Comparison of Long-term outcomes between Lynch sydrome and sporadic colorectal cancer: a propensity score matching analysis

Yun Xu
Fudan University Shanghai Cancer Center
Cong Li
Fudan University Shanghai Cancer Center
Charlie Zhi-Lin Zheng
UCLA: University of California Los Angeles
Yu-Qin Zhang
Fudan University Shanghai Cancer Center
Tian-An Guo
Fudan University Shanghai Cancer Center
Fang-Qi Liu
Fudan University Shanghai Cancer Center
Ye Xu (yexu@shmu.edu.cn)
Fudan University Shanghai Cancer Center

Research article

Keywords: Colorectal cancer, Lynch syndrome, Mismatch repair, Survival, Chemotherapy

Posted Date: December 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-130271/v1

Version of Record: A version of this preprint was published on January 9th, 2021. See the published version at https://doi.org/10.1186/s12885-020-07771-8.

Abstract

Background

Lynch syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome. Comparison of prognosis between LS and sporadic CRC (SCRC) were rare,with conflicting results. This study aimed to compare the long-term outcomes between patients with LS and SCRC.

Methods

Between June 2008 and September 2018, a total of 47 patients were diagnosed with LS by genetic testing at Fudan University Shanghai Cancer Center. A 1:2 propensity score matching was performed to obtain homogeneous cohorts from SCRC group. Thereafter, 94 SCRC patients were enrolled as control group. The long-term survival rates between the two groups were compared, and the prognostic factors were also analyzed.

Results

The 5-year OS rate of LS group was 97.6%, which was significantly higher than of 82.6% for SCRC group ($p = 0.029$). The 5-year PFS rate showed no significant differences between the two groups (78.0% for LS group vs. 70.6% for SCRC patients; $p = 0.262$). The 5-year TFS rates in LS group was 62.1% for LS patients, which were significantly lower than of 70.6% for SCRC group ($p = 0.039$). By multivariate analysis, we found that tumor progression of primary CRC and TNM staging were independent risk factors for OS.

Conclusion

LS patients have better long-term survival prognosis than SCRC patients. Strict regular follow-up monitoring, detection at earlier tumor stages, and effective treatment are key to ensuring better long-term prognosis.

Background

Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies worldwide and the second leading cause of cancer-related death globally [1]. CRC has been recognized as a heterogeneous disease based on different molecular mechanisms, therefore presenting heterogeneous outcomes and drug responses [2–4]. Lynch syndrome (LS) is the most common hereditary CRC syndrome. It results from heterozygous pathogenic germline variants in the mismatch repair (MMR) genes (path_*MLH1*, path_*MSH2*, path_*MSH6*, and path_*PMS2*), accounting for approximately 3–5% of all cases of CRC [5, 6]. In addition to higher risk for CRC, LS presents significantly higher risks for cancers in organs including the

endometrium, ovaries, stomach, small bowel, bile duct, pancreas and upper urinary tract [5, 6]. The consequent tumors present the phenotypes of MMR protein deficiency (dMMR) under immunohistochemistry (IHC) and microsatellite instability (MSI).

During the last decade, increasing multi-gene cancer panel tests on suspected LS patients have allowed the identification LS positive CRC patients at an increasing rate. Correspondingly, the introduction of tailored policies of management and treatment, different from those for sporadic colorectal cancer (SCRC), has largely contributed to the long-term prognosis of LS patients and their affected relatives. In theory, CRC with dMMR should have better prognosis and therapeutic responses because the MMR pathway is involved in triggering cell death after chemotherapy-induced DNA damage [4, 7–10].

Analysis of LS patients' survival prognoses has been reported by several western studies [7–17]. Nevertheless, because of the phenotype difference between LS and SCRC, research comparing the long-term prognoses of these two subgroups is rare and has conflicting results. In addition, almost all previous findings on LS were derived from Western medical centers, so data from Asian populations remain lacking. The aim of this study is to compare the long-term survival outcome of LS associated CRC patients with that of SCRC patients. A propensity score matching (PSM) analysis was used to balance the baseline of the two groups. Afterwards, overall survival (OS), progression-free survival (PFS), and tumor-free survival (TFS) were compared, and prognostic factors associated with survival were also analyzed. To our knowledge, the current article is the first report on survival of LS from one of the largest colorectal surgery centers in China, and thus may better inform the comprehensive understanding of LS and clarify the differences between LS and SCRC.

Materials And Methods

Patients

Between June 2008 and September 2018, a total of 22833 consecutive CRC patients underwent curative surgeries, depending on the location of tumors, at the Fudan University Shanghai Cancer Centre.

Multi-gene panel testing that included 139 genes was recommended for suspected LS patients and some of their affected relatives. All patients gave informed consent for genetic analyses. Germline variants were defined as variants carried by both patients and their respective family members, for whom genetic counseling was recommended. Through genetic testing, a total of 47 patients who identified as carrying a pathogenic variant (PV) in MMR genes were diagnosed with LS and classified as LS group. SCRC was defined as patients without neither family history nor dMMR phenotype. Base on the baseline of LS group, we matched 94 SCRC patients (1:2) by propensity score matching, a total of 94 patients were recruited as SCRC group.

Immunohistochemistry

MMR deficiency was determined according to the absence of protein expression for any one of several genes including *hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2*. IHC was performed using the fully automated BenchMark ULTRA platform (Ventana Medical Systems, Inc., Tucson, AZ, United States). Normal tissues adjacent to the tumor or lymphocytes in the stroma served as internal positive controls. Each result was confirmed by at least two experienced pathologists.

Mutation screening of KRAS and BRAF

Then methods of mutation detection in *KRAS* and *BRAF* were the same as our previous report [18]. All results were confirmed according to the criterion suggested by the manufacturer.

Next-generation sequencing

Peripheral blood (10 mL) was collected, stored in ethylenediaminetetraacetic acid tubes, and allowed to stand at 25 °C for 2 h. The supernatant was transferred to a 15-mL centrifuge tube and then centrifuged for 10 min at 2200 g at 4 °C. Thereafter, the intermediate white blood cells were transferred to a 1.5-ml centrifuge tube. The DNA was recovered using the MagPure FFPE DNA LQ Kit (Magen). NGS was conducted on the germline DNA as a standard genetic testing for germline analysis.

Sequence data were mapped to the reference human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization was performed using GATK 3.2. Germline SNVs were identified using Varscan with default parameters. Germline indels were identified using Varscan and GATK. Pathogenic variants were determined by a clinical molecular geneticist according to the guidelines of the American College of Medical Genetics [19]. ClinVar and Enigma were used during manual curation for final confirmation of the results.

Clinical data acquisition and follow-up evaluation

For the 141 enrolled patients, the baseline information on tumor characteristics, pathological results, and treatment were retrospectively obtained from medical charts. Follow-ups conducted for LS patients were according to clinical practice guidelines [20], and regular follow-ups were performed for SCRC patients. During the follow-up evaluations, any occurrence of tumor progression of primary CRC, metachronous CRC, and extra-colonic cancer was recorded.

