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Original Article

Higher LNM rate and poorer prognosis of early-onset compared to late-onset T1 stage colorectal cancer: a large-population based study

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Abstract: As for T1 stage CRC, there is little knowledge of differences in lymph node metastasis (LNM) and prognosis between early-onset and late-onset CRC. To know that, we included 13,084 patients from the SEER database and 476 patients in T1 stage from our hospital to analyze difference of LNM and prognosis. Univariate and multivariate logistic analyses revealed that early-onset CRC was more likely than late-onset CRC to be positive for LNM. In addition, we found that T1b stage, poor differentiation and lymphatic invasion were risk factors for LNM. Specifically, we found that black race was a risk factor. Before propensity-score matching (PSM), we also found that early-onset CRC patients had better survival, as demonstrated by SEER data. After adjusting for confounding factors by PSM, we found that early onset remained a risk factor for LNM. Moreover, we found that patients diagnosed with early-onset CRC had a poorer prognosis than those diagnosed with late-onset CRC, which was demonstrated by analysis of SEER data and our own data. In conclusion, our study was the first to find that early-onset T1 stage CRC more frequently developed LNM, suggesting that endoscopic submucosal resection should be performed more carefully in these patients. Moreover, early-onset patients in the T1 stage also had poorer survival, suggesting that clinical doctors should pay more attention to early-onset patients.

Keywords: Colorectal cancer, T1 stage, LNM, early-onset, late-onset

Introduction

According to cancer statistics from 2020, globally, colorectal cancer (CRC) ranks second in mortality and third in incidence [1]. Luckily, the rates of mortality and incidence have declined in recent decades [1]. Regarding the change in incidence rate, most data have shown that it has decreased among patients aged over 50 but increased slightly in patients aged less than 50 [2, 3]. Moreover, the mortality rate also showed this opposite trend between patients aged over 50 and patients younger than 50 [2, 4]. According to the prediction model, the incidence of CRC in patients younger than 50 could increase by as much as 142% by 2030 [2]. Early-onset CRC is defined as CRC diagnosed in patients earlier than 50 years of age, while late-onset CRC is defined as CRC diagnosed in patients over 50 years of age [5]. Usually, CRC is diagnosed in older patients; however, over the past decades, new cases of early-onset CRC have increased sharply worldwide. Accordingly, new cases of early-onset CRC account for 30-40% of all new cases of CRC [6]. As reasons for this trend, genetic alterations and lifestyle factors were most important. Some observational studies revealed that family history, obesity and smoking were potential risk factors associated with early-onset CRC [7, 8]. Moreover, early-onset CRC was more associated with polygenic risk factors such as MMR gene and APC mutations, especially for patients with a family history of CRC [9]. Several studies have found that early-onset CRC was more likely to be an advanced tumor at first diagnosis; for instance, 50%-70% of early-onset CRC cases were diagnosed as stage III-IV, while 40%-60% of late-onset CRC cases were diagnosed as stage III-IV. To date, for T1 stage CRC, there has

been a lower awareness of differences in lymph node metastasis (LNM) and prognosis between early-onset and late-onset cases.

From this perspective, we described the difference in LNM characteristics and analyzed the prognosis in early- and late-onset CRC in T1 stage. In our study, data extracted from the SEER database and from our hospital were used to perform propensity score matching (PSM) and logistic regression analysis, both of which demonstrated that early-onset patients had a greater risk of LNM and a poorer prognosis than later-onset CRC patients.

Methods

Patient extraction

All patients were selected from the SEER database and the First Hospital of Nanchang University. Patients were selected from the SEER database by the National Cancer Institute's SEER * Stat software (version 8.3.6) according to the following inclusion criteria: (1) patients who were over 20 years old and were diagnosed with T1 stage CRC with histological examination; (2) patients with detailed records of survival information; (3) patients with information on characteristics such as race, histological grade, examined lymph nodes (LNs), positive LNs and tumor size; and (4) all patients who underwent surgery without chemotherapy before surgery. The exclusion criteria were as follows: patients with no recorded information about our included clinical features, such as tumor site, T stage and N stage. To select patients from our center, we chose patients who were diagnosed from January 2010 through December 2019 to collect the clinical characteristics. The inclusion criteria were as follows: (1) patients who were diagnosed with T1 stage CRC by pathology and who were aged over 20; and (2) patients who did not receive preoperative adjuvant therapy. The exclusion criteria included (1) patients without recorded T stage, N stage and lymphatic invasion; and (2) patients with severe diseases such as cirrhosis, renal failure and cardiac failure. All of our patients were followed up by telephone, and those who were lost to follow-up were excluded when we analyzed the difference in prognosis. The characteristic information of patients from the SEER database is provided in **Table 1**, while information on patients selected from our center is shown in **Table 2**.

Definitions of variables

In this study, the clinical features extracted from the SEER database included sex, race, primary tumor site, pathological grade, N stage, M stage, examined LNs and liver metastasis. Additionally, our patients were divided into early- and late-onset group according to the definitions. Sex was recorded as male or female. Race was separated into white, black and other race. Primary site included the right side of the colorectum, the left side of the colorectum and overlap or NOS, of which the right side of the colorectum was defined as the right part of the colon, including the cecum, ascending colon, liver flexure and transverse colon, while the left colorectum included the splenic flexure, the descending and sigmoid colon, and the rectum [10]. Pathological grade was divided into four groups: well, moderately, or poorly differentiated and undifferentiated. N and M stages were recorded as negative (No) and positive (Yes). Tumor size was divided into ≤3 cm, ≤5 cm and >5 cm according to previous studies [11]. Examined LNs were divided into <12 and ≥12 according to some published guidelines [12, 13]. As for the classification of MLH1, MSH2, EGFR and C-erbB2, we divided into three groups: negative, positive and unknown. The main observation features included LNM, overall survival (OS) and cumulative events.

