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Synchronous Resectable Metastatic Colorectal Cancer

Lymph Node Involvement Predicts Poor Outcome

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Abstract: To evaluate the value of lymph node status of primary tumors in predicting the prognosis of synchronous resectable metastatic colorectal cancer (mCRC).

The characteristics of resectable mCRC are substantially different from other cancers, and the prognostic factors of resectable mCRC are still controversial.

The data of 2007 patients with mCRC who received resection of the primary tumors and metastatic lesions synchronously were reviewed from the Surveillance, Epidemiology and End-Result database. The Kaplan–Meier method was used to evaluate the capacity of different prognostic factors. Univariate and multivariate logistic regression models were used to evaluate the relationship between the lymph node status and other factors. The mRNA profiles of primary resectable mCRC tumors were obtained by microarray at our center.

The median survival times were 50, 36, 32, 27, and 19 months in the N0-stage, N1a-stage, N1b-stage, N2a-stage, and N2b-stage subgroups

according to the 7th American Joint Committee on Cancer (AJCC) Tumor Lymph Node Metastasis (TNM) N-classification ($P = 0.000$), and 40, 29, 22, and 15 months in patients with metastatic lymph node ratio (LNR) <0.25 , $0.25–0.49$, $0.5–0.74$, and ≥ 0.75 subgroups ($P = 0.000$). In the COX model, the 7th AJCC TNM N-stage and LNR were independent prognostic factors. The mRNA profile was not associated with lymph node involvement.

Both the N-stage according to the 7th AJCC TNM staging system and LNR had the capacity to subclassify synchronous resectable mCRC with different prognoses. The lymph node might be integrated into the AJCC staging system as a diagnose-delay prognostic factor for stage IV disease.

(*Medicine* 94(30):e1215)

Abbreviations: CI = confidence intervals, LNR = lymph node ratio, mCRC = metastatic colorectal cancer.

Editor: Wafi Attaallah.

Received: March 27, 2015; revised: June 26, 2015; accepted: June 30, 2015.

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Key Projects in the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period (2014BAI09B07).

Training Program of the Major Research Plan of the National Natural Science Foundation of China (91229104).

The National High Technology Research and Development Program of China (863 Program) (2012AA02A506).

The National Science and Technology Major Project (2011ZX09307-001-05).

The National High Technology Research and Development Program of China (863 Program) (2012AA02A204).

The authors have no conflicts of interest to disclose.

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ISSN: 0025-7974

DOI: 10.1097/MD.0000000000001215

INTRODUCTION

Colorectal cancer is the 4th most common cancer and the second-leading cause of cancer-related death worldwide, making it a serious threat to public health. Approximately 20% of patients are diagnosed with metastatic colorectal cancer (mCRC, or stage IV colorectal cancer), and more than 1/3 of those initially diagnosed with localized disease will develop mCRC.^{1,2} In the 7th American Joint Committee on Cancer (AJCC) Tumor Lymph Node Metastasis (TNM) staging system, stage IV is subclassified into stage IVa (metastasis confined to one organ or site) and stage IVb (metastasis in more than one organ/site or the peritoneum).³ However, the clinical application of this classification has not been further validated and was called into question by Kobayashi et al.⁴ Patients with mCRC who do not undergo surgery have a shorter survival time.^{5,6} Radical resection is the only known method to cure the disease, and this technique could achieve a 5-year overall survival rate of 30% to 60%. There is a lack of strong evidence supporting a good clinical outcome following surgical resection, but both the European Society for Medical Oncology and National Comprehensive Cancer Network guidelines recommend radical resection as the standard therapy.⁷ Moreover, obvious heterogeneity exists in the results of resectable mCRC. Approximately 2/3 of patients with resectable mCRC will suffer recurrence and treatment failure.^{8–10} The most effective strategy to improve the outcome is to stratify resectable mCRC accurately and to personalize treatment. In previous studies, several risk score models were proposed to predict outcomes, but all of the models were complicated and in discord. Therefore, the prognostic factors of resectable mCRC remain controversial, and a simple and reliable factor to predict the prognoses of resectable mCRC is needed. The status of the lymph node of the primary tumor was confirmed as a prognostic indicator in localized CRC.³ Furthermore, the lymph node ratio (LNR) was recognized as a prognostic factor in localized

CRC.^{11,12} However, the value of lymph node status of primary tumors in predicting the prognosis of resectable mCRC remains unclear.

The current population-based analysis using the Surveillance, Epidemiology and End-Result (SEER) database was performed to confirm the predicted value of lymph node status of the primary tumor in resectable mCRC.

METHODS

Origins of Materials

The SEER registry sponsored by the National Cancer Institution collects information on cancer incidence and survival. The current SEER database (from 2004–2007) consists of 18 population-based cancer registries that represent approximately 27.8% of the population of the United States. The SEER data contain no identifiers and are publicly available for studies of cancer-based epidemics and health policy. We obtained the permission to access the research data (Reference Number: 10937-Nov2013). The study was approved by the review board of the Second Affiliated Hospital of Zhejiang University School of Medicine. The SEER. Stat software was used to identify patients with synchronous resection of both the primary tumor and distant metastatic lesions during the period 2004 to 2007. Patients diagnosed after 2007 were excluded to ensure adequate duration of follow-up. Then, a total of 2007 obtained cases were regrouped according to the 7th AJCC TNM staging system.