Tumor progression was defined as any recurrent tumor at the anastomotic site, invasion of adjacent tissues, lymph node metastasis, or distant metastasis that developed within 5 years after surgery. Synchronous tumors were defined as two colorectal tumors that were discovered simultaneously or within 6 months of each other, and the metachronous colorectal tumors were discovered more than 6 months apart [21]. For patients with synchronous tumors, the tumor with the higher stage was documented as the primary tumor [17]. Extra-colonic cancers were defined as primary cancers within the disease spectrum of LS and presenting dMMR under IHC. This study was censored on July 31, 2020. The mean follow-up period was (80.3 ± 41.2) months for SCRC patients and (82.0 ± 57.2) months for LS patients, no significant difference was observed between the two groups in follow-up period ($\chi^2 = 0.238$, $p = 0.626$).

Analysis of outcome

The outcomes of this analysis were oncologic outcomes including OS, PFS, and TFS. OS time was defined as the period between the date of surgery to the date of death or last follow-up; the PFS time was defined as the period between the date of surgery and the date of tumor progression or last follow-up; and the TFS time was defined as the period between the date of surgery and the date of tumor progression, metachronous CRC, extra-colonic cancer or last follow-up. Treatment options involving repeat resection, chemotherapy, radiotherapy, immunotherapy, conservative treatment for these events were formulated based on the recommendations of our multidisciplinary team. The primary endpoint was the 5-year OS rate; the secondary endpoints were TFS and PFS rate.

Statistical Analysis

All analyses were performed using the R software package (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (statistical software (version 20.0; Chicago, Ill).

Propensity score matching analysis was performed using the R software package. We used propensity score matching to balance the assignment of the included patients. Patients were matched using the following baseline characteristics as covariates: age, gender, CEA, tumor location, tumor size, pathologic result (classification, differentiation grade, cancerous node, vascular invasion, perineural invasion), TNM stage, KRAS (wild type vs. variant type), NRAS (wild type vs. variant type), adjuvant chemotherapy (received or not) (Table 1). Each variable was multiplied by a coefficient that was calculated using logistic regression analysis, and the sum of these values was taken as the propensity score for individual patients. For matching, complex LS and SCRC pairs with an equivalent propensity score were selected by a 1: 2 matching. Thereafter, we matched 47 LS patients with 94 SCRC patients using the nearest neighbor method (caliber = 0.02).

Table 1
Demographic and clinical characteristics of 141 colorectal cancer patients.

Variables	LS group (N = 47)	SCRC group (N = 94)	χ^2 value	p value
Age (years)			0.070	0.792
< 50	35(74.5%)	68(72.3%)		
≥50	12(2.5.5%)	26(27.7)		
Gender			0.058	0.810
Male	26(55.3%)	54(57.4%)		
Female	21(44.7%)	40(42.6%)		
CEA (ng/ml)			0.104	0.747
≥ 5.2	7(14.9%)	16(17.0%)		
< 5.2	40(85.1%)	78(83.0%)		
Location			3.177	0.365
Right colon	18(38.3%)	40(42.6%)		
Left colon	20(42.6%)	41(43.6%)		
Rectal	5(10.6%)	11(11.7%)		
Multiple	4(8.5%)	2(2.1%)		
Tumor size [a](cm)	5.17 ± 2.61	5.21 ± 2.79	0.106	0.754
Pathological classification			0.474	0.789
Adenocarcinoma	34(72.3%)	71(75.5%)		
Partial mucinous	5(10.7%)	11(11.7%)		
Mucinous adenocarcinoma	8(17.0%)	12(12.8%)		
Differentiation grade			0.259	0.878
Well	1(2.1%)	1(1.1%)		
Moderately	28(59.6%)	56(59.5%)		
Poorly	18(38.3%)	37(39.4%)		
Cancerous node			0.534	0.465
Occurrence	2(4.3%)	7(7.4%)		
Absence	45(95.7%)	87(92.6%)		

Variables	LS group (N = 47)	SCRC group (N = 94)	χ^2 value	p value
Vascular invasion			0.534	0.461
Occurrence	8(17.0%)	21(22.3%)		
Absence	39(83.0%)	73(77.7%)		
Perineural invasion			0.252	0.616
Occurrence	6(12.8%)	15(16.0%)		
Absence	41(87.2%)	79(84.0%)		
T stage			0.130	0.937
T1	7(14.9%)	12(12.8%)		
T2	8(17.0%)	17(18.1%)		
T3	32(68.1%)	65(69.1%)		
N stage			1.840	0.399
N0	34(72.3%)	57(60.6%)		
N1	9(19.1%)	23(24.5%)		
N2	4(8.6%)	14(14.9%)		
Metastasis			0.265	0.607
Occurrence	2(4.3%)	6(6.4%)		
Absence	45(95.7%)	88(93.6%)		
TNM stage			1.200	0.753
I	13(27.7%)	27(28.7%)		
II	17(36.2%)	30(31.9%)		
III	15(31.8%)	31(33.0%)		
IV	2(4.3%)	6(6.4%)		
KRAS mutant	21(44.6%)	49 (52.1%)	0.695	0.404
NRAS mutant	2(4.3%)	3 (3.2%)	0.104	0.747
Adjuvant chemotherapy			1.420	0.233
Received	26(55.3%)	42(44.7%)		

We assessed the balance of all baseline covariates in Table 1 between the two groups after propensity score matching. Continuous variables were compared using the Student t test between the two groups. Categorical variables were compared using the Chi square test or Fisher's exact test. OS, TFS, and PFS curves were evaluated using Kaplan–Meier curves and compared using the log-rank test. Variables with p-values less than 0.05 in the univariate analysis were entered into a Cox proportional hazards model for multivariate analysis. For all statistical tests, two-tailed p-values less than 0.05 were considered statistically significant.

Results

Molecular Characteristics

In the LS group, PVs of *MLH1* were identified in 17 (36.2%) probands and those of *MSH2*, *MSH6*, and *PMS2* were identified in 18 (38.3%), 10 (21.3%), and 2 (4.2%) probands, respectively. Variants from LS patients and frequency of each variant in the Asian population are summarized in Table 2. In patients identified with PVs in MMR genes, the results of IHC MMR staining were consistent with those of gene detection.

Table 2
Variants in the LS patients and frequency of each variant in Asian population.