Statistical analysis

For basic statistical analysis, all extracted patients in T1 stage were divided into early- and late-onset group according to age at diagnosis, and then the included clinical characteristics were compared via Pearson's chi-squared test. Univariate and multivariate logistic regression analyses were used to investigate the potential risk factors associated with LNM, while Cox regression analysis was utilized for the analysis of prognostic factors. All results are shown by odds ratios (ORs) and hazard ratios (HRs) with 95% confidence intervals (CIs). Regarding the imbalance between the two groups, we performed PSM to obtain new data for analysis, and the caliper value was set as 0.001, the effect of which was balanced when the *P* value was more than 0.05. As our previous study

Table 1. Basic information of patients with colorectal cancer in T1 stage from 2010 through 2015 in SEER database

	Total	Early-onset CRC	Late-onset CRC	*P* value
Total	13084	1040	12044	
Gender				0.003
Male	6766 (51.71%)	492 (47.31%)	6274 (52.09%)	
Female	6318 (48.28%)	548 (52.69%)	5770 (47.91%)	
Race				0.099
White	10380 (79.33%)	802 (77.12%)	9578 (79.53%)	
Black	1514 (11.57%)	141 (13.56%)	1373 (11.40%)	
Another	1190 (9.10%)	97 (9.33%)	1093 (9.08%)	
Primary Site				<0.001
Right colorectum	5133 (39.23%)	195 (18.75%)	4938 (41.00%)	
Left colorectum	6795 (51.93%)	793 (76.25%)	6002 (49.83%)	
Overlap/NOS	1156 (8.84%)	52 (5.00%)	1104 (9.17%)	
Pathological grade				0.014
Well differentiated	2463 (18.82%)	159 (15.29%)	2304 (19.13%)	
Moderately	9611 (73.46%)	788 (75.77%)	8823 (73.26%)	
Poorly	861 (6.58%)	80 (7.70%)	781 (6.48%)	
Undifferentiated	149 (1.14%)	13 (1.25%)	136 (1.13%)	
N stage				<0.001
No	11433 (87.38%)	822 (79.04%)	10611 (88.10%)	
Yes	1651 (12.62%)	218 (20.96%)	1433 (11.90%)	
M stage				
No	12950 (98.98%)	1011 (97.21%)	11939 (99.13%)	<0.001
Yes	134 (1.02%)	29 (2.79%)	105 (0.87%)	
Tumor size				0.085
≤3 cm	914 (6.99%)	56 (5.38%)	858 (7.12%)	
≤5 cm	1121 (8.57%)	73 (7.02%)	1048 (8.70%)	
>5 cm	11049 (84.45%)	911 (87.60%)	10138 (84.17%)	
Examined LNs				<0.001
<12	3046 (23.28%)	206 (19.81%)	2840 (23.58%)	
≥12	10038 (76.72%)	834 (80.19%)	9204 (76.42%)	
Liver metastasis				<0.001
No	12988 (99.27%)	1022 (98.27%)	11966 (99.35%)	
Yes	96 (0.73%)	18 (1.73%)	78 (0.65%)	
Median Survival months (Quartile)	30	30 (13-71)	29 (11-68)	

described [14], we completed the analyses in R software and determined the *P* values by Pearson's chi-squared test. Finally, we explored the correlations of LNM and survival between early- and late-onset CRC by univariate logistic and Cox analyses, respectively. All statistical analyses were performed in R software, and all associated packages were obtained from the R software program website (https://cran.r-project.org/web/packages/). Student's t-test was used for continuous variables with a Gaussian distribution, and the nonparametric Kruskal-Wallis rank-sum test was used for non-normally distributed continuous variables or ordinal categorical variables. The chi-squared test was carried out with SPSS (version 24.0). The results were considered statistically significant when the *P* value was less than 0.05.

Results

Basic information of patients from the SEER database and our hospital

According to the inclusion criteria, we performed a stepwise extraction of satisfactory

Table 2. Basic information of patients with colorectal cancer in T1 stage from our own hospital diagnosed from 2011 through 2019

	Total	Early-onset CRC	Late-onset CRC	*P* value
Total	476	119	357	
Gender				0.037
Male	275 (57.77%)	59 (49.58%)	216 (60.50%)	
Female	201 (42.23%)	60 (50.42%)	141 (39.50%)	
T stage				0.651
T1a	101 (21.22%)	27 (22.69%)	74 (20.73%)	
T1b	375 (78.78%)	92 (77.31%)	283 (79.27%)	
Primary Site				0.545
Right-side colorectum	68 (14.29%)	19 (15.97%)	49 (13.73%)	
Left-side colorectum	408 (85.71%)	100 (84.03%)	308 (86.27%)	
Pathological grade				0.547
Well differentiated	42 (8.82%)	14 (11.76%)	28 (7.84%)	
Moderately	320 (67.23%)	77 (64.71%)	243 (68.07%)	
Poorly differentiated	47 (9.87%)	13 (10.92%)	34 (9.52%)	
Undifferentiated	67 (14.08%)	15 (12.61%)	52 (14.57%)	
N stage				0.032
No	425 (89.29%)	100 (84.03%)	325 (91.04%)	
Yes	51 (10.71%)	19 (15.97%)	32 (8.96%)	
Lymphatic invasion				0.072
No	448 (94.12%)	108 (90.76%)	340 (95.24%)	
Yes	28 (5.88%)	11 (9.24%)	17 (4.76%)	
M stage				0.635
No	470 (98.74%)	117 (98.32%)	353 (98.88%)	
Yes	6 (1.26%)	2 (1.68%)	4 (1.12%)	
Tumor size				0.189
≤3 cm	335 (70.38%)	76 (63.87%)	259 (72.55%)	
≤5 cm	104 (21.85%)	31 (26.05%)	73 (20.45%)	
>5 cm	37 (7.77%)	12 (10.08%)	25 (7.00%)	
Examined LNs				0.848
<12	347 (72.90%)	84 (70.59%)	263 (73.67%)	
≥12	129 (27.10%)	35 (29.41%)	94 (26.33%)	
Chemotherapy				0.024
No	448 (94.12%)	107 (89.92%)	341 (95.52%)	
Yes	28 (5.88%)	12 (10.08%)	16 (4.48%)	
Treatment methods				0.384
Robot	66 (13.87%)	12 (10.08%)	54 (15.13%)	
Laparoscopy	301 (63.24%)	79 (66.39%)	222 (62.18%)	
Surgery	109 (22.90%)	28 (23.53%)	81 (22.69%)	
Radiotherapy				0.712
No	466 (97.90%)	116 (97.48%)	350 (98.04%)	
Yes	10 (2.10%)	3 (2.52%)	7 (1.96%)	
Smoking				0.039
No	354 (74.37%)	97 (81.51%)	257 (71.99%)	
Yes	122 (25.63%)	22 (18.49%)	100 (28.01%)	
Drinking				0.414
No	380 (79.83%)	98 (82.35%)	282 (78.99%)	
Yes	96 (20.17%)	21 (17.65%)	75 (21.01%)	

