. ScientificWorldJournal 2014; 2014: 524045.

[12] Zhang Y, Xie J, Wang J, Yang D, Jiang Z, Han G, Fu Q, Zhang Y. Clinicopathological and prognostic analysis of neuroendocrine carcinoma of the colorectum. Adv Clin Exp Med 2016; 25: 719-24.

[13] Cai YJ, Dong JJ, Dong JZ, Chen Y, Lin Z, Song M, Wang YQ, Chen YP, Shi KQ, Zhou MT. A nomogram for predicting prognostic value of inflammatory response biomarkers in decompensated cirrhotic patients without acute-on-chronic liver failure. Aliment Pharmacol Ther 2017; 45: 1413-26.

[14] Simon R. Confidence intervals for reporting results of clinical trials. Ann Intern Med 1986; 105: 429-35.

[15] Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996; 15: 361-87.

[16] Colon cancer treatment (PDQ(R)): patient version. PDQ Cancer Information Summaries. Bethesda (MD) 2002.

[17] Starzyńska T, Szawłowski A, Kos-Kudła B, Królicki L, Kunikowska J, Nasierowska-Guttmejer A, Rudzki S, Zgliczyński W; oraz Pozostali Uczestnicy Konferencji Okrągłego Stołu. Neuroendocrine tumors of the colon - management guidelines (recommended by The Polish Network of Neuroendocrine Tumors). Endokrynol Pol 2008; 59: 97-104.

[18] Coriat R, Walter T, Terris B, Couvelard A, Ruszniewski P. Gastroenteropancreatic well-differentiated grade 3 neuroendocrine tumors: review and position statement. Oncologist 2016; 21: 1191-9.

[19] Kulke MH, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, Jensen RT; North American Neuroendocrine Tumor Society (NANETS). NANETS treatment guidelines: well-differentiated neuroendocrine tumors of the stomach and pancreas. Pancreas 2010; 39: 735-52.

[20] Strosberg JR, Coppola D, Klimstra DS, Phan AT, Kulke MH, Wiseman GA, Kvols LK; North American Neuroendocrine Tumor Society (NANETS). The NANETS consensus guidelines for the diagnosis and management of poorly differentiated (high-grade) extrapulmonary neuroendocrine carcinomas. Pancreas 2010; 39: 799-800.

[21] Yabuki H, Suto T, Inoue K, Fujimoto H, Sato T, Ikeda E, Iizawa H. A case of colorectal neuroendocrine carcinoma effectively treated with bevacizumab+levofolinate+5-FU chemotherapy. Gan To Kagaku Ryoho 2012; 39: 1139-42.

[22] Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, Dueland S, Hofsli E, Guren MG, Ohrling K, Birkemeyer E, Thiis-Evensen E, Biagini M, Gronbaek H, Soveri LM, Olsen IH, Federspiel B, Assmus J, Janson ET, Knigge U. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. Ann Oncol 2013; 24: 152-60.

[23] Miyamoto H, Kurita N, Nishioka M, Ando T, Tashiro T, Hirokawa M, Shimada M. Poorly differentiated neuroendocrine cell carcinoma of the rectum: report of a case and literal review. J Med Invest 2006; 53: 317-20.

[24] Patta A, Fakih M. First-line cisplatin plus etoposide in high-grade metastatic neuroendocrine tumors of colon and rectum (MCRC NET): review of 8 cases. Anticancer Res 2011; 31: 975-8.

[25] Anthony LB, Strosberg JR, Klimstra DS, Maples WJ, O'Dorisio TM, Warner RR, Wiseman GA, Benson AB 3rd, Pommier RF; North American Neuroendocrine Tumor Society (NANETS). The NANETS consensus guidelines for the diagnosis and management of gastrointestinal neuroendocrine tumors (nets): well-differentiated nets of the distal colon and rectum. Pancreas 2010; 39: 767-74.

[26] Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, Emerson L, Engstrom PF, Fanta P, Giordano T, Goldner WS, Halfdanarson TR, Heslin MJ, Kandeel F, Kunz PL, Kuvshinoff BW 2nd, Lieu C, Moley JF, Munene G, Pillarisetty VG, Saltz L, Sosa JA, Strosberg JR, Vauthey JN, Wolfgang C, Yao JC, Burns

J, Freedman-Cass D; National comprehensive cancer network. Neuroendocrine tumors, version 1.2015. J Natl Compr Canc Netw 2015; 13: 78-108.