Gene	Variants (HGVS)	Clinical Significance	Frequency
MLH1	*NM_001167618.2(MLH1):c.-33del(p.Ile231fs)*	Pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.1976G > C (p.Arg659Pro)*	Pathogenic	0.00002
MLH1	*NM_000249.3(MLH1):c.883A > G (p.Ser295Gly)*	Pathogenic	No data
MLH1	*NM_000249.4(MLH1):c.244A > G (p.Thr82Ala)*	Likely pathogenic	0.00000
MLH1	*NM_000249.3(MLH1):c.979C > T (p.Gln327Ter)*	Pathogenic	0.0000
MLH1	*NM_000249.3(MLH1):c.1489_1490insCG(p.Arg497fs)*	Pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.199G > C (p.Gly67Arg)*	Pathogenic	0.0000
MLH1	*NM_000249.3(MLH1):c.2101C > A (p.Gln701Lys)*	Likely pathogenic	0.00249
MLH1	*NM_001167618.2(MLH1):c.-131_-130GA(p.Glu199fs)*	Pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.116 + 1G > A(5 prime UTR)*	Likely pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.1990-2A > G*	Pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.453 + 1G > T*	Likely pathogenic	No data
MLH1	*NM_000249.3(MLH1):c.250A > G (p.Lys84Glu)*	Likely pathogenic	No data
MSH2	*NM_000251.3(MSH2):c.1165C > T (p.Arg389Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.244A > T (p.Lys82Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.877A > G (p.Thr293Ala)*	Likely pathogenic	0.0000
MSH2	*NM_000251.3(MSH2):c.2038C > T (p.Arg680Ter)*	Pathogenic	0.0000
MSH2	*NM_000251.2(MSH2):c.1528C > T (p.Gln510Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.1963G > A (p.Val655Ile)*	Likely pathogenic	0.00012
MSH2	*NM_000251.2(MSH2):c.859G > T (p.Gly287Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.1077A > T (p.Arg359Ser)*	Pathogenic	No data
MSH2	*NM_000251.3(MSH2):c.1710T > G (p.Tyr570Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.352dup (p.Tyr118fs)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.1009C > T (p.Gln337Ter)*	Pathogenic	No data
MSH2	*NM_000251.3(MSH2):c.2131C > T (p.Arg711Ter)*	Pathogenic	0.0000
MSH2	*NM_000251.2(MSH2):c.1042C > T (p.Gln348Ter)*	Pathogenic	No data
MSH2	*NM_000251.2(MSH2):c.2021G > A (p.Gly674Asp)*	Likely pathogenic	No data

Gene	Variants (HGVS)	Clinical Significance	Frequency
MSH6	*NM_000179.2(MSH6):c.3252dup (p.Thr1085fs)*	Pathogenic	No data
MSH6	*NM_000179.2(MSH6):c.718C > T (p.Arg240Ter)*	Pathogenic	0.0000
MSH6	*NM_000179.2(MSH6):c.2294dup (p.Cys765fs)*	Pathogenic	No data
MSH6	*NM_000179.2(MSH6):c.3515G > C (p.Arg1172Thr)*	Pathogenic	No data
MSH6	*NM_000179.3(MSH6):c.3202C > T (p.Arg1068*)*	Pathogenic	No data
MSH6	*NM_000179.2(MSH6):c.652A > T (p.Lys218Ter)*	Pathogenic	0.00000
MSH6	*NM_000251.2(MSH2):c.518T > C (p.Leu173Pro)*	Likely pathogenic	No data
PMS2	*large intragenic in EXON9*	Pathogenic	No data
PMS2	*NM_000535.7:c.2 T > G(p.Met1Arg)*	Pathogenic	0.000

Patient groups

After propensity score 1:2 matching analysis, 94 SCRC patients were enrolled as control group. The baseline characteristics of these two groups were compared and summarized in the Table 1, and no significant difference was found between the two groups in any variables. In the LS group, synchronous CRC were observed in four (4/47, 8.5%) patients. The occurrence of synchronous tumors did not serve as a baseline since they are rarely observed in SCRC patients.

Progression of primary CRC and progression free survival

During the follow up, a total of 26 (27.7%) SCRC patients developed tumor progression including 14 (14.9%) of liver metastases, 4 (4.3%) abdominal lymph node metastasis, 3 (3.2%) of lung metastases, 3 (3.2%) of extensive invasion, and 2 (2.1%) of extensive metastasis. In LS group, 9 (19.1%) patients developed tumor progression including 4 (8.5%) of liver metastases, 2 of (4.3%) of extensive invasion, and 2 (4.3%) of abdominal lymph node metastasis. No significant differences were observed between the two groups (χ^2 = 1.216, p = 0.270). The 1-, 3-, and 5-year PFS rates for the SCRC group were 85.1%, 77.6%, and 70.6%, respectively, whereas those for the LS group were 95.7%, 86.9%, and 78.0%, respectively. No significant differences were observed between the two groups in PFS (χ^2 = 1.260, P = 0.262, Fig. 1).

All patients who developed tumor progression in both groups received 5-fluorouracil-based chemotherapy (XELOX or mFOLFOX6). In LS group, all patients received targeted agents, including cetuximab for 3 patients with wild-type KRAS and bevacizumab for 6 patients with variant-type KRAS. In SCRC group, cetuximab combined with chemotherapy was performed for 4 patients with wild-type KRAS and bevacizumab for 7 patients with variant-type KRAS.

Metachronous tumor and tumor free survival

During the follow-up period, 34.0% (16/47) of the patients in the LS group developed metachronous CRC, with an average period of (28.78 ± 29.14) months between the occurrence of primary and metachronous CRC. In addition, 11 patients developed 15 cases of primary extra-CRC, including 5 cases of endometrial cancer, 5 cases of gastric cancer, 2 cases of small intestinal cancer, and 1 case each of ovarian, breast, and cutaneous cancer. Therefore, in LS group, the 1-, 3-, and 5-year TFS rates were 89.4%, 71.5%, and 62.1% respectively, which were significantly lower than those in SCRC group (85.1%, 77.6%, and 70.6%, respectively; $\chi^2 = 4.258$, $p = 0.039$) (Fig. 2).

All patients with metachronous cancers received radical resection. Of the 16 patients who developed metachronous CRC, 14 patients underwent extended resection, including 9 cases of subtotal colectomy, 4 of extended left hemicolectomy, 1 of total colectomy, and 1 of extended right hemicolectomy; the other 2 patients underwent standard radical resection. 5-fluorouracil-based adjuvant chemotherapy was performed for 2 patients in stage II and 5 patients in stage III.

Overall survival

During the follow-up period, 4 (8.5%) LS patients, including 2 *MLH1* variants carriers and 2 *MSH2* variants carrier, died of tumor progression. No significant differences in OS were found among the four genotypes ($\chi^2 = 3.803$ $p = 0.430$). In SCRC group, 24 (25.5%) SCRC patients died of tumor progression.

For LS patients, the 1-, 3-, and 5-year OS rates were 100.0%, 97.6%, and 97.6%, respectively, which were significantly higher than those of SCRC patients (95.7%, 88.3%, and 82.6%, respectively; $\chi^2 = 4.745$; $p = 0.029$) (Fig. 3).