A total of 15 patients registered in our center with resectable mCRC were selected to extract RNA for further analysis, with 7 patients in the lymph node negative subgroup and 8 patients in the lymph node positive subgroup. The tissue samples were obtained from the tumor tissue bank in our laboratory, which was approved by the reviews board of the Second Affiliated Hospital of Zhejiang University School of Medicine.

Inclusion and Exclusion Criteria

The specific inclusion criteria were as follows: the years of diagnosis ranged from 2004 to 2007; site record ICD-O-3 was limited to colon and rectum; the “surgery therapy of others regional/distance” field of SEER. Stat was limited to surgical procedures at distant sites so that only the synchronous resectable mCRC was included; histological type ICD-O-3 was limited to 8140(adenocarcinoma), 8480(mucinous adenocarcinoma), and 8490 (signet ring cell cancer); and the stage was confirmed to be stage IV according to the 7th AJCC TNM staging system, including stage IVa and stage IVb. The exclusion criteria were as follows: primary tumor or regional lymph nodes were not removed; patients lacking documentation of race and age at diagnosis; patients younger than 18 years or older than 90 years; patients with multiple primary tumors were excluded to make the analyses of cancer-specific survival more accessible; and the patients surviving less than 1 month were excluded because such patients may die of surgical complications or rapidly progress after actually palliative resection.

Extraction of Total RNA

Freshly frozen tissue samples of primary colorectal cancers from 15 patients with resectable mCRC were obtained. All tissue samples were collected, immediately snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Written informed consent from each patient was obtained according to the institutional regulations. Total RNA isolation was performed with TRIzol (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. The RNA concentration was determinate

using the NanoDrop-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) was used to assess the integrity of the RNA. RNA samples used in this study all had a 260/280 ratio above 1.8 and an RNA integrity number greater than 5.0.

mRNA Profiles

Cyanine-3 (Cy3)-labeled cRNA was prepared from 0.5 μg total RNA using the One-Color Low RNA Input Linear Amplification PLUS kit (Agilent), followed by RNeasy column purification (QIAGEN, Valencia, CA). A total of 1.65 μg of Cy3-labeled cRNA (specific activity $>6.0\text{ pmol}$) was fragmented and hybridized to Agilent 4 \times 44K Whole Human Genome Oligo Microarrays (G2600D) using the Gene Expression Hybridization Kit (Agilent). After hybridization, the microarrays were washed with the Gene Expression Wash Buffer Kit (Agilent) and scanned with Agilent's Feature Extraction 9.1 software with default parameters. The microarray data have been deposited in NCBI's Gene Expression Omnibus with the series accession number GSE63596.

Analysis of mRNA Profiles

The statistical analysis of microarray data was performed with the GeneSpring GX Analysis Software v11.5.1 (Agilent). Raw data were preprocessed by log 2 transformation, and normalization between all arrays was performed using the 75th percentile method. Analyses where 100% of the samples in any condition had values were included. The significance of differential expression between 2 groups was estimated using the *t*-test. Only those entities with an adjusted *P*-value (*q*-value) < 0.05 (Benjamini correction for multiple testing) were considered to be significant. Heat-map and clustering were performed in Mev4.8.0.¹³ Hierarchical clustering with average linkage using the Pearson correlation as a distance metric was applied to cluster the samples according to their mRNA expression levels.

Statistical Analyses

The year and age at diagnosis, gender, race, site record, histological type, differentiated grade, 7th TNM T-stage, number of metastatic lymph nodes, number of regional lymph nodes examined, the level of carcinoembryonic antigen (CEA), survival months, and cause of death were retrieved from the SEER database. Site of tumor was coded as colon and rectum. Race was divided into white, black, and other. Age was classified into young (≤ 50 -years old) and old (> 50 -years old) groups. All cases were regrouped according to the 7th AJCC TNM staging system.³ Cancer-specific overall survival was calculated from the date of diagnosis to the date of death of cancer. Death attributed to other causes was defined as a censored observation.

