Ki67 testing in the clinical management of patients with non-metastatic colorectal cancer: Detecting the optimal cut-off value based on the Restricted Cubic Spline model

HONG TAO LEI[1*], SHAN YAN[2*], YIN HUA HE[3*], NING XU[3], MIN ZHAO[4], CHUN JIAO YU[5], HONG LIN LI[2], SAI KUANG[1], ZHAN HANG CUI[1] and JING FANG[1]

[1]School of Public Health; [2]Yunnan Key Laboratory of Stem Cell and Regenerative Medicine, Institute of Biomedical Engineering, Kunming Medical University; [3]Department of Oncology, The First Affiliated Hospital of Kunming Medical University; [4]Department of Medical Records, The Third Affiliated Hospital of Kunming Medical University; [5]School of Basic Medicine, Kunming Medical University, Kunming, Yunnan 650500, P.R. China

Received June 29, 2022; Accepted September 23, 2022

DOI: 10.3892/ol.2022.13540

Abstract. The proliferation of the biomarker Ki67 has been extensively studied in colorectal cancer (CRC). Although numerous Ki67 cut-off values have previously been reported, the optimal cut-off value remains unclear with previous studies providing contrasting results. The present retrospective cohort study aimed to determine the optimal cut-off value for CRC. Ki67 levels and the prognosis of patients with non-metastatic CRC were obtained from the Electronic Health Information System of a tertiary hospital in Kunming City. The Restricted Cubic Spline (RCS) model was used to analyze the non-linear association between Ki67 levels and the risk of patient death and metastasis. Moreover, the RCS model was used to determine the optimal cut-off value of Ki67. Cox proportional hazards models were used to verify the effects of the cut-off value. In total, 210 patients with CRC and a median age of 62.5 years (age range, 23.0-88.0 years) were studied. Results of the present study demonstrated a non-linear association between Ki67 levels and the risk of patient death based on the RCS model, and at Ki67 levels ≥60%, the hazard ratio (HR) of patient death gradually increased. Using multivariate-adjusted Cox proportional hazards models, the results of the present study demonstrated that Ki67 ≥60% indicated a high-risk ratio for both distant metastasis and death [HR, 2.640; 95% confidence interval (CI), 1.066-6.539], compared with Ki67 <60% (HR, 2.558; 95% CI, 1.079-6.064). Therefore, Ki67 ≥60% may be the optimal cut-off value for the prediction of death and metastasis in patients with CRC. Thus, Ki67 may act as a biomarker for predicting the prognosis of patients with CRC, and the optimal cut-off value for the prediction of both death and metastasis of patients with CRC is 60%.

Correspondence to: Dr Jing Fang, School of Public Health, Kunming Medical University, 1168 Chunrong West Road, Chenggong, Kunming, Yunnan 650500, P.R. China
E-mail: fangjing07@126.com

*Contributed equally

Key words: Ki67, cut-off value, Restricted Cubic Spline model, prognosis, colorectal cancer

Introduction

Colorectal cancer (CRC) exhibits the third highest incidence rate and the second highest cancer-related mortality rate worldwide (1). The global disease burden of CRC is expected to increase by 60% by 2035, with new cases rising to 2.5 million (2,3). The increasing incidence of CRC will be accompanied by an increase in mortality (4), particularly for patients experiencing recurrence and metastasis following curative surgery (5). At present, clinical settings and prognostic indicators, including pathological indicators, are used to guide the clinical management of patients with CRC. Notably, pathological indicators include classic TNM stage, differentiation, invasion, and blood and stool proteins, such as carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, and CA-195. Pathological indicators also include molecular markers, such as microsatellite instability, chromosome 18q loss of heterozygosity, P53, KRAS, BRAF, epidermal growth factor receptor, and vascular endothelial growth factor (6,7). However, the effect of these indicators on the accurate prediction of patient prognosis remains unsatisfactory (8,9). Therefore, it is necessary to explore new biomarkers for the improved prediction of patient prognosis (9).