Prognostic factor analysis for OS and PFS

Univariate analysis showed that sex, etiology, pathological classification, cancerous node, vascular invasion, perineural invasion, TNM stage, and tumor progression of primary CRC were significantly associated with OS. Multivariate analysis showed that etiology (LS vs. SCRC), TNM stage, and tumor progression primary CRC were independent prognostic factors in OS (Table 3). In addition, univariate analysis showed that pathological classification, differentiation grade, cancerous node, vascular invasion, perineural invasion, and TNM stage were significantly associated with PFS. Multivariate analysis showed that cancerous node, vascular invasion, and TNM stage were the independent prognostic factors in PFS (Table 4).

Table 3
Factors associated with overall survival in the patients of LS and SCRC group (univariate and multivariate analysis).

Variable	Nb. patients	Nb. events	Univariate analysis		Multivariate analysis	
Total	141	28	χ^2	p value	Risk ratio (95% CI)	p value
Sex			4.745	0.028	1.360 (0.525–0.770)	0.527
Male	80	24				
Female	61	4				
Age			0.022	0.883		
<50	103	21				
≥50	38	7				
CEA			0.482	0.488		
<5.2	118	21				
≥5.2	23	7				
Etiology			4.745	0.029	0.106(0.025–0.446)	0.002
Lynch syndrome	47	4				
Sporadic CRC	94	24				
Location			3.235	0.357		
Right colon	58	8				
Left colon	61	14				
Rectal	16	3				
Multiple	6	3				
Pathological classification			11.122	0.004	1.504(0.770–2.937)	0.233
Adenocarcinoma	105	21				
Partial mucinous	16	0				
Mucinous adenocarcinoma	20	7				
Differentiation grade			2.611	0.271		
Well	2	0				

Variable	Nb. patients	Nb. events	Univariate analysis		Multivariate analysis	
Moderately	84	15				
Poorly	55	13				
Cancerous node			13.123	< 0.001	3.285(0.860-12.546)	0.082
Occurrence	9	5				
Absence	132	23				
Vascular invasion			10.163	0.001	3.404(0.915–12.662)	0.068
Occurrence	112	17				
Absence	29	11				
Perineural invasion			41.204	< 0.001	0.707(0.247–2.028)	0.519
Occurrence	21	13				
Absence	120	15				
TNM stage			57.546	< 0.001	2.968(1.478–5.964)	0.002
I	40	2				
II	47	4				
III	46	16				
IV	8	6				
KRAS			0.225	0.636		
Wild type	71	9				
Variant type	70	19				
NRAS			0.011	0.916		
Wild type	136	27				
Variant type	5	1				
Recurrence or metastasis of primary CRC			106.81	< 0.001	48.917(9.866-242.539)	< 0.001
Occurrence	35	25				
Absence	106	3				

Table 4
Factors associated with progression free survival in the patients of LS and SCRC group (univariate and multivariate analysis).

Variable	Nb. patients	Nb. events	Univariate analysis		Multivariate analysis	
Total	141	35	χ^2	p value	Risk ratio (95% CI)	p value
Sex			1.173	0.314		
Male	80	21				
Female	61	14				
Age						
<50	103	26	0.092	0.762		
≥50	38	9				
CEA			0.220	0.639		
<5.2	118	28				
≥5.2	23	7				
Etiology			1.260	0.262		
Lynch syndrome	47	9				
Sporadic CRC	94	26				
Location			2.018	0.569		
Right colon	58	11				
Left colon	61	17				
Rectal	16	5				
Multiple	6	2				
Pathological classification			19.274	< 0.001	1.345(0.865–2.090)	0.188
Adenocarcinoma	105	24				
Partial mucinous	16	0				
Mucinous adenocarcinoma	20	11				
Differentiation grade			7.260	0.027	0.761(0.343–1.689)	0.502
Well	2	0				

Variable	Nb. patients	Nb. events	Univariate analysis		Multivariate analysis	
Moderately	84	15				
Poorly	55	20				
Cancerous node			12.595	< 0.001	3.011(1.067–8.498)	0.037
Occurrence	9	6				
Absence	132	29				
Vascular invasion			43.571	< 0.001	0.236(0.109–0.512)	< 0.001
Occurrence	29	21				
Absence	112	14				
Perineural invasion			13.654	< 0.001	1.552(0.679–3.546)	0.297
Occurrence	21	15				
Absence	120	20				
TNM stage			52.055	< 0.001	2.841(1.619–4.986)	< 0.001
I	40	1				
II	47	8				
III	46	19				
IV	8	7				
KRAS			0.007	0.934		
Wild type	71	16				
Variant type	70	19				
NRAS			0.368	0.544		
Wild type	136	33				
Variant type	5	2				

Subgroup survival

For early-onset (< 50 years) CRC patients, the 1-, 3-, and 5-year OS rates for LS patients were 100%, 96.8%, and 96.8%, respectively, whereas those for the SCRC group were 95.6%, 89.7%, and 82.0%, respectively. No significant differences were observed between the two subgroups ($\chi^2 = 3.332$, P = 0.068, Fig. 4a).

For patients who develop tumor progression, the 1-, 3-, and 5-year OS rates for LS patients were 100.0%, 88.9%, and 88.9%, respectively, which were significantly higher than those of SCRC patients (84.6%, 57.7%, and 39.1%, respectively; $\chi^2 = 9.354$; $p = 0.002$) (Fig. 4b).

Discussion

As the most common hereditary CRC, LS has received increasing attention from colorectal surgeons, because hereditary background and molecular subtypes are significant factors in the prognosis of CRC patients [22]. However, due to LS's greater difficulty of diagnosis and its different behavior from sporadic CRC, it is difficult to compare the long term prognoses for LS and SCRC. Using propensity score matching analysis, with the clinical characteristics of LS as baseline and a 1:2 matching ratio between LS and SCRC patients, we achieved comparability between the two groups in the current study. We found that OS period in LS patients was significant longer than that in SCRC patients, which may indicate that long-term prognosis for LS patients is better than that of SCRC patients. We used tumor progression of primary CRC to calculate PFS and included metachronous tumor occurrence into the calculations for TFS, because although LS patients have higher metachronous tumor occurrence rates, the occurrence of such second primary tumors is different from primary tumor progression in clinical significance. We found that PFS for LS patients is comparable to that for SCRC group, whereas the high proportion of metachronous CRCs and extra-colonic cancers in LS group have remarkably shortened TFS time, resulting in higher TFS rates for the SCRC group.

By analyzing genetic testing results for suspected LS patients, we found that the Chinese population's LS genotype distribution is similar to distributions reported in western review,[6] with *MLH1* and *MSH2* being the majority. However, due to insufficient sample size, no significant differences in the phenotypes and long-term prognosis of each genotype can be found. The insufficient sample size relates to the wider issue that, the detection rate and genetic counseling of LS in the Chinese population are lower than those in western populations, which is already considered insufficient [23]. There are a few possible reasons. Firstly, for patients with high hereditary CRC risk, existing screening policies and procedures largely originate from Western guidelines and thus may not be fully suited for Asian populations. Secondly, hereditary CRC research in Asia is in its early stages, so there are only a few retrospective reports on the subject. Therefore, prospective studies with larger samples are needed to better understand the clinical characteristics, genotypes, and phenotypes of Asian LS patients. This would improve screening and follow-up policies for Asian patients, which would allow higher detection rates and even larger samples for more future prospective studies.