Survival curves were generated using the Kaplan–Meier methods, and the log-rank test was performed to evaluate the differences in survival. Adjusted hazard ratios along with 95% confidence intervals (CI) were calculated using the Cox proportional hazards regression model. Differentiated grade, T-stage, and N-stage were considered as ordered categorical covariates, whereas race and histological type were considered as nonordered categorical covariates. All missing values were transformed by the method of median of nearby points.¹⁴ We evaluated the impacts of T-stage, race, age, histological type, differentiated grade, and location on lymph node metastasis using univariate and multivariate logistic regression models. When the 2-side *P*-value was less than 0.05, the difference was

TABLE 1. The Characteristics of 2007 Patients With Resectable Metastatic Colorectal Cancer

Risk Factors	N, %	5-year OS, %	Median (months)	P (Univariate Analysis)
Race				0.005
White	1578 (78.6)	25.6	28	
Black	258 (12.9)	15.1	24	
Others	171 (8.5)	25.5	33	
Age				0.000
≤50 years	476 (23.7)	28.1	35	
>50 years	1531 (76.3)	23.1	26	
Gender				0.029
Male	957 (47.7)	25.9	30	
Female	1050 (52.3)	22.9	27	
Year of diagnosis				0.632
2004	510 (25.4)	24.1	29	
2005	488 (24.3)	25.0	28	
2006	505 (25.2)	22.9	28	
2007	504 (25.1)	0	29	
Location of primary tumor				0.000
Colon	1772 (88.3)	23.5	27	
Rectum	235 (11.7)	34.1	43	
Histological type				0.000
Adenocarcinoma	1689 (84.2)	26.1	31	
Mucinous adenocarcinoma	264 (13.2)	19	22	
Signet ring cell carcinoma	54 (2.7)	7.1	16	
Differentiated grade				0.000
Well	85 (4.2)	34.9	40	
Moderate	1263 (62.9)	27.5	33	
Poor	531 (26.5)	17.1	19	
Undifferentiated	40 (2.0)	16.4	16	
Unknown	88 (4.4)	24.2	28	
T-stage*				0.000
T1	29 (1.4)	42.2	43	
T2	68 (3.4)	33.9	43	
T3	1240 (61.8)	26.7	32	
T4	644 (32.1)	17.8	21	
Unknown	26 (1.3)	57.7	70	
N-stage*				0.000
N0	362 (18.0)	41.8	50	
N1a	263 (13.1)	31.7	36	
N1b	389 (19.4)	25.2	32	
N2a	411 (20.5)	20.9	27	
N2b	508 (25.3)	12.9	19	
Unknown	74 (3.7)	15.6	23	
LNR				0.000
<0.25	943 (47.0)	35.1	40	
0.25–0.49	421 (21.0)	21.5	29	
0.50–0.74	279 (13.9)	13.2	22	
≥0.75	281 (14.0)	8.8	15	
Unknown	83 (4.1)	13.8	22	
The level of CEA [†]				0.000
Positive	1026 (51.1)	21.6	27	
Negative	344 (17.1)	35.9	40	
Unknown	637 (31.8)	23.5	27	

CEA = carcinoembryonic antigen, LNR = ratio of metastatic lymph node, OS = overall survival.

*T-stage and N-stage according to the 7th edition of AJCC TNM staging.

†The reference value of CEA: nonsmoker <2.5 ng/mL, smoker <5 ng/mL.

considered to be statistically significant. The SPSS 16.0 (SPSS Chicago IL) software was used for data analysis.

RESULTS

Characteristics of 2007 Patients

The cut-off date of follow-up was November 2013, with a median follow-up of 27.0 months (range from 1 to 95 months). A total of 2007 eligible patients were analyzed, with a median survival of 27.0 months and 5-year overall survival of 24.3%. A total of 1571 (78.3%) patients were diagnosed with lymph node involvement, whereas 362 (18.0%) patients were free of lymph node metastasis. The status of the lymph node of 74 (3.7%) patients was unknown. Only 235 (11.7%) patients were diagnosed with rectal cancer. The median age was 61-years old (range from 18 to 90-years old). The detailed characteristics of the patients are provided in Table 1.

Univariate Predictors of Outcome

The univariate analysis showed that the median survival times were 50, 36, 32, 27, and 19 months in the N0-stage, N1a-stage, N1b-stage, N2a-stage, and N2b-stage subgroups, respectively, according to the 7th TNM N-classification, with significant difference ($P=0.000$). The 5-year overall survival rates were 41.8, 31.7, 25.2, 20.9, and 12.9% in the N0-stage, N1a-stage, N1b-stage, N2a-stage, and N2b-stage subgroups according to the 7th TNM N-classification, respectively (Figure 1A). The median survival times were 40, 29, 22, and 15 months in the LNR <0.25, 0.25–0.49, 0.5–0.74, and ≥ 0.75 subgroups, respectively, with significant differences ($P=0.000$). The 5-

year overall survival rates were 34.3%, 21.5%, 13.2%, and 8.8% in the LNR <0.25, 0.25–0.49, 0.5–0.74, and ≥ 0.75 subgroups, respectively (Figure 1B).

Additionally, race, gender, age, location, histological type, differentiated grade, T-stage, and the level of CEA could predict the outcome, whereas the year of diagnosis could not (Table 1) (Appendix 1, <http://links.lww.com/MD/A347>).

Multivariate Analyses of Outcome

All of the factors associated with survival based on the univariate analysis were included in the COX model. The multivariate analysis showed that race, age, tumor location, histological type, differentiated grade, T-stage, N-stage, and the level of CEA were independent prognostic factors. Gender was not an independent prognostic factor. In the COX model, the N-stage was the most weighted factor, with a Wald of 104.63 (Table 2). When the N-stage was replaced by the LNR in the COX model, the LNR was also the most weighted factor, with a Wald of 167.61.