In 1983, Gerdes *et al* (10) discovered the Ki67 antigen and determined that Ki67 was associated with the active proliferation of cells (10). Ki67 is only expressed in the interphase and mitotic phases of mitosis and is not expressed in the resting phase (G0). During mitosis, Ki67 expression gradually increases until expression reaches a peak (11). Thus, Ki67 expression may reflect the growth fraction of cell populations, and results of numerous previous studies have demonstrated

that Ki67 may serve as a prognostic or predictive biomarker for different types of tumors (12,13). Moreover, numerous previous studies and meta-analyses demonstrated that high expression levels of Ki67 are associated with adverse overall survival and disease-free survival of patients with CRC, and may therefore be used as a valuable marker of CRC prognosis (14-16).

Investigating Ki67 expression in tumor tissues using immunohistochemistry is a routine and reliable examination strategy for the determining the proliferative activity of tumor cells following therapeutic surgery (17,18). However, the results of testing have not yet been used to guide the clinical management of patients with CRC, as the optimal cut-off value remains undetermined (14,19). Miller *et al* (20) demonstrated that Ki67 expression plays a key role in the cell cycle following the tracking of Ki67 expression in a single cell over time (20). Based on the different expression levels of Ki67 throughout the cell cycle, and the results obtained by Miller *et al* (20), it was hypothesized that Ki67 may possess a non-linear association with CRC prognosis, rather than a linear one, as previously suggested (21). Thus, the present immunohistochemical study aimed to use a cohort of patients with non-metastatic CRC and the Restricted Cubic Spline (RCS) model to analyze the association between Ki67 levels and the risk of patient death and metastasis. The present study aimed to obtain an evidence-based cut-off value of Ki67, to guide the clinical management of patients with CRC.

Materials and methods

Patients. A retrospective cohort study was employed for the present analysis. Data were collected from patient medical records stored in the Electronic Health Information System of the First Affiliated Hospital of Kunming Medical University. Patients with CRC included in the present study underwent therapeutic surgery at the aforementioned hospital between January 2014 and December 2020. The inclusion criteria for patients were as follows: i) Patients with stage I-III CRC who received therapeutic surgery, but did not receive preoperative chemoradiation, and ii) patients with complete demographic, clinical treatment, associated laboratory test, and follow-up data, whose tumor tissue was tested for Ki67. Patients with CRC who did not meet the aforementioned criteria were excluded from the present analysis. The covariates included in the present analysis consisted of demographic features, including patient sex, age at diagnosis, race, body mass index (BMI), CEA and Ki67, and tumor pathological features, including type, location, differentiation, stage, vascular invasion, and perineural invasion of CRC.

Immunohistochemistry. Immunohistochemistry was used to detect Ki67 expression in tumor tissue following therapeutic surgery, and this was performed according to the manufacturer's instructions for the antibody (ProteinTech Group, Inc.). Briefly, tumor tissue was embedded in paraffin wax and subsequently dewaxed using xylene and hydrated, and antigen retrieval was performed. Endogenous peroxidase activity was blocked using 3% H_2O_2 and then the slides were blocked with 5% BSA blocking buffer at room temperature for 1 h. Subsequently, tumor tissue was incubated with Ki67 polyclonal antibody (ProteinTech Group, Inc.; cat. no. 27309-1-AP, RRID: AB_2756525, 1:2,000) at 37˚C for 1 h followed by incubation with anti-rabbit IgG (ProteinTech Group, Inc.; cat. no. SA00004-2, RRID: AB_2890944; 1:200) at 37˚C for 1.5 h. Immunostaining was detected using DAB substrate solution and samples were counterstained using hematoxylin at room temperature for 10-30 sec. Immunohistochemistry was performed following surgery. A score was assigned to the Ki67 testing result of each patient to represent the Ki67 level, and the guidelines recommended by the International Ki67 in Breast Cancer Working Group were adopted as the scoring method (22). Immunohistochemical scoring was performed independently by two specialists in pathology who were blinded to the clinicopathological characteristics and prognosis of the patients. A total of five non-overlapping high-power fields (objective, x40, Leica Microsystems GmbH) were randomly selected in the chromatically homogeneous area, and Ki67 expression was calculated as the proportion of tumor cells with positive nuclear staining in all tumor cells.