While comparing primary tumor progression rates, we found that the liver, lungs, and abdominal lymph nodes remain the most common sites for CRC metastasis. Studies have confirmed that LS patients have lower risk of tumor recurrence or distant metastasis, which implies that LS patients should have longer PFS [24, 25]. However, in the current study, the LS and SCRC groups show no statistical difference in PFS, despite showing a significant tendency of dispersion in their PFS survival curves (Fig. 1). A possible reason is that because the tumor stages in both samples are relatively early, both groups have longer

TFS. As a result, our follow-up time is not long enough to record tumor progression beyond TFS and find further statistical difference in PFS. Therefore, future studies should increase patient sample size and extend follow-up time to yield more accurate comparative results.

A higher probability of metachronous tumor occurrence, including endometrial cancers and other cancers in the LS tumor spectrum, is the most prominent feature of LS [6]. Thus, treating the first primary tumor is often only the first step of treatment for LS patients. In the current study, metachronous tumor occurrence was present in a large portion of patients in the LS group, which significantly shortened the LS group's TFS. Standardized postoperative monitoring and follow-ups enabled us to detect these metachronous tumors at earlier stages, most of which were cured after complete resection. Especially after any second primary tumor occurrence, we largely performed extensive radical resections and individualized postoperative adjuvant treatments, including chemotherapy, targeted therapy, etc., according to each patient's condition. As a result, in terms of survival rate, metachronous tumor occurrence should have no significant impact on OS if it can be cured through resection. This result highlights the importance of establishing and improving the registration system for hereditary CRC in clinical work. Creating standardized and individualized diagnosis and treatment plans with respect to each patients' heterogeneity, including in age, tumor, and molecular characteristics, etc., is key to ensuring good prognoses. Only through strict monitoring and follow-ups can we detect and treat metachronous tumors in a timely manner, and minimize their impact on the long-term survival of LS patients after treatment.

OS is the most important indicator for evaluating long-term prognosis, and the current study confirms what many studies have already proposed, that LS patients have better long-term prognosis than SCRC patients [7–17].The current study has confirmed that apart from factors intrinsic to the initial primary tumor, such as tumor characteristics, pathological type, and differentiation grade, tumor progression such as recurrence and metastasis is independent prognostic factor affecting long-term survival. Thus, given that microsatellite instability (MSI) tumors experience lower rates of local tumor recurrence, especially at distant sites, than do microsatellite-stable (MSS) tumors [24].CRC displaying MSI that are diagnosed at early stages have a better prognosis compared to CRC displaying MSS [24]. In line with these expectations, our results show that the 5-year survival rate of LS patients is 97.6%, which is significantly higher than the 82.6% of SCRC patients. In addition, our multivariate analyses show that the etiology of the two groups (LS vs. Sporadic) is an independent factor in long-term survival after surgery. Thus, the current study further confirms that LS's long-term prognosis is better than that of SCRC.

With respect to oncologic outcomes, LS-associated tumors are associated with better prognoses and therapeutic responses, in part because the DNA MMR system, which is missing in CRCs with MSI, is involved in triggering cell death after chemotherapy-induced DNA damage [7]. We did not include chemotherapy into our analysis of prognostic factors, however, because during survival analysis, we found that chemotherapy and TNM staging overlapped in their effects on prognosis, since only patients with late staging (II/III) received chemotherapy. Nonetheless, during subgroup analysis, we found that for patients with distant metastasis, although all received chemotherapy combined with targeted therapy such as bevacizumab and cetuximab, the LS group had longer OS than the SCRC group. This

phenomenon further affirms the aforementioned explanation that LS patients, who has dMMR and thus MSI, should be more responsive to chemotherapy than SCRC patients. In fact, several prospective-retrospective analyses collecting data from different randomized trials demonstrated that the use of adjuvant 5-FU-based chemotherapy was effective in stage II CRC displaying MSI [24–27].In particular, stage III MSI cases did benefit from adjuvant 5-FU-based chemotherapy, albeit only if the MSI was associated with LS [24].Although numerous western guidelines on LS treatment exist today, there is no recognized consensus on treatment for recurrence and metastasis, due to significant differences between individual patients. Therefore, targeted combined chemotherapy should at least be offered to LS patients with recurrence or metastasis, regardless of whether complete resection can be performed. Simultaneous, corresponding prospective clinical trials and basic research will also contribute significantly to future treatment selection for preventing postoperative LS tumor progression and improving long-term survival.

During the analysis of factors in long-term survival, we found that microvascular invasion, cancer nodule occurrence, and the late TNM staging of the first primary CRC are three independent risk factors that affect PFS and OS. These factors have already been widely recognized as adverse prognostic factors [28, 29], which this study has once again confirmed. Given that all three factors occur later in a tumor's course of disease, early diagnosis and treatment can mitigate these adverse factors and ensure better survival prognoses. Thus, after an LS patient is diagnosed, the patient's relatives (esp. first- and second-degree relatives) should undergo genetic counseling and standardized follow-up monitoring in accordance to guidelines, as they have a higher risk of cancer than the general population.

Lastly, because early-onset tumors (< 50 y/o) is another important characteristic of LS, we conducted a subgroup analysis and comparison of LS and SCRC early-onset CRC patients. However, we found no significant difference in comparison of OS, which indicates that early-onset CRC is an indicator of hereditary CRC in general. Moreover, current domestic epidemic reports in China show a trend of CRC incidence at an increasingly younger age, which indicates that early-onset CRC is a growing concern [30]. Thus, early-onset cancer patients are recommended to undergo genetic testing in order to increase the detection rate of hereditary CRCs, including LS, so that more precise treatment and follow-ups can be performed for the patient and his/her relatives

The current study has the following limitations. Firstly, this study is retrospective. Even though propensity score matching was used, selection bias is still hard to avoid. Secondly, the LS group contains patients with synchronous primary tumors, a factor which could not be balanced on the baseline and thus may affect the results. Lastly, the sample size for the LS group is relatively small, and would ideally require further accumulation and longer follow-up times.

Conclusion

In conclusion, the results of the current study indicate that LS patients have better long-term OS than SCRC patients, even though the two groups have comparable PFS. This indicates that LS patients with dMMR may be more responsive to chemotherapy than SCRC patients. Therefore, in treating LS, especially

for LS patients who developed progression of primary CRC, targeted combined chemotherapy may be the standard treatment. In addition, strict regular follow-up monitoring, detection at earlier tumor stages and effective treatment for tumor progression and metachronous tumors are key to ensuring better long-term prognosis. Lastly, establishing a database for LS patients across Asian populations would allow a deeper understanding of the clinical-pathological, molecular-pathological and familial characteristics of LS in Asia, and would provide a stronger theoretical basis for the screening, treatment, and follow-up monitoring of these LS patients and their relatives.