Factors Associated With Lymph Node Metastasis

The univariate analysis showed that age, grade, histology type, T-stage, and the level of CEA were correlated to the status of lymph node. The young group, the undifferentiated grade, mucinous adenocarcinoma, T4-stage, and CEA positive status were associated with increased lymph node metastases. When multivariate analysis was performed with the factors listed above, the factors of age, differentiated grade, T-stage, and CEA status could independently predict lymph node metastasis (Table 3).

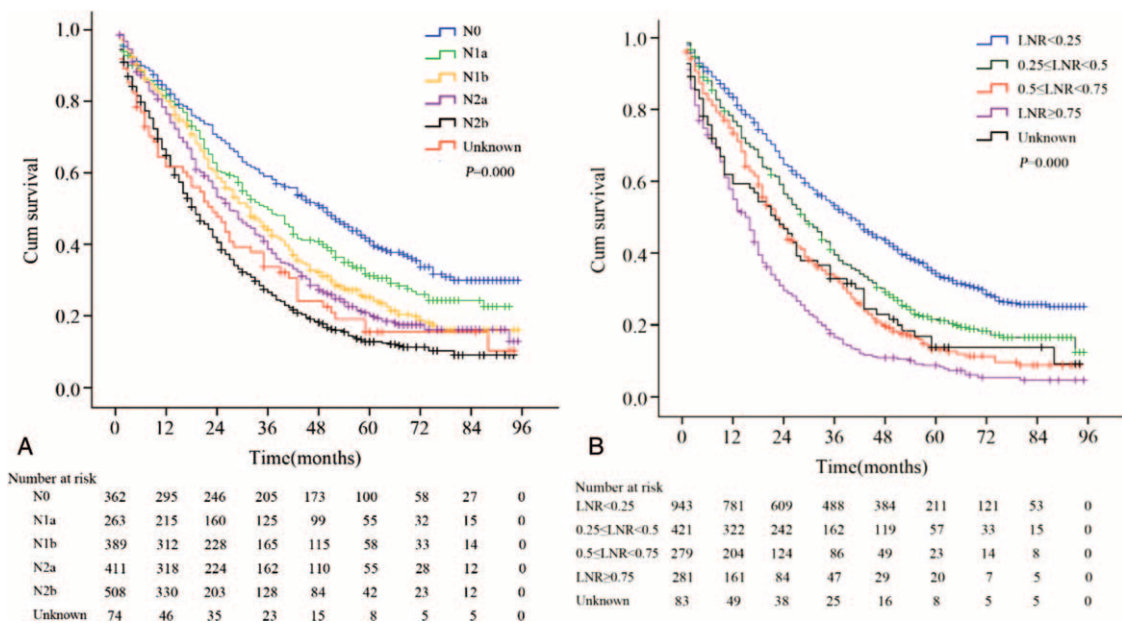


FIGURE 1. The overall survival in subgroup analysis with lymph node status. (A) The 5-year overall survival rate was 41.8%, 31.7%, 25.2%, 20.9%, and 12.9% for the N0-stage, N1a-stage, N1b-stage, N2a-stage, and N2b-stage subgroups, respectively ($P=0.000$). According to the 7th AJCC TNM staging system, N0-stage: no regional lymph node metastasis; N1a-stage: metastasis in 1 regional lymph node; N1b-stage: metastasis in 2–3 lymph nodes; N2a-stage: metastasis in 4–6 lymph nodes; and N2b-stage: metastasis in 7 or more regional lymph nodes. Unknown regional lymph nodes could not be accessed. (B) The 5-year overall survival rate was 34.3%, 21.5%, 13.2%, and 8.8% in patients in the ratios of metastatic lymph nodes <0.25, 0.25–0.49, 0.5–0.74, and ≥ 0.75 subgroups, respectively ($P=0.000$). Lymph node ratio (LNR): lymph node metastasis rate, calculated by the metastatic lymph nodes divided by the total evaluated lymph nodes.

TABLE 2. Multivariate Analysis (Cox Proportional Hazard Model) of Overall Survival for 2007 Patients with Synchronous Resectable Metastatic colorectal Cancer (the Status of the Lymph Was Determined Based on the N-Stage)

Variable (reference)	Wald	HR	95.0% CI for HR	P
Age (≤50-year old)	29.204	1.400	1.239–1.581	0.000
Gender (male)	1.682	1.070	0.966–1.186	0.195
Race (other)	10.616			0.005
White	0.494	1.069	0.887–1.289	0.482
Black	6.846	1.346	1.077–1.682	0.009
Location (colon)	10.678	0.753	0.635–0.893	0.001
Histological type (signet ring cell cancer)	20.323			0.000
Adenocarcinoma	8.180	0.641	0.473–0.869	0.004
Mucinous	0.976	0.848	0.612–1.176	0.323
Differentiated grade (well)	25.086	1.266	1.154–1.388	0.000
T-stage (T1)*	21.724	1.251	1.138–1.374	0.000
N-stage (N0)*	104.629	1.216	1.171–1.262	0.000
CEA (positive)†	28.239	0.679	0.589–.783	0.000

CEA = carcinoembryonic antigen, CI = confidence interval, HR = hazard ratio.