MLH1				0.622
Negative	33 (5.73%)	9 (7.56%)	24 (9.34%)	
Positive	89 (15.45%)	23 (19.33%)	66 (18.49%)	
unknown	351 (60.94%)	84 (70.59%)	267 (74.79%)	
MSH2				0.754
Negative	27 (5.67%)	7 (5.88%)	20 (5.6%)	
Positive	97 (20.38%)	27 (22.69%)	70 (19.61%)	
unknown	352 (73.95%)	85 (71.42%)	267 (74.79%)	
EGFR				0.214
Negative	82 (17.23%)	24 (20.17%)	58 (16.25%)	
Positive	35 (7.35%)	12 (10.08%)	23 (6.44%)	
unknown	359 (75.42%)	83 (69.75%)	276 (77.31%)	
C-erbB1				0.327
Negative	97 (20.38%)	25 (21.01%)	72 (20.17%)	
Positive	62 (13.02%)	20 (16.81%)	42 (11.76%)	
unknown	317 (66.6%)	74 (62.18%)	243 (68.07%)	

patients (Supplementary Figure 1). Ultimately, we included 13,084 patients from the SEER database, of which 1040 patients were diagnosed with early-onset CRC and 12,044 patients were identified as late-onset CRC. As summarized in the basic information shown in **Table 1**, we found that early-onset patients were more likely to be female than late-onset CRC patients (52.69% vs 47.91%, P=0.003), while the distribution of race was not significantly different (P>0.05). The majority of early-onset patients had lesions in the left colorectum (76.25% 18.75%), while late-onset CRC exhibited lesions in the left colorectum as often as in the right colorectum (49.83% vs 41.00%). In addition, early-onset CRC tended to be poorly differentiated (P=0.014) and inclined to be advanced stage (LNM, 20.96% vs 11.9%; metastasis, 2.79% vs 0.87%; P<0.05). The tumor sizes were similar in early- and late-onset CRC; however, the number of examined LNs seemed to be greater in early-onset patients than in late-onset patients (≥12, 80.19% vs 76.42%, P<0.05). The median survival time of early-onset patients was 30 months, while that of late-onset CRC was 29 months. Regarding our own patients, we ultimately extracted 476 patients, including 119 early-onset CRC and 357 late-onset CRC patients, as described in Supplementary Figure 2 and as summarized in the basic information in **Table 2**. Similarly, the distribution of sex in our own data showed that early-onset CRC was more common in female patients (50.42% vs 39.5%, P≤0.037). Furthermore, there were more cases of early-onset CRC with positive LNM than of late-onset CRC (15.97% vs 8.96%, P=0.032). Although it was not significantly different, we found that early-onset CRC had more cases with lymphatic invasion (9.24% vs 4.76%). With regard to treatment, interestingly, more patients with early-onset CRC than patients with late-onset CRC received chemotherapy after surgery (10.08% vs 4.48%, P=0.024); however, the distributions of other treatment methods, such as surgery and radiotherapy, were not different (P>0.05). Additionally, the distributions of other variables, such as T stage, tumor site, pathological grade, tumor size and examined LNs, were similar between the two groups. Also, the distribution of some genomic genes' expression such as MLH1, MSH2, EGFR and C-erbB2 had no significant difference between early-onset CRC and late-onset CRC in T1 stage.

Identifying risk factors for LNM in both centers

To investigate the risk factors for LNM, we first analyzed the information from patients from the SEER database via logistic regression analyses. For the results from the SEER database (**Table 3**), we found that black patients and patients of other races had a higher risk of LNM than white patients (P<0.01), as demonstrated by univariate and multivariate logistic regression analyses. Additionally, tumor location in the right colon was a risk factor for LNM compared to tumor location in the left colorectum (OR, 1.546, 95% CI, 1.373-1.74, P<0.001). As expected, tumors with poor differentiation and

Table 3. Univariate and multivariate logistic regression model for exploring the potential risk factors for lymph node metastasis in patients from SEER database

Variables (LNM Yes/No)	Univariate analysis	*P* Value	Multivariate analysis	*P* Value
Gender				
Male (820/5946)	Reference	-		
Female (831/5487)	1.098 (0.99-1.218)	0.075		
Race				
White (1239/9141)	Reference	-	Reference	-
Black (223/1291)	1.274 (1.092-1.487)	0.002	1.348 (1.15-1.581)	<0.001
Another (189/1001)	1.393 (1.179-1.645)	<0.001	1.354 (1.141-1.606)	0.001
Primary Site				
Left colorectum (529/4604)	Reference	-	Reference	-
Right colorectum (1023/5772)	1.543 (1.379-1.725)	<0.001	1.546 (1.373-1.74)	<0.001
Overlap/NOS (99/1057)	0.815 (0.651-1.021)	0.075	0.867 (0.689-1.091)	0.222
Pathological grade				
Well (185/2278)	Reference	-	Reference	-
Moderately (1219/8392)	1.789 (1.522-2.102)	<0.001	1.716 (1.457-2.022)	<0.001
Poorly (212/649)	4.022 (3.242-4.99)	<0.001	3.881 (3.114-4.837)	<0.001
Undifferentiated (35/114)	3.78 (2.515-5.681)	<0.001	3.822 (2.519-5.788)	<0.001
CRC type				
Early-onset (218/822)	Reference	-	Reference	-
Late-onset (1433/10611)	0.509 (0.434-0.579)	<0.001	0.621 (0.525-0.733)	<0.001
Tumor size				
≤3 cm (1322/9639)	Reference	-	Reference	-
≤5 cm (192/1255)	1.115 (0.948-1.312)	0.187	1.037 (0.877-1.228)	0.669
>5 cm (137/1539)	1.853 (1.523-2.255)	<0.001	1.691 (1.373-2.082)	<0.001
Examined LNs				
<12 (458/3652)	Reference	-	Reference	-
≥12 (1193/7781)	1.223 (1.09-1.371)	0.001	1.276 (1.133-1.438)	<0.001