Patient follow-up. Patient follow-up following therapeutic surgery included death, distant metastasis, and survival without metastasis. The outcome data were obtained from the Electronic Health Information System, or by contacting patients or family members via telephone. The survival time was calculated from the time of diagnosis of CRC to the time of metastasis or death. The date of the last follow-up was recorded if metastasis or death did not occur. The last date of follow-up was 31st August 2021.

Statistical analysis. Quantitative data was presented as the mean ± standard deviation or the median (interquartile range, IQR), and comparisons between two groups was performed using an independent Student's t-test or Mann-Whitney U test, based on the distribution of data. Categorical data are presented as the frequency (percentage), and a comparison between groups were performed using χ^2 or Fisher's exact test. The RCS model was used to determine the non-linear association between Ki67 levels in the tumor tissue and the risk ratio of metastasis and death of patients with CRC. The association between Ki67 expression with distant metastasis or death of patients was analyzed using univariate and multivariate Cox proportional hazards regression models, using hazard ratios (HR), 95% confidence intervals (CIs) to describe the risk ratio, and a test level $\alpha \geq 0.05$. SPSS (version, 24.0; IBM Corp) was used for statistical analysis, baseline data comparisons, and Cox regression analysis. R (version, 4.1.2; R Project for Statistical Computing) and R packages ('rms', 'survival', and 'ggplot2') were used to perform RCS analysis and visualization.

Results

A total of 293 patients with CRC who met the inclusion criteria were first included in the present study; however, a total of 83 patients were lost during the follow-up period. A total of 210 (71.67%) patients with CRC with an age range of 23-88 years old, median age of 62.5 years old, and a BMI at diagnosis of 22.1 kg/m^2 (IQR, 20.3-24.2 kg/m^2) were included in the present research. These included 107 male patients (51.0%) and 103 female patients (49.0%). The majority (206 patients, 98.1%) of the included patients with CRC were

Table I. Characteristics of the 210 patients with non-metastatic colorectal cancer stratified by Ki67 levels.

Characteristic	Total, n=210	Ki67 level		P-value
		<60%, n=97, 46.2%	≥60%, n=113, 53.8%	
Age at diagnosis, median (IQR), (y)	62.5 (54.0, 71.3)	64.0 (54.0, 71.5)	62.0 (53.0, 71.5)	0.708
Age, n (%)				0.524
<60	85	37 (38.1)	48 (42.5)	
≥60	125	60 (61.9)	65 (57.5)	
Sex, n (%)				0.343
Male	107	46 (47.4)	61 (54.0)	
Female	103	51 (52.6)	52 (46.0)	
Race, n (%)				0.725
Han	206	96 (99.0)	110 (97.3)	
Ethnic minority	4	1 (1.0)	3 (2.7)	
BMI, median (IQR), kg/m^2	22.1 (20.3, 24.2)	22.2 (20.8, 24.0)	22.0 (20.2, 24.3)	0.660
BMI, n (%)				0.389
≤18.4	25	10 (10.3)	15 (13.3)	
18.5-23.9	124	61 (62.9)	63 (55.8)	
24.0-27.9	49	23 (23.7)	26 (23.0)	
≥28.0	12	3 (3.1)	9 (8.0)	
CEA, median (IQR), ng/ml	3.2 (1.7, 8.4)	3.2 (1.7, 8.4)	3.4 (1.7, 8.4)	0.958
Type, n (%)				0.501
Adenocarcinoma	208	97 (100.0)	111 (98.2)	
Other	2	0 (0.0)	2 (1.8)	
Location, n (%)				0.194
Right colon	112	57(58.8)	55 (48.7)	
Left colon	53	19 (19.6)	34 (30.1)	
Rectal	45	21 (21.6)	24 (21.2)	
Differentiation, n (%)				0.176
High	26	16 (16.5)	10 (8.8)	
Middle	163	70 (72.2)	93 (82.3)	
Low	21	11 (11.3)	10 (8.8)	
Stage, n (%)				0.229
I	46	18 (18.6)	28 (24.8)	
II	93	49 (50.5)	44 (38.9)	
III	71	30 (30.9)	41 (36.3)	
Vascular invasion, n (%)				0.054
No	166	71 (73.2)	95 (84.1)	
Yes	44	26 (26.8)	18 (15.9)	
Perineural invasion, n (%)				0.282
No	140	61 (62.9)	79 (69.9)	
Yes	70	36 (37.1)	34 (30.1)	