* T-stage was subclassified as T1, T2, T3, and T4; N-stage was subclassified as N0, N1a, N1b, N2a, and N2b according to the 7th AJCC TNM staging system.

† The reference value of CEA: nonsmoker <2.5 ng/mL, smoker <5 ng/mL.

TABLE 3. Multivariate Analysis of the Factors Associated With Lymph Node Metastasis

	LN Negative	LN Positive	Univariate Analysis		Multivariate Analysis
			HR*	P	P
Race				0.651	NA
White	290 (19.2)	1224 (80.8)	0.887	0.576	
Black	43 (17.1)	209 (82.9)	1.021	0.936	
Others	29 (17.4)	138 (82.6)	1		
Age				0.001	0.001
Young (≤50 years)	61 (13.4)	393 (86.6)	1.646		
Old (>50 years)	301 (20.4)	1178 (79.6)	1		
Gender				0.558	NA
Male	179 (19.3)	750 (80.7)	0.934		
Female	183 (18.2)	821 (81.8)	1		
Location				0.108	NA
Colon	311 (18.2)	1397 (81.8)	1.317		
Rectum	51 (22.7)	174 (77.3)	1		
Histological type				0.060	0.227
Adenocarcinoma	302 (18.5)	1330 (81.5)	0.383	0.068	
Mucinous	56 (22.3)	195 (77.7)	0.303	0.028	
Signet ring cell	4 (8.0)	46 (92.0)	1		
Differentiated grade				0.000	0.000
Well	24 (28.9)	59 (71.8)	0.447	0.111	
Moderate	254 (20.7)	975 (79.3)	0.698	0.424	
Poor	60 (11.7)	452 (88.3)	1.370	0.498	
Undifferentiated	6 (15.4)	33 (84.6)	1		
T-stage†				0.000	0.003
T1	11 (45.8)	13 (54.2)	0.001	0.238	
T2	22 (32.4)	46 (67.6)	0.002	0.421	
T3	219 (18.1)	993 (81.9)	0.484	0.912	
T4	103 (16.7)	512 (83.3)	1		
CEA‡				0.015	0.023
Positive	166 (16.7)	829 (83.3)	1.460		
Negative	76 (22.6)	260 (77.4)	1		

CEA = carcinoembryonic antigen, HR = hazard ratio, NA = nonapplicable.

* The HR of positive lymph nodes.

† T-stage was subclassified as T1, T2, T3, and T4 according to the 7th AJCC TNM staging system.

‡ The reference value of CEA: nonsmoker <2.5 ng/mL; smoker <5 ng/mL.