more examined LNs more commonly experienced LNM. Unexpectedly, late-onset CRC was a protective factor for LNM (OR, 0.621, 95% CI, 0.525-0.733, P<0.01). Regarding tumor size, we found that the rate of LNM was not obviously different in tumors less than 5 cm; however, tumors with a size of more than 5 cm had an increased rate of LNM (OR, 1.691; 95% CI, 1.373-2.082, P<0.01). Similarly, the results of our own data also showed that late-onset CRC more rarely had LNM (HR, 0.572, 95% CI, 0.294-0.959, P=0.042) (**Table 4**). Furthermore, we found that tumors in stage T1b or with lymphatic invasion were more likely to be positive for LNM, while examined LNs were not independent factors for LNM, as demonstrated by multivariate logistic regression analysis (**Table 4**). Some genomic genes' expression such as MLH1, MSH2, EGFR and C-erbB2 were not associated with LNM (**Table 4**). As there were too many confounding factors associated with our observational features, we performed PSM to adjust the imbalance. As shown in **Table 5**, we found that early-onset CRC remained a higher risk factor (P<0.001) after correction, which was consistent with that before PSM.

Comparison of survival between early-onset and late-onset patients

With regard to the prognosis between early- and late-onset CRC, we generated K-M survival and cumulative event curves (**Figure 1**). As shown in **Figure 1A**, early-onset CRC patients from SEER database had better survival than late-onset CRC patients (P<0.0001). Additionally, the cumulative event curve also suggested that the prognosis of late-onset patients was poorer (**Figure 1B**). We matched 1009 early-onset patients with 1009 late-onset patients

Table 4. Univariate and multivariate logistic regression model for exploring the potential risk factors for lymph node metastasis in patients from our hospital

Variables (LNM Yes/No)	Univariate analysis	*P* Value	Multivariate analysis	*P* Value
Gender				
Male (25/250)	Reference	-		
Female (26/175)	1.486 (0.830-2.659)	0.182		
T stage				
T1a (4/97)	Reference	-	Reference	-
T1b (47/328)	3.475 (1.221-9.887)	0.020	2.963 (0.993-8.839)	0.041
Primary Site				
Left-side colorectum (40/368)	Reference	-		
Right-side colorectum (11/57)	1.601 (0.612-4.185)	0.337		
Pathological grade				
Well differentiated (2/40)	Reference	-		
Moderately (42/278)	3.022 (0.704-12.968)	0.137		
Poorly differentiated (6/41)	2.927 (0.557-15.370)	0.204		
Undifferentiated (1/66)	0.303 (0.027-3.45)	0.336		
Lymphatic invasion				
No (36/412)	Reference	-	Reference	-
Yes (15/13)	13.205 (5.83-29.89)	<0.001	12.683 (5.43-29.62)	<0.001
CRC type				
Early-onset CRC (19/100)	Reference	-	Reference	-
Late-onset CRC (32/325)	0.518 (0.281-0.954)	0.035	0.572 (0.294-0.959)	0.042
Tumor size				
≤3 cm (39/296)	Reference	-		
≤5 cm (10/94)	0.807 (0.388-1.680)	0.567		
>5 cm (2/35)	0.434 (0.100-1.874)	0.263		
Examined LNs				
<12 (31/316)	Reference	-	Reference	-
≥12 (20/109)	1.870 (1.024-3.418)	0.042	1.893 (0.98-3.658)	0.057
Smoking				
No (42/313)	Reference	-		
Yes (9/113)	0.592 (0.279-1.254)	0.171		
Drinking				
No (43/337)	Reference	-		
Yes (8/88)	0.712 (0.323-1.570)	0.400		
MLH1				
Negative (6/27)	Reference	-		
Positive (14/78)	0.808 (0.282-2.312)	0.691		
Unknown (31/320)	0.436 (0.167-1.137)	0.09		
MSH2				
Negative (5/22)	Reference	-		
Positive (15/82)	0.805 (0.264-2.457)	0.703		
Unknown (31/321)	0.425 (0.15-1.201)	0.106		
EGFR				
Negative (16/66)	Reference	-		
Positive (8/27)	1.222 (0.468-3.191)	0.682		
Unknown (27/332)	0.335 (0.171-0.657)	0.001		
C-erbB1				
Negative (16/81)	Reference	-		
Positive (11/51)	1.092 (0.47-2.539)	0.838		
Unknown (24/293)	0.415 (0.21-0.817)	<0.001		

Table 5. Basic information of patients from SEER database with colorectal cancer in T1 stage from 2010 through 2015 after propensity-score-matching