BMI, body mass index.

Han ethnic group (Table I). A total of 29 patients died and 32 patients experienced metastases during the follow-up period; the median survival time following surgery for those who died was 45.3 months. In addition, the median follow-up time following surgery for patients with CRC was 42.6 months. Patients in the Ki67 ≥60% group predominantly exhibited a younger age, were male and of an ethnic minority, exhibited a low BMI, a high CEA, non-adenocarcinoma, moderate differentiation, stage III CRC, no vascular infiltration, and no nerve infiltration; however, differences between groups were not statistically significant (Table I).

RCS model analysis demonstrated a non-linear association between Ki67 expression levels and the HR of death in patients with CRC (P=0.039). When Ki67 was <60%, the HR of death in

Table II. Relationship between Ki67 levels and overall survival stratified based on a cutoff value of 60%.

			Multivariate models					
	Univariate model		Model A		Model B		Model C	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years								
Continuously	1.036 (1.002-1.072)	0.038[a]	1.040 (1.005-1.077)	0.024[a]	1.039 (1.003-1.075)	0.031[a]	1.038 (1.002-1.075)	0.039[a]
Sex								
Male	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Female	1.004 (0.484-2.083)	0.992	1.218 (0.577-2.571)	0.606	1.133 (0.531-2.417)	0.748	1.074 (0.492-2.345)	0.858
Ethnicity								
Han	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Ethnic minority	1.713 (0.233-12.608)	0.597	1.558 (0.206-11.771)	0.667	1.516 (0.199-11.545)	0.688	1.884 (0.226-15.716)	0.558
Body mass index, kg/m^2								
Continuously	0.942 (0.841-1.055)	0.301	0.935 (0.834-1.049)	0.252	0.937 (0.833-1.054)	0.278	0.928 (0.811-1.062)	0.276
CEA, ng/ml								
Continuously	1.016 (0.999-1.033)	0.065			1.018 (1.000-1.036)	0.045[a]	1.016 (0.997-1.036)	0.1
Type								
Adenocarcinoma	1 [Reference]				1 [Reference]		1 [Reference]	
Other	/	0.766			/	0.979	/	0.978
Location								
Right colon	1 [Reference]				1 [Reference]		1 [Reference]	
Left colon	0.835 (0.327-2.136)	0.707			0.993 (0.371-2.662)	0.989	0.738 (0.244-2.230)	0.59
Rectal	1.096 (0.450-2.670)	0.84			1.122 (0.453-2.781)	0.804	1.811 (0.615-5.330)	0.281
Differentiation								
High	1 [Reference]						1 [Reference]	
Middle	4.283 (0.556-31.616)	0.154					2.397 (0.296-19.389)	0.412
Low	3.840 (0.399-36.965)	0.244					2.545 (0.232-27.953)	0.445
Stage								
I	1 [Reference]						1 [Reference]	
II	0.763 (0.215-2.707)	0.676					1.069 (0.251-4.559)	0.928
III	3.550 (1.203-10.476)	0.022[a]					4.714 (1.174-18.931)	0.029[a]
Vascular invasion								
No	1 [Reference]						1 [Reference]	
Yes	2.555 (1.220-5.354)	0.013[a]					2.062 (0.833-5.104)	0.118

Table II. Continued.