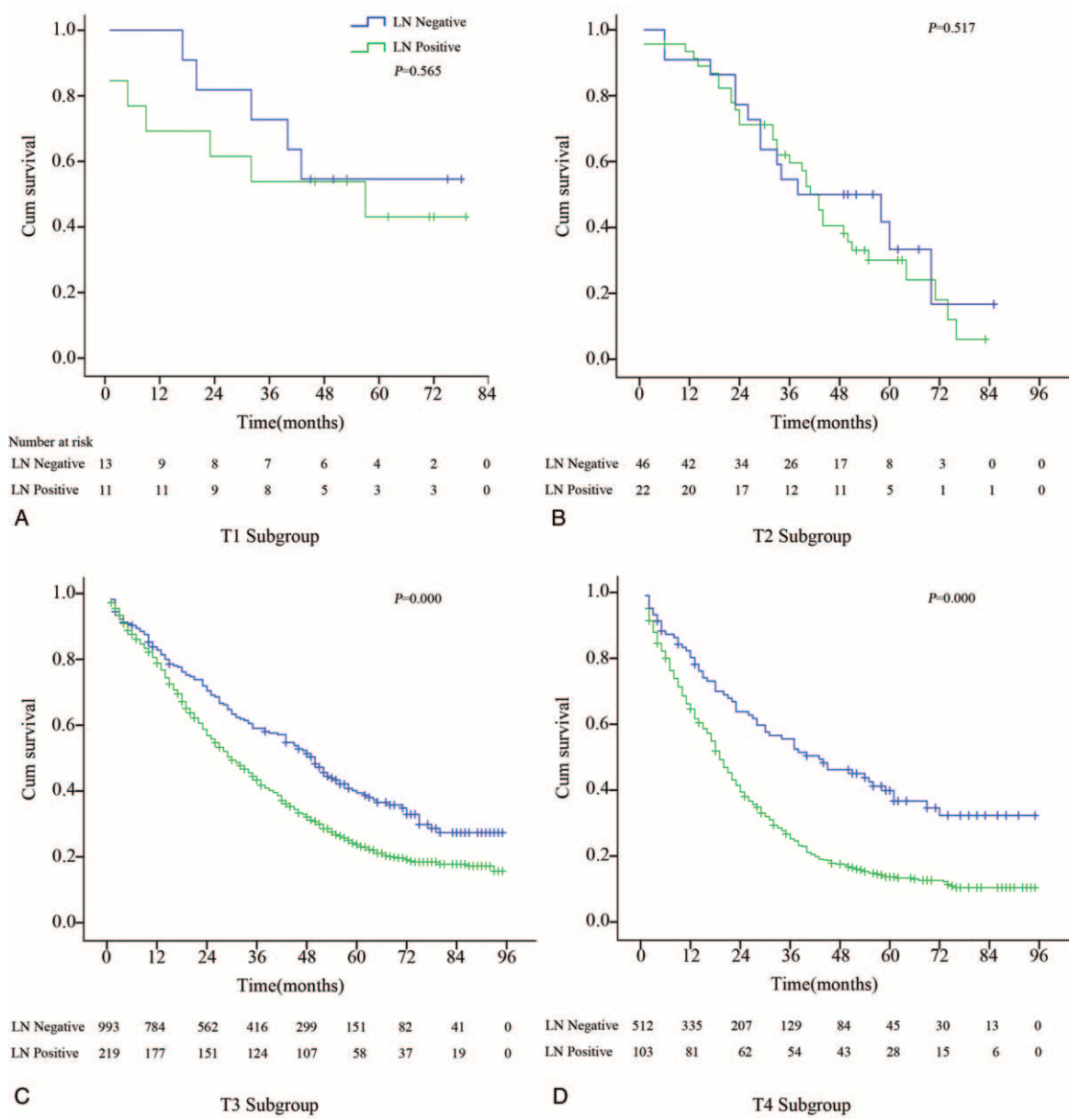


FIGURE 2. The overall survival in subgroup analysis with T-stage. (A) In the T1-stage subgroup, the lymph node could not predict survival, $P=0.565$. (B) In the T2-stage subgroup, the lymph node could not predict survival, $P=0.517$. (C) In the T3-stage subgroup, the patients with lymph node metastasis had worse survival, $P=0.000$. (D) In the T4-stage subgroup, the patients with lymph node metastasis had a worse survival, $P=0.000$.

The stratified analysis with T-stage showed that lymph node metastasis could predict poor prognosis only in the T3-stage ($P=0.000$) and T4-stage ($P=0.000$) (Figure 2C,D), but not in T1-stage ($P=0.565$) and T2-stage ($P=0.517$) (Figure 2A,B). The outcomes of the stratified analyses using other factors were shown in Figure 3.

The Distinction of the mRNA Profile

The detailed characteristics of 15 patients with resectable mCRC selected in our center are provided in Appendix 2, <http://links.lww.com/MD/A347>. The mRNA profiles of the primary

tumor tissues were analyzed. No obvious difference was found between the lymph node positive subgroup and the lymph node negative subgroup based on the heat maps of mRNA profiles (Figure 4). There was no distinct mRNA expression profile identified among 27,598 eligible entities.

DISCUSSION

In the current study, the 5-year overall survival rate for 2007 cases with synchronous resectable metastatic lesions was 24.3%. This was lower than the survival rate reported by the study of Thelen et al, who found a 5-year overall survival rate of