	Total	Early-onset CRC	Late-onset CRC	*P* value
Total	2070	1035	1035	
Gender				1.000
Male	980 (47.34%)	490 (47.34%)	490 (47.34%)	
Female	1090 (52.66%)	545 (52.66%)	545 (52.66%)	
Race				1.000
White	1604 (77.49%)	802 (77.49 %)	802 (77.49%)	
Black	276 (13.33%)	138 (13.33%)	138 (13.33%)	
Another	190 (9.18%)	95 (9.18%)	95 (9.18%)	
Primary Site				1.000
Right colorectum	390 (18.84%)	195 (18.84%)	195 (18.84%)	
Left colorectum	1578 (76.23%)	789 (76.23%)	789 (76.23%)	
Overlap/NOS	102 (4.93%)	51 (4.93%)	51 (4.93%)	
Pathological grade				1.000
Well	318 (15.36%)	159 (15.36%)	159 (15.36%)	
Moderately	1574 (76.04%)	787 (76.04%)	787 (76.04%)	
Poorly	158 (7.63%)	79 (7.63%)	79 (7.63%)	
Undifferentiated	20 (0.97%)	10 (0.97%)	10 (0.97%)	
N stage				<0.001
No	1708 (82.51%)	817 (78.94%)	891 (86.09%)	
Yes	362 (17.49%)	218 (21.06%)	144 (13.91%)	
Tumor size				1.000
≤3 cm	1694 (81.84%)	847 (81.84%)	847 (81.84%)	
≤5 cm	244 (11.79%)	122 (11.79%)	122 (11.79%)	
>5 cm	132 (6.38%)	66 (6.38%)	66 (6.38%)	
Examined LNs				1.000
<12	548 (26.47%)	274 (26.47%)	274 (26.47%)	
≥12	1522 (73.53%)	761 (73.53%)	761 (73.53%)	
Liver metastasis				0.128
No	2042 (98.65%)	1017 (98.26%)	1025 (99.03%)	
Yes	28 (1.35%)	18 (1.74%)	10 (0.97%)	
Median Survival months (Quartile)	35	35	34	

to investigate the association of survival with age at diagnosis (Supplementary Table 1). The K-M survival curve results showed that early-onset patients had poorer survival than late-onset patients (P<0.001) (**Figure 2**). In our data from the first affiliated hospital of Nanchang university, we excluded patients without survival information and showed the basic information of patients in Supplementary Table 2. In Supplementary Table 2, we observe that the distributions of lymphatic invasion and chemotherapy were significantly different (P<0.05), while most of the variables were not different. Additional analysis of survival revealed that the prognosis of early-onset patients was poorer (P=0.045) (**Figure 3**), in agreement with the result of the analysis with SEER data.

Discussion

Over the past several decades, early-onset CRC has received increasing attention because of its rising incidence. Worldwide, many countries, such as the USA, Australia, Canada and China, have documented disturbing and alarming trends [15]. Even the American Cancer Society has recommended that patients undergo examination for CRC screening at 45 years rather than 50 years, suggesting that the trend of younger patients with CRC is without doubt

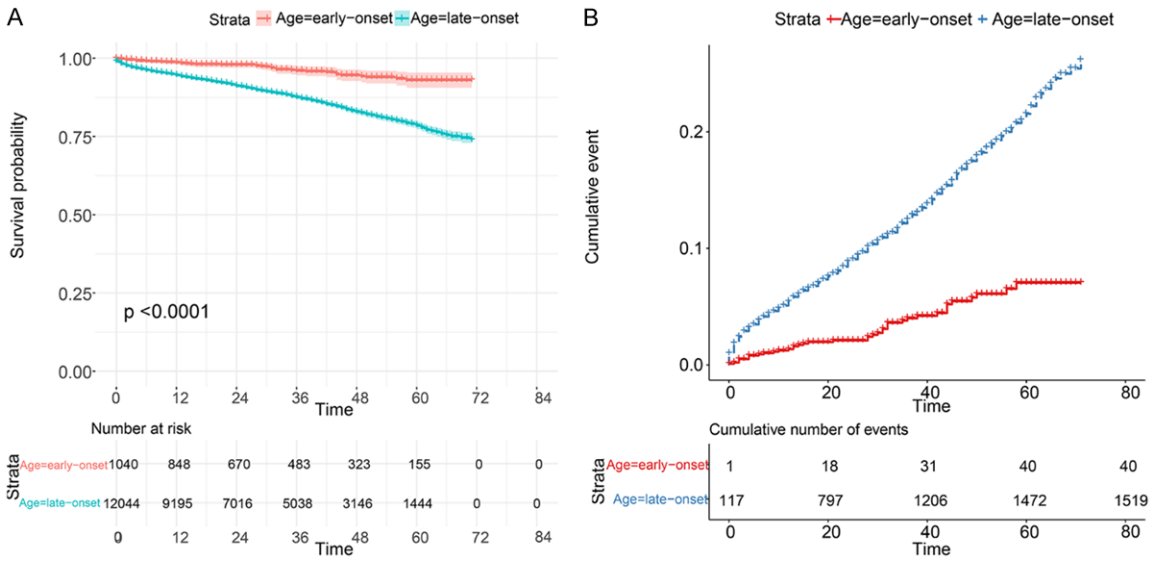

Figure 1. Comparison of survival between early- and late-onset CRC in T1 stage. A, B. K-M survival and cumulative events curves between the two groups.

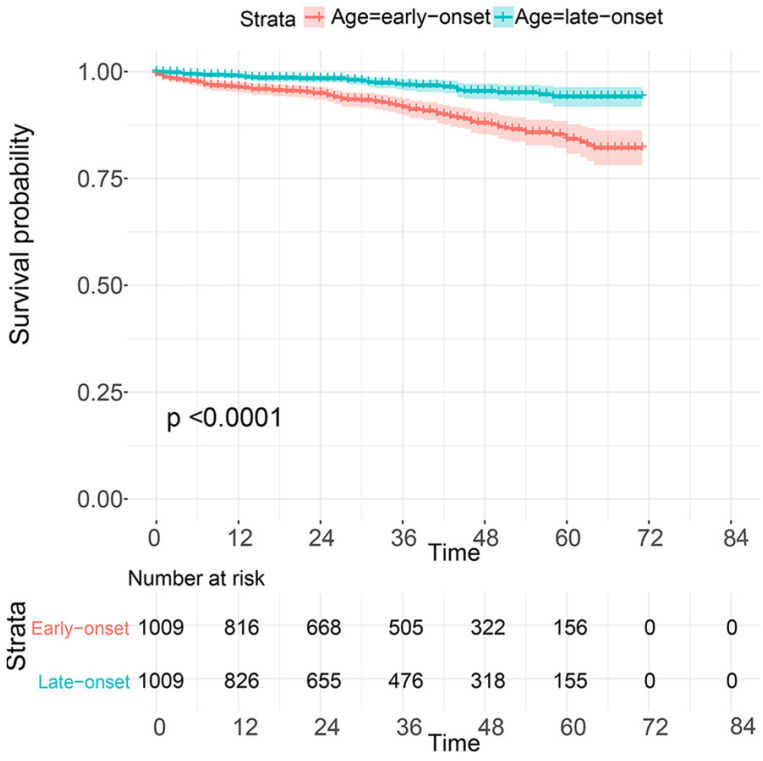

Figure 2. K-M survival curve between early- and late-onset CRC in T1 stage after PSM, performed using SEER data.