[27] Aytac E, Ozdemir Y, Ozuner G. Long term outcomes of neuroendocrine carcinomas (high-grade neuroendocrine tumors) of the colon, rectum, and anal canal. J Visc Surg 2014; 151: 3-7.

[28] Modrek AS, Hsu HC, Leichman CG, Du KL. Radiation therapy improves survival in rectal small cell cancer - analysis of surveillance epidemiology and end results (SEER) data. Radiat Oncol 2015; 10: 101.

[29] Brieau B, Lepère C, Walter T, Lecomte T, Guimbaud R, Manfredi S, Tougeron D, Desseigne F, Lourenco N, Afchain P, El Hajbi F, Terris B, Rougier P, Coriat R. Radiochemotherapy versus surgery in nonmetastatic anorectal neuroendocrine carcinoma: a multicenter study by the association des gastro-enterologues oncologues. Medicine (Baltimore) 2015; 94: e1864.

[30] Sinha N, Gaston D, Manders D, Goudie M, Matsuoka M, Xie T, Huang WY. Characterization of genome-wide copy number aberrations in colonic mixed adenoneuroendocrine carcinoma and neuroendocrine carcinoma reveals recurrent amplification of PTGER4 and MYC genes. Hum Pathol 2018; 7316-25.

[31] Knigge U, Hansen CP. Surgery for GEP-NETs. Best Pract Res Clin Gastroenterol 2012; 26: 819-31.

[32] Fendrich V, Bartsch DK. Surgical treatment of gastrointestinal neuroendocrine tumors. Langenbecks Arch Surg 2011; 396: 299-311.

[33] Kakuta S, Takayama W, Kou T, Satou M, Sugaya M. A case of descending colon adenocarcinoma with neuroendocrine differentiation successfully treated with FOLFIRI plus bevacizumab. Gan To Kagaku Ryoho 2016; 43: 129-32.

Table S1. Multivariable Cox regression for all-cause mortality among patients with different treatment in colorectal cancer

	Multivariable Cox Regression			Multivariable Cox Regression			Multivariable Cox Regression		
	With Surgery	Without Surgery HR (95% CI)	p value	With Chemotherapy	Without Chemotherapy HR (95% CI)	p value	With Radiation	Without Radiation HR (95% CI)	p value
Stage 0+I									
ADEC	1 [Reference]	9.669 (8.521-10.971)	0.000	1 [Reference]	1.952 (1.565-2.434)	0.000	1 [Reference]	0.720 (0.547-0.948)	0.019
NEC	1 [Reference]	NA	0.256	1 [Reference]	0.043 (0.009-0.200)	0.000	1 [Reference]	NA	0.384
MANEC/ANED	1 [Reference]	NA	0.317	1 [Reference]	NA	0.083	1 [Reference]	NA	NA
Stage II									
ADEC	1 [Reference]	5.788 (5.133-6.527)	0.000	1 [Reference]	2.263 (2.091-2.449)	0.000	1 [Reference]	NA	0.612
NEC	1 [Reference]	NA	0.671	1 [Reference]	0.285 (0.098-0.829)	0.021	1 [Reference]	NA	0.542
MANEC/ANED	1 [Reference]	NA	NA	1 [Reference]	NA	0.316	1 [Reference]	NA	0.378
Stage III									
ADEC	1 [Reference]	3.063 (2.704-3.469)	0.000	1 [Reference]	3.505 (3.325-3.695)	0.000	1 [Reference]	1.184 (1.087-1.290)	0.000
NEC	1 [Reference]	NA	0.186	1 [Reference]	0.441 (0.298-0.652)	0.000	1 [Reference]	NA	0.331
MANEC/ANED	1 [Reference]	NA	NA	1 [Reference]	4.377 (1.958-9.786)	0.000	1 [Reference]	NA	0.557
Stage IV									
ADEC	1 [Reference]	1.982 (1.878-2.092)	0.000	1 [Reference]	3.196 (3.050-3.350)	0.000	1 [Reference]	1.282 (1.160-1.417)	0.000
NEC	1 [Reference]	1.566 (1.186-2.068)	0.002	1 [Reference]	1.350 (1.025-1.778)	0.033	1 [Reference]	NA	0.547
MANEC/ANED	1 [Reference]	2.627 (1.297-5.322)	0.007	1 [Reference]	4.396 (2.059-9.384)	0.000	1 [Reference]	NA	0.677

Abbreviations: HR, hazard ratio; NA, not applicable.