Abbreviations

CRC: colorectal cancer; CEA: carcinoembryonic antigen; IHC: immunohistochemistry; LS: Lynch syndrome; MMR: mismatch repair; MSI: microsatellite instability; MSS: microsatellite stability; OS: overall survival; PFS: progression-free survival; PSM: propensity score matching; PV: pathogenic variant; TFS: tumor-free survival; SCRC: sporadic colorectal cancer;

Declarations

Ethics approval and consent to participate

All examinations and treatments were conducted at the Fudan University Shanghai Cancer Center (Shanghai, China) and were in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of the Fudan University Shanghai Cancer Center. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

Consent for publication: All authors approved the final version of the manuscript.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests*:* The authors declare that they have no competing interests.

Funding*:* Funding for this study was provided by the National Natural Science Foundation of China (No. 81472620), and the Shanghai Natural Science Foundation (No. 16ZR1406700).

Authors' Contributions*:* (I) Conception and design: YX, FQL, and YX; (II) Administrative support: YX, FQL, and YX; (III) Provision of study materials or patients: YX, CL, CZL, and YQZ; (IV) Collection and assembly of data: YX, CL, TAG, and FQL; (V) Data analysis and interpretation: YX and TAG; (VI) Manuscript writing: YX; (VII) Final approval of manuscript: All authors.

Acknowledgments*:* Not applicable

Authors' information: 1 Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Dong'an road, 270, Shanghai 200032, China; 2 Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China; 3 Mechanical & Aerospace Engineering, University of California, 7400 Boelter Hall Los Angeles, CA 90095, The United States of America.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68: 394-424..
2. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015; 21(11):1350-6.
3. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008;135:1079-99.
4. Alex AK, Siqueira S, Coudry R, Santos J, Alves M, Hoff PM, et al. Response to Chemotherapy and Prognosis in Metastatic Colorectal Cancer With DNA Deficient Mismatch Repair. Clin Colorectal Cancer. 2017;16:228-39.
5. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005;352:1851-60.
6. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer. 2015; 15: 181-94.
7. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000;342:69-77.
8. Koopman M, Kortman GA, Mekenkamp L, Ligtenberg MJ, Hoogerbrugge N, Antonini NF, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer. 2009 ;100:266-73.
9. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005; 23: 609-18.
10. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003; 349:247-57.
11. Cohen R, Buhard O, Cervera P, Hain E, Dumont S, Bardier A, et al. Clinical and molecular characterisation of hereditary and sporadic metastatic colorectal cancers harbouring microsatellite instability/DNA mismatch repair deficiency. Eur J Cancer. 2017;86:266-74.
12. Lautrup CK, Mikkelsen EM, Lash TL, Katballe N, Sunde L. Survival in familial colorectal cancer: a Danish cohort study. Fam Cancer. 2015;14:553-9.
13. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first

report from the prospective Lynch syndrome database. Gut. 2017; 66:464-72.

14. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective Lynch syndrome database. Gut. 2017;66:1657-64.
15. Dominguez-Valentin M, Seppälä TT, Sampson JR, Macrae F, Winship I, Evans DG, et al. Survival by colon cancer stage and screening interval in Lynch syndrome: a prospective Lynch syndrome database report. Hered Cancer Clin Pract. 2019;17:28.
16. Liu GC, Liu RY, Yan JP, An X, Jiang W, Ling YH, et al. The Heterogeneity Between Lynch-Associated and Sporadic MMR Deficiency in Colorectal Cancers. J Natl Cancer Inst. 2018;110:975-84.
17. Haraldsdottir S, Hampel H, Wu C, Weng DY, Shields PG, Frankel WL, et al. Patients with colorectal cancer associated with Lynch syndrome and MLH1 promoter hypermethylation have similar prognoses. Genet Med. 2016 ;18:863-8.
18. Guo TA, Wu YC, Tan C, Jin YT, Sheng WQ, Cai SJ, et al. Clinicopathologic features and prognostic value of KRAS, NRAS and BRAF mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with Stage I-IV colorectal cancer. Int J Cancer. 2019 ;145:1625-34.
19. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
20. Gupta S, Provenzale D, Llor X, Halverson AL, Grady W, Chung DC, et al. NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 2.2019. J Natl Compr Canc Netw. 2019;17:1032-41.
21. Moertel CG. Multiple primary malignant neoplasms: historical perspectives. Cancer. 1977; 40 (4 Suppl):1786-92.
22. Koh PK, Kalady M, Skacel M, Fay S, McGannon E, Shenal J, et al. Familial colorectal cancer type X: polyp burden and cancer risk stratification via a family history score. ANZ J Surg. 2011;81(7-8):537-42.
23. Faust N, Muller C, Prenner J, Lee SM, Kupfer SS. Low Rates of Genetic Counseling and Testing in Individuals at Risk for Lynch Syndrome Reported in the National Health Interview Survey. Gastroenterology. 2020; 158: 1159-61.
24. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst. 2011 ;103:863-75.
25. Hemminki A, Mecklin JP, Järvinen H, Aaltonen LA, Joensuu H. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology. 2000; 119:921-8.

26. Liang JT, Huang KC, Lai HS, Lee PH, Cheng YM, Hsu HC, et al. High-frequency microsatellite instability predicts better chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV sporadic colorectal cancer after palliative bowel resection. Int J Cancer. 2002;101:519-25.
27. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010;28: 3219-26.
28. Dienstmann R, Villacampa G, Sveen A, Mason MJ, Niedzwiecki D, Nesbakken A, et al. Relative contribution of clinicopathological variables, genomic markers, transcriptomic subtyping and microenvironment features for outcome prediction in stage II/III colorectal cancer. Ann Oncol. 2019; 30:1622-29.
29. Roxburgh CS, McMillan DC, Richards CH, Atwan M, Anderson JH, Harvey T, et al. The clinical utility of the combination of T stage and venous invasion to predict survival in patients undergoing surgery for colorectal cancer. Ann Surg. 2014; 259:1156-65.
30. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016 ;66:115-32.