and needs a call to action [16]. However, there is little knowledge about the tumor characteristics of early- compared with late-onset CRC in the T1 stage. Our study is the first to compare the LNM and survival between early- and late-onset CRC in T1 stage.

Late-onset CRC has been stable or has declined slightly in developed countries, while early-onset cases have increased continuously in both high- and low-income countries, which might be attributable to factors such as genetic differences, environmental factors, diet, sedentary lifestyle or increased incidence of inflammatory bowel disease [17]. Because of these special exposure factors, the clinical features of early-onset CRC were different from those of late-onset CRC. In our study, both SEER data and our hospital data showed that early-onset CRC in T1 stage was common in female patients. Some studies found that male sex was a risk factor for early-onset CRC; however, this was unknown for T1 stage CRC [17, 18]. Several studies on T1 stage CRC did not illustrate the sex ratio between early onset and late onset, suggesting that our findings were new [19, 20]. In line with other studies, our study also found that early-onset CRC most commonly occurred in the left colorectum, whereas late-onset CRC had a similar frequency across the colorectum [9, 21]. As shown

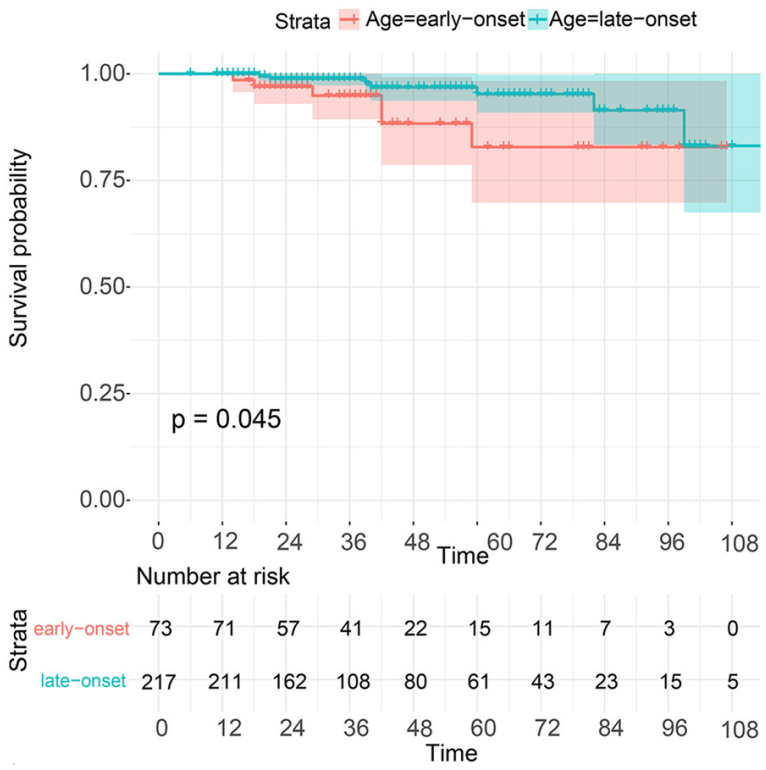

Figure 3. K-M survival curve between early- and late-onset CRC in T1 stage, performed with data from our hospital.

in other studies [22], we found that early-onset patients more often received chemotherapy. As for the genomic genes' expression such as MLH1, MSH2, EGFR and C-erbB2, some previous studies showed MLH1/MSH2-negative tumors were characterized by LNM, which was not consistent with our results [23, 24]. However, due to the limited sample in our study, it may not be very credible. Some studies also reported that occurrence of deficient mismatch repair genes including MLH1 and MSH2 were not correlated with LNM, but with perineural or lymphatic vascular invasion [25, 26]. These contradictory results could be explained by different samples and different detection methods. Of course, as for the association of EGFR and C-erbB2 with LNM, different studies had different results [27, 28]. Some factors associated with invasion, such as poor differentiation and lymphatic invasion, were more frequent in early-onset CRC, consistent with other studies [21, 29]. Similarly, we also directly found that early-onset CRC in T1 stage was associated with a higher prevalence of advanced stage, which was demonstrated by the higher ratios of LNM and distant metastasis. Several studies also showed that younger age was correlated with a higher risk of LNM [19, 30]. There are several possible reasons for this phenomenon. Our results and other studies showed that early-onset CRC was mainly located in the left colon; additionally, a site in the left side of the colorectum was considered a risk factor for LNM [20]. Lymphatic invasion and poor histological grade were also risk factors for LNM [31]. Furthermore, the degree of SM invasion and female sex were reported as risk factors [32]. All referenced risk factors were more frequent in early-onset CRC, which could explain why early-onset CRC in T1 stage was prone to positive LNM. Considering these confounding factors, we further performed PSM, because PSM has been effectively applied in many clinical studies [14]. The results revealed that early-onset CRC in the T1 stage had a higher rate of LNM. In addition, genetic alterations and gut bacteria profile were also important factors. Approximately 30% of early-onset patients had a family history of tumors, and mutations in mismatch repair genes such as MLH1 and PMS2 were more commonly observed. For instance, consensus molecular subtype (CMS) family members, such as CMS2 and CMS4, were mainly expressed in early-onset patients; these proteins could activate the WNT and MYC signaling pathways and further promote epithelial-to-mesenchymal differentiation [33].