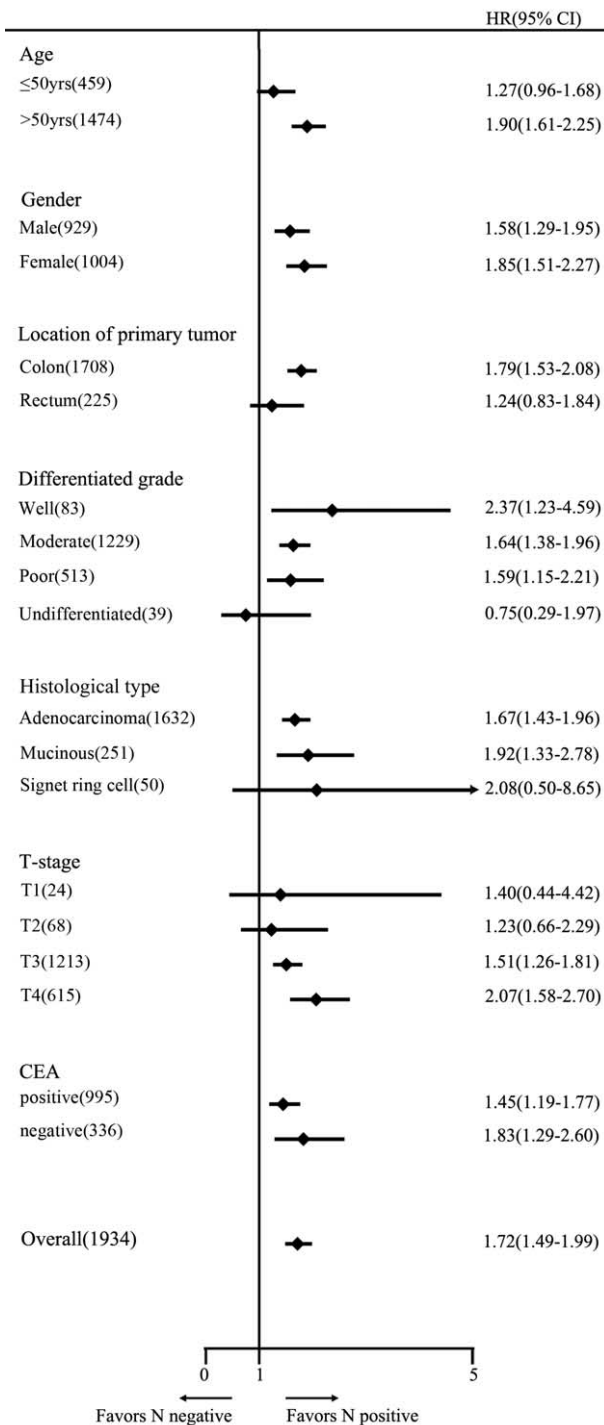


FIGURE 3. Hazard ratios and 95% CIs in different subgroups (Forest plot analysis). CI = confidence index, HR = hazard ratio.

53%.¹⁵ The following reasons partially contributed to this disparity. Firstly, resection of both the primary tumor and distant metastatic lesions were not equal to radical resection. In other words, some patients may receive palliative resection. Secondly, previous studies on colorectal cancer with liver

metastases indicated that patients with extra hepatic metastases had poor survival,^{16,17} and some patients with extra hepatic metastases were included in the current study. Furthermore, synchronous liver metastases were considered to be a poor prognostic factor.¹⁷⁻²² All patients in the current study had synchronous metastases.

Several factors can influence the outcome of resectable mCRC, including primary tumor characteristics, metastatic lesion feature, the condition of therapy, and the selection of patients.⁸⁻¹⁰ In recent years, surgery complications have obviously decreased, with an operation mortality of less than 2%.⁹ Therefore, the extent of resection was further expanded.²³ Advanced imaging methods such as enhanced magnetic resonance imaging and positron emission tomography-computed tomography have aided in the more accurate selection of patients.^{24,25} Therefore, tumor characteristics were considered to play a key role in the prediction of the prognosis of resectable mCRC.

Either N-stage or LNR can differentiate the heterogeneity of resectable mCRC. The multivariate analyses also showed that N-stage and LNR were independent prognostic factors. The lymph node status of primary tumor is easy to obtain in clinical practice. Therefore, prediction using N-stage or LNR is easy to perform and more reliable than other complicated prognostic models.

Lymph node metastasis was correlated with poor outcome in most studies,^{16,17,19,21,26,27} but several studies showed contradictory results.^{22,28,29} The different sample size used in various studies might result in this discrepancy. For example, 3 studies including more than 1000 patients showed that lymph node metastasis was correlated with poor prognosis.^{16,17,19} However, in those studies the status of the lymph node was categorized as positive or negative rather than into the 5 levels used in the current study. Additionally, the LNR was first applied to subclassify resectable mCRC, an approach that was proven to be feasible and effective in our study.

Many of the studies listed above were confined to patients with resectable liver metastases. By contrast, there have only been rare studies focused on mCRC. In recent years, patients with extra hepatic metastases have also been permitted to undergo surgical resection.³⁰⁻³² Therefore, the conception of resectable mCRC will be applied more extensively. Huh et al³³ chose 468 consecutive patients with curatively resectable stage IV colorectal cancer for analysis. The 3 and 5-year overall survival rates were 66.5% and 52.1%, respectively. The univariate analysis showed that the N-stage was related to survival, but this finding did not translate into an independent risk factor for survival according to the Cox regression model. Only adjuvant chemotherapy and the preoperative serum CEA levels were independent prognostic factors for overall survival.³³ Stratified analysis with the status of the lymph node showed that CEA status could predict prognosis only in the N0-stage and the N1-stage ($P=0.046$ and 0.013 , respectively). For patients with the N2-stage, CEA status could not predict the outcome ($P=0.948$). In our study, both the level of CEA and status of lymph node were independent prognostic predictors.

A study by Huh et al³³ showed that T-stage and differentiated grade were related to lymph node metastases, which is similar to our result.³³ In their study,³³ rectal cancer increased the potential to present lymph node metastasis. This result contradicted our finding that colonic cancer was associated with increased lymph node metastasis, although the association did not reach statistical significance. However, in the current study we found that the level of CEA was linked to lymph node metastasis, which was not mentioned in their study.