Figures

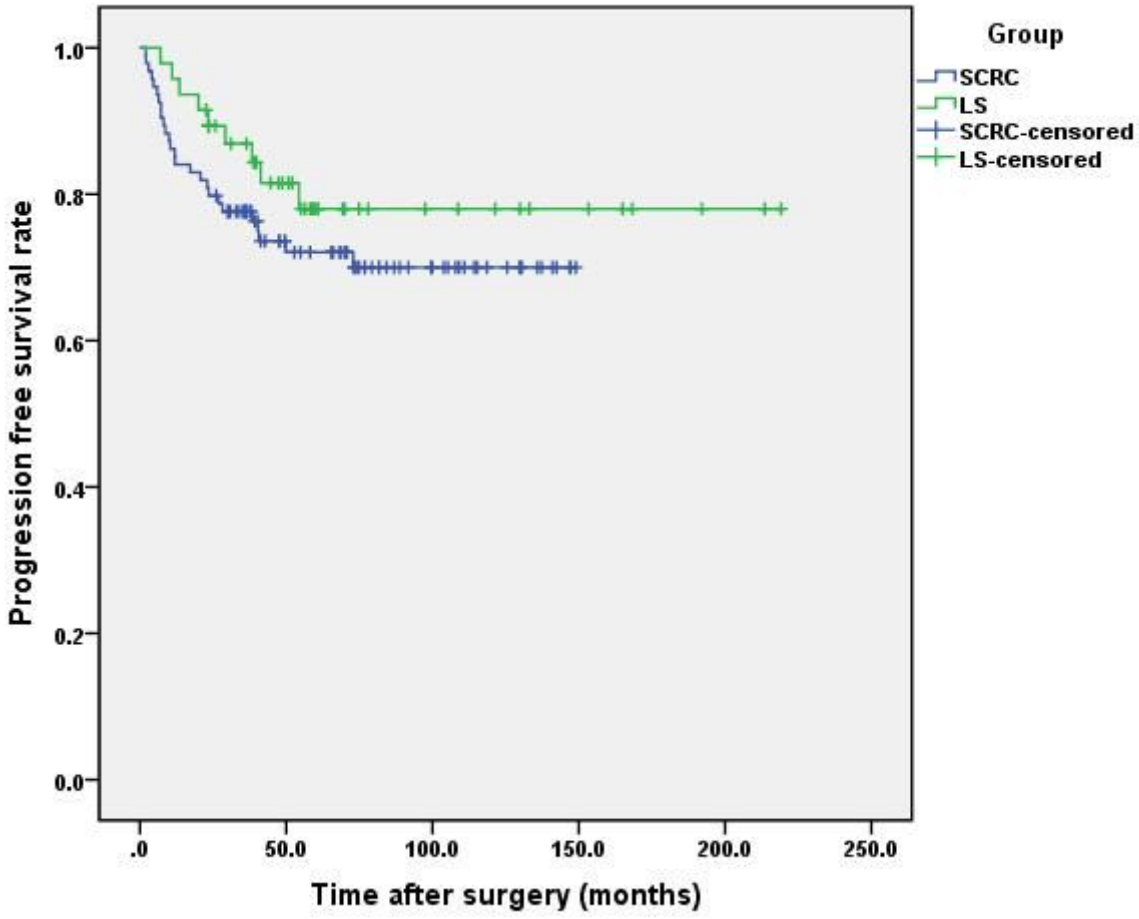

Figure 1

Progression free survival curves for patients in LS group and SCRC group. Survivals were evaluated using Kaplan–Meier curves and compared with the log-rank test.

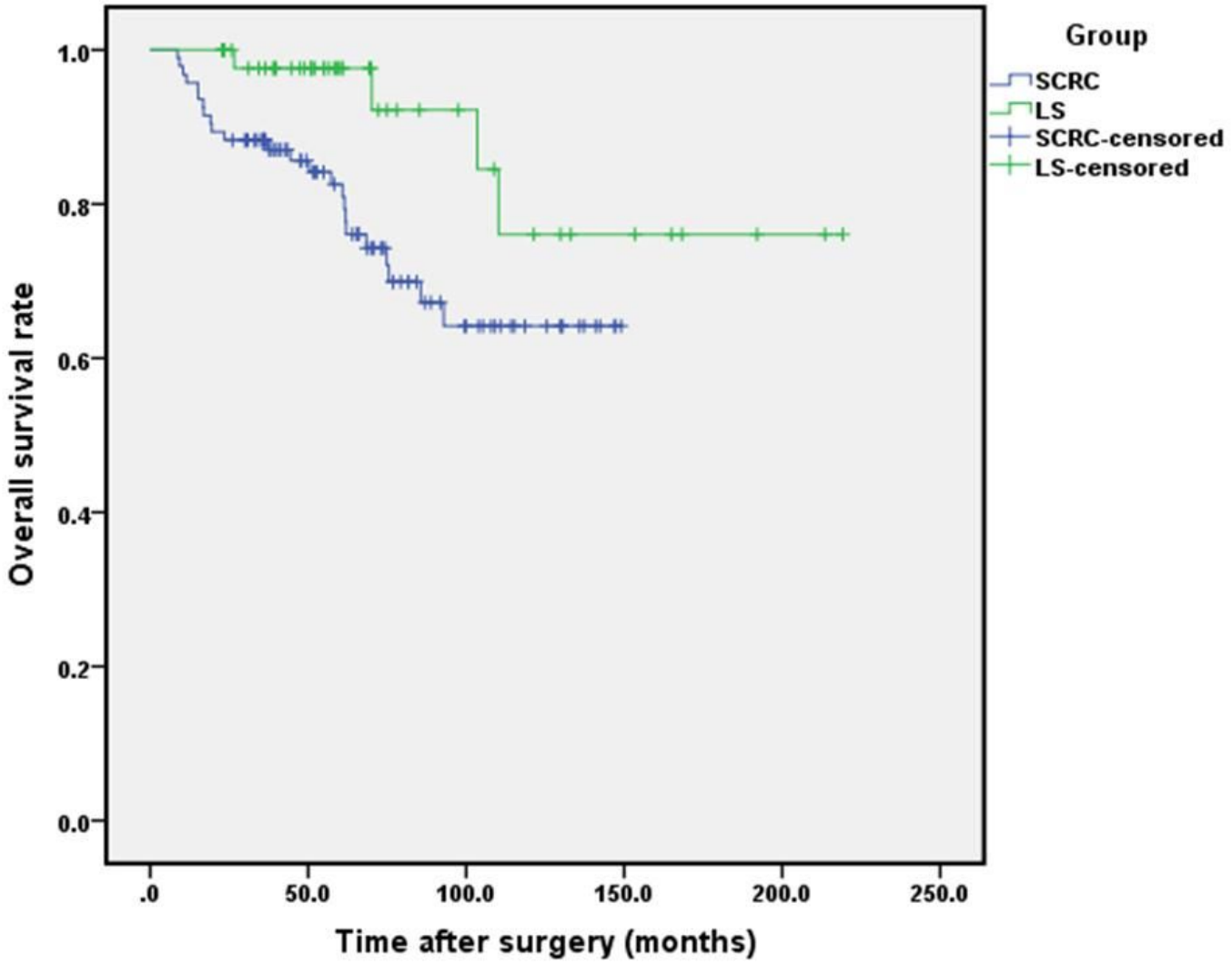

Figure 1

Overall survival curves for patients in LS group and SCRC group. Survivals were evaluated using Kaplan–Meier curves and compared with the log-rank test.