For the survival analysis, there were some contradictory results in previous studies. Chen et al found that early-onset CRC in the T1 stage favored survival, as demonstrated via PSM and other comprehensive analyses [34]. However, some other studies considered early-onset CRC patients to have equivalent survival [35-37]. Our study showed that early-onset CRC in the T1 stage had poorer survival than late-onset CRC, which was demonstrated by data from two centers. Some studies also supported our

results [38-40]. Notably, findings across studies are often incomparable owing to the heterogeneity in study designs and populations.

In conclusion, according to data from two centers, we found that early-onset CRC in T1 stage had a higher risk of LNM than late-onset CRC of the same stage, especially for tumors with poor differentiation, larger tumor size (>5 cm) or deeper SM invasion. Therefore, in light of these results, more care should be taken when endoscopic treatments such as ESD and EMR are performed on younger patients with T1 stage CRC. More accurate assessments of LNM and R0 resection will be imperative for reducing recurrence in early-onset T1 stage CRC. Furthermore, the poorer prognosis of early-onset patients may require us to more frequently perform preventive CRC screening and immediate follow-up.

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Disclosure of conflict of interest

None.

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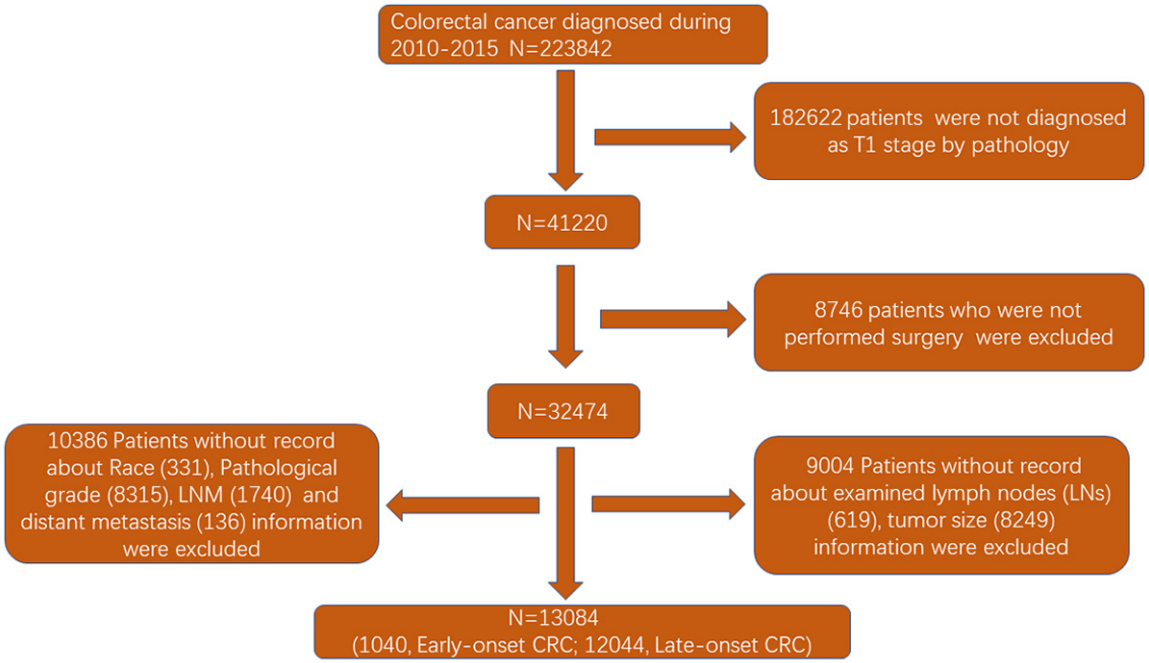
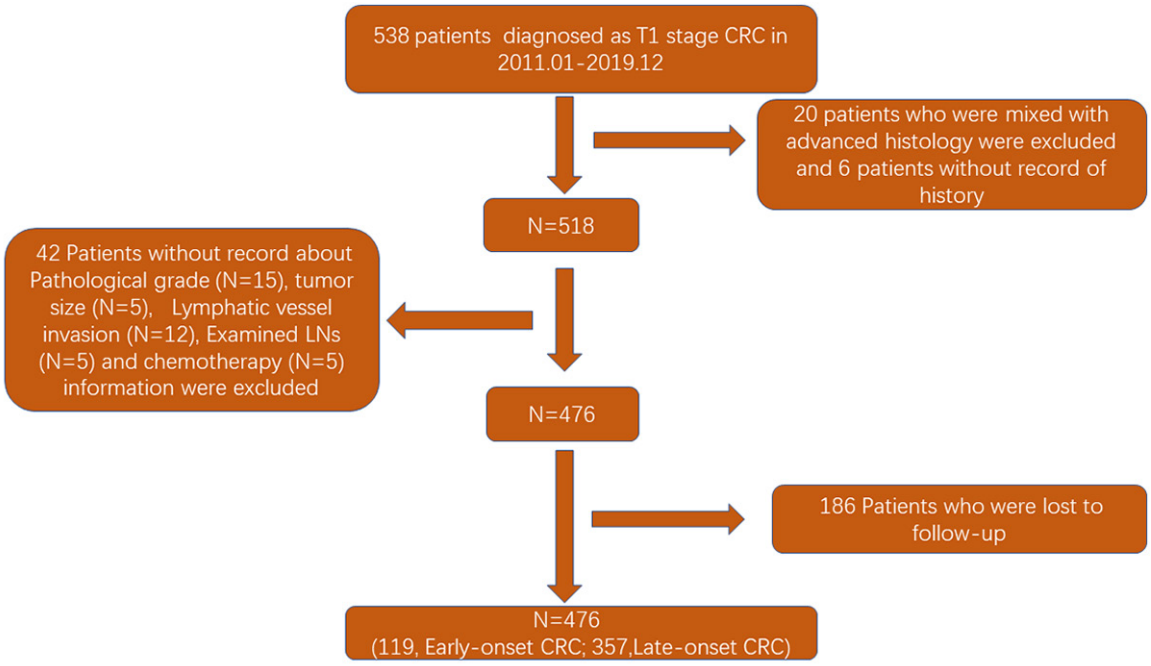


Supplementary Figure 1. The flow chart of extracting information of patients from SEER database.