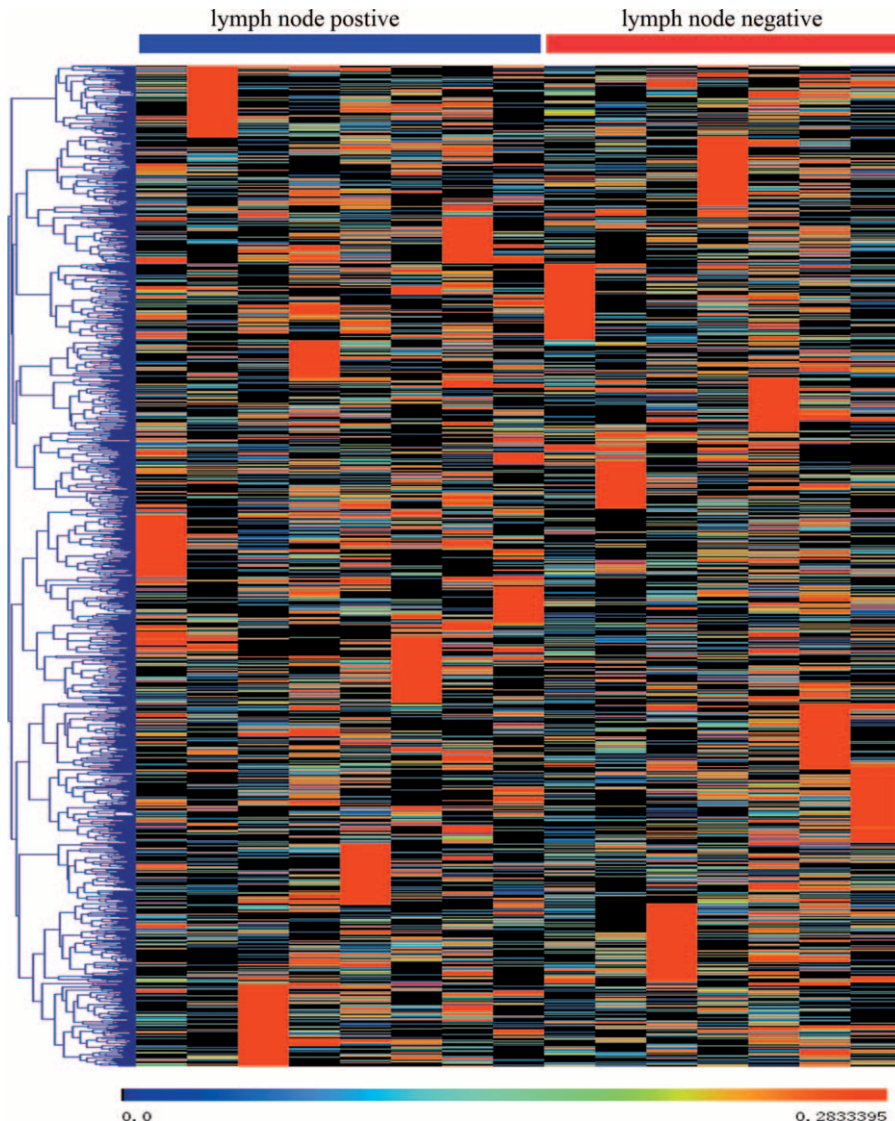


FIGURE 4. Clustering analysis of 15 samples from primary tumors of resectable metastatic colorectal cancers representing 8 patients with positive lymph node and 7 patients with negative lymph node. mRNAs were included in the tree when normalized expression values ranged from 20% to 70% of all mRNAs. A total of 1240 mRNAs were retained for clustering. Hierarchical clustering with average linkage using Pearson correlation as the distance metrics was applied to cluster the samples according to their mRNA-normalized expression values.

It is well-known that 2 reasons may contribute to distant site metastasis. First, the “seed” is so aggressive that metastases can occur as an early event. The biological behavior of the “seed” plays a key role in the process of metastasis. Second, the “seed” may not be aggressive, but metastasis may occur due to failure to diagnose with localized disease at an early stage; therefore, metastasis resulted from delayed diagnoses.

High levels of CEA and later T-stage predict a delayed diagnose. Based on the phenomenon that CEA positivity and advanced T-stage were correlated with lymph node metastasis, we proposed the hypothesis that lymph node metastases may result from delayed diagnose rather than the biological behavior of the tumor. The mRNA array analysis supported this hypothesis, in that minimal differences existed between the lymph node positive and negative subgroups.

The stratified analysis showed that a positive lymph node can predict the prognosis of patients in T3-stage and T4-stage cancers, but not in T1-stage and T2-stage disease. Thus, for patients with tumors in the T1-stage and T2-stage diagnosed with distant metastases, inherent biological factors of the “seed” may contribute to the tumor metastatic process regardless of the presence of lymph node metastasis, thereby playing a key role in the prediction of prognosis.

CONCLUSION

Extremely heterogeneous prognoses are associated with synchronous resectable mCRC. The status of the lymph node can identify such heterogeneity, using either the 7th TNM N-stage or LNR. Based on our finding, the lymph node status

serves as an important diagnostic and available prognostic factor for resectable mCRC and should be considered for integration into the Staging System as a stratified item for mCRC.

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