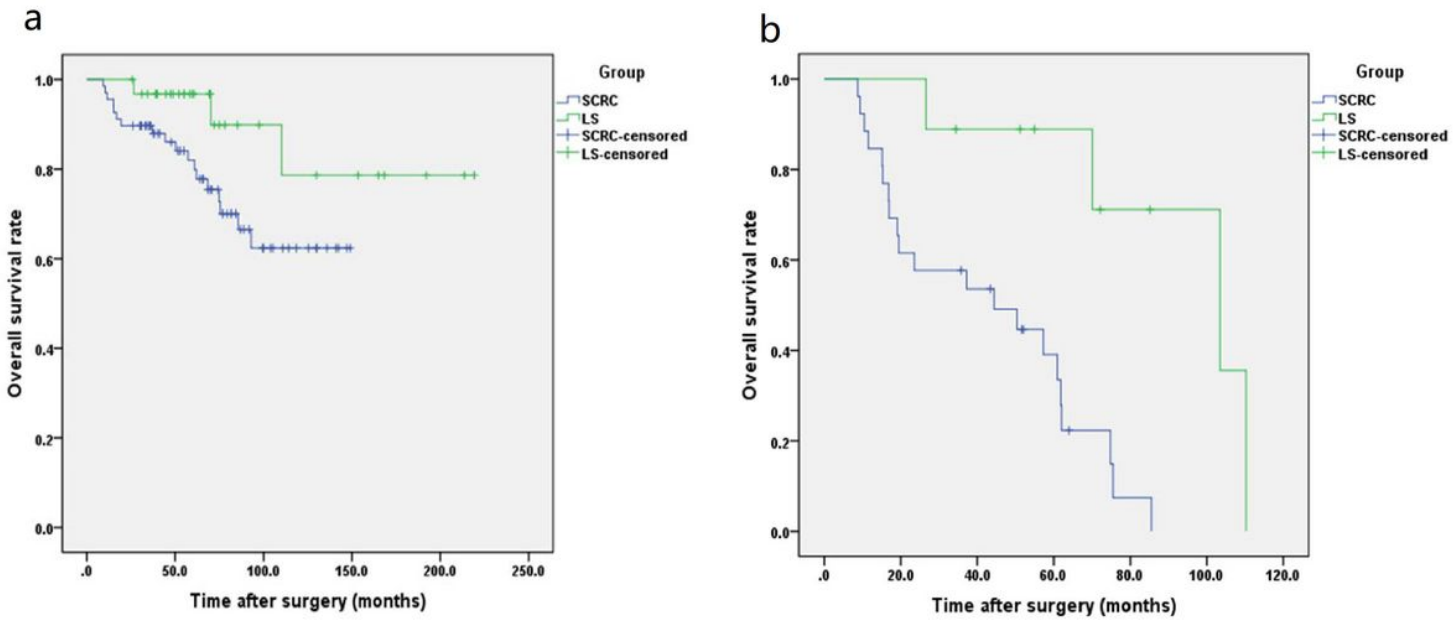

Figure 1

Overall survival curves for patients in subgroup of LS group and SCRC group. Survivals were evaluated using Kaplan–Meier curves and compared with the log-rank test. a. early onset CRC patients; b. CRC patients who developed tumor progression.

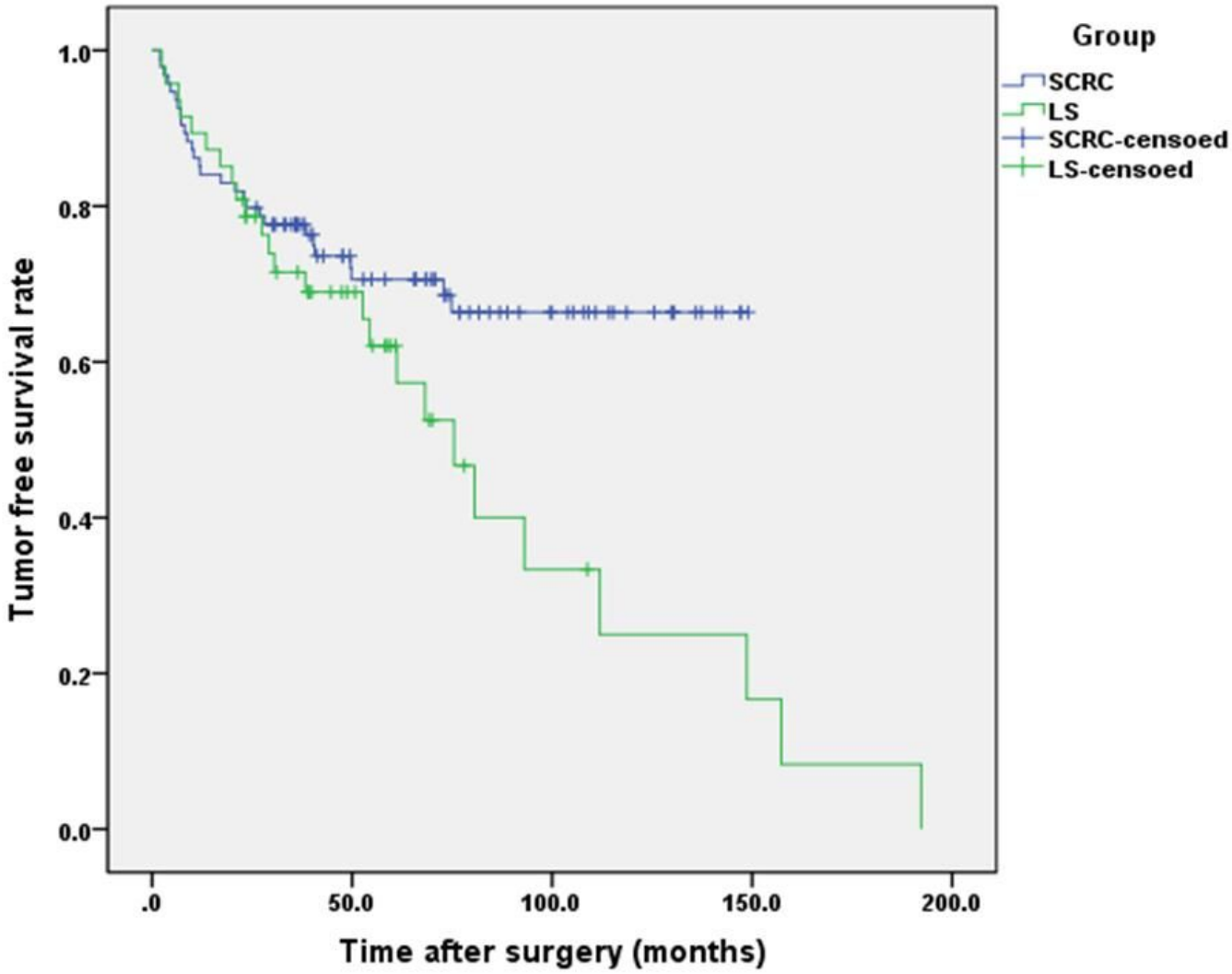

Figure 1

Tumor free survival curves for patients in LS group and SCRC group. Survivals were evaluated using Kaplan–Meier curves and compared with the log-rank test.