Supplementary Figure 2. The flow chart of extracting information of patients from the First affiliated hospital of Nanchang university.

Supplementary Table 1. Basic information of patients with colorectal cancer in T1 stage from 2010 through 2015 after propensity-score-matching

	Total	Early-onset CRC	Late-onset CRC	*P* value
Total	2018	1009	1009	
Gender				1.000
Male	958 (47.47%)	479 (47.47%)	479 (47.47%)	
Female	1060 (52.53%)	530 (52.53%)	530 (52.53%)	
Race				0.997
White	1569 (77.75%)	784 (77.70 %)	785 (77.80%)	
Black	266 (13.18%)	133 (13.18%)	133 (13.18%)	
Another race	183 (9.07%)	92 (9.12%)	91 (9.02%)	
Primary Site				0.989
Left colorectum	376 (18.63%)	187 (18.53%)	189 (18.73%)	
Right colorectum	1547 (76.66%)	774 (76.71%)	773 (76.61%)	
Overlap/NOS	95 (4.71%)	48 (4.76%)	47 (4.66%)	
Pathological grade				1.000
Well differentiated	308 (15.26%)	154 (15.26%)	154 (15.26%)	
Moderately	1549 (76.76%)	774 (76.71%)	775 (76.81%)	
Poorly differentiated	145 (7.19%)	73 (7.23%)	72 (7.14%)	
Undifferentiated	16 (0.79%)	8 (0.79%)	8 (0.79%)	
N stage				0.955
No	1625 (80.53%)	813 (80.57%)	812 (80.48%)	
Yes	393 (19.47%)	196 (19.43%)	197 (19.52%)	
M stage				
No	1997 (98.96%)	999 (99.01%)	998 (98.91%)	0.826
Yes	21 (1.04%)	10 (0.99%)	11 (1.09%)	
Tumor size				0.987
≤3 cm	1669 (82.71%)	834 (82.66%)	835 (82.76%)	
≤5 cm	236 (11.69%)	119 (11.79%)	117 (11.60%)	
>5 cm	113 (5.60%)	56 (5.55%)	57 (5.65%)	
Examined LNs				0.960
<12	537 (26.61%)	269 (26.66%)	268 (26.56%)	
≥12	1481 (73.39%)	740 (73.34%)	741 (73.44%)	
Liver metastasis				1.000
No	2004 (99.31%)	1002 (99.31%)	1002 (99.31%)	
Yes	14 (0.69%)	7 (0.69%)	7 (0.69%)	
Median Survival months (Quartile)	35	35	35	

Supplementary Table 2. Basic information of patients who has survival information with colorectal cancer in T1 stage from our hospital

	Total	Early-onset CRC	Late-onset CRC	*P* value
Total	290	73	217	
Gender				0.168
Male	167 (57.58%)	37 (50.68%)	130 (59.91%)	
Female	123 (42.41%)	36 (49.32%)	87 (40.09%)	
T stage				0.074
T1a	69 (23.79%)	23 (31.51%)	46 (21.20%)	
T1b	221 (76.21%)	50 (68.49)	171 (78.80%)	
Primary Site				0.494
Right-side colorectum	37 (12.76%)	11 (15.07%)	26 (11.98%)	
Left-side colorectum	253 (87.24%)	62 (84.93%)	191 (88.02%)	
Pathological grade				0.348
Well differentiated	18 (6.21%)	7 (9.59%)	11 (5.07%)	
Moderately	196 (67.59%)	44 (60.27%)	152 (70.05%)	
Poorly differentiated	32 (11.03%)	10 (13.70%)	22 (10.14%)	
Undifferentiated	44 (15.17%)	12 (16.44%)	32 (14.75%)	
N stage				0.401
No	258 (88.97%)	63 (86.30%)	195 (89.86%)	
Yes	32 (11.03%)	10 (13.70%)	22 (10.14%)	
Lymphatic invasion				0.012
No	272 (93.79%)	64 (87.67%)	208 (95.85%)	
Yes	18 (6.21%)	9 (12.33%)	9 (4.15%)	
M stage				0.084
No	289 (99.66%)	72 (98.63%)	217 (100.00%)	
Yes	1 (0.34%)	1 (1.37%)	0 (0.00%)	
Tumor size				0.804
≤3 cm	209 (72.07%)	53 (72.60%)	156 (71.89%)	
≤5 cm	56 (19.31%)	15 (20.55%)	41 (18.89%)	
>5 cm	25 (8.62%)	5 (6.85%)	20 (9.22%)	
Examined LNs				0.618
<12	212 (73.10%)	55 (75.34%)	157 (72.35%)	
≥12	78 (26.90%)	18 (24.66%)	60 (27.65%)	
Chemotherapy				0.019
No	274 (94.48%)	65 (89.04%)	209 (96.31%)	
Yes	16 (5.52%)	8 (10.96%)	8 (3.69%)	
Treatment methods				0.218
Robot	41 (14.14%)	6 (8.22%)	35 (16.13%)	
Laparoscopy	191 (65.86%)	50 (68.49%)	141 (64.98%)	
Surgery	58 (20.00%)	17 (23.29%)	41 (18.89%)	
Radiotherapy				0.275
No	283 (97.59%)	70 (95.89%)	213 (98.16%)	
Yes	7 (2.41%)	3 (4.11%)	4 (1.84%)	
Smoking				0.066
No	215 (74.14%)	60 (82.19%)	155 (71.43%)	
Yes	75 (25.86%)	13 (17.81%)	62 (28.57%)	
Drinking				0.892
No	233 (80.34%)	59 (80.82%)	174 (80.18%)	
Yes	57 (19.66%)	14 (19.18%)	43 (19.82%)	