Variable	Univariate model		Multivariate models					
			Model A		Model B		Model C	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Perineural invasion								
No	1 [Reference]						1 [Reference]	
Yes	1.077 (0.508-2.284)	0.846					0.641 (0.281-1.467)	0.293
Ki67								
<60%	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
≥60%	2.328 (1.061-5.109)	0.035[a]	2.428 (1.094-5.390)	0.029[a]	2.637 (1.163-5.977)	0.02[a]	2.640 (1.066-6.539)	0.036[a]

[a]P<0.05. HR, hazard ratio; CI, confidence interval.

patients with CRC remained low. The HR of death increased as Ki67 expression levels increased, and when Ki67 levels were ≥60%, the HR of death was markedly increased (Figs. 1 and 2). However, the association between Ki67 expression and the HR of metastasis in patients with CRC was not non-linear (P=0.068).

Results of the univariate Cox regression analysis demonstrated that increased age, stage III cancer, vascular invasion, and Ki67 ≥60% (P<0.05) were risk factors for death in patients with CRC. After adjusting for different confounding factors, Ki67 ≥60% was considered a risk factor for death (HR, 2.640 and 95% CI, 1.066-6.539; P=0.036; Table II).

Moreover, results of the univariate Cox regression analysis demonstrated that increased CEA, stage III cancer, neural invasion, and Ki67 ≥60% were risk factors for distant metastasis in patients with CRC (P<0.05). After adjusting for different confounding factors, Ki67 ≥60% was considered a risk factor for metastasis (HR, 2.558; 95% CI, 1.079-6.064; P=0.033; Table III).

Discussion

Ki67 has been widely used as a marker of cell proliferation in numerous types of tumors (22-24). However, there is still heterogeneity in the use of Ki67 as a biomarker in CRC (25,26), which is comparable to its use in breast cancer (22). Numerous previous studies have demonstrated that increased Ki67 expression is unfavorable in the progression and prognosis of patients with CRC (19,27-32); however, these results differ from other previous studies (14,15,33). Notably, patients included in different studies may possess different characteristics, such as undergoing preoperative chemoradiotherapy or different tumor stages. In addition, results of previous studies demonstrated the use of different cut-off values of Ki67, varying from 5 to 62% (16,34,35). A previous meta-analysis including 34 studies and 6,180 patients confirmed that increased Ki67 expression was associated with unfavorable disease-free survival and overall survival in patients with CRC (16). Notably, the present study included patients with stage I-III CRC who did not receive preoperative chemoradiotherapy, and the results of the present study demonstrated that increased Ki67 expression was independently associated with distant metastasis and death in patients with CRC.

Notably, numerous cut-off values were reported in previous studies, and these studies selected a median or alternative value to allocate patients into different groups (34,36-39). However, these values may obscure important clinical features or prognostic outcomes. The optimal cut-off value should be derived from maximizing the difference in HRs between groups (34). Results of the present study demonstrated a bell-shaped association between Ki67 levels and prognostic outcomes of CRC, which may be attributed to Ki67 expression only occurring in interphase and mitotic phases of mitosis (G0). During mitosis, Ki67 expression is low in interphase (G1, S, and G2) and gradually increases in the pre-mitotic phase and metaphase. Ki67 expression reaches a peak and is markedly decreased in anaphase and telophase (40), exhibiting a graded longitudinal change (20). The RCS model is a powerful tool in the analysis of non-linear dose-effect associations between continuous exposure and outcome (41). In the present

Table III. Relationship between Ki67 levels and metastasis stratified based on a cutoff value of 60%.

			Multivariate models					
	Univariate model		Model A		Model B		Model C	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years								
Continuously	0.999 (0.971-1.028)	0.962	1.003 (0.975-1.032)	0.826	1.005 (0.975-1.035)	0.76	1.008 (0.976-1.040)	0.642
Sex								
Male	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Female	1.460 (0.720-2.959)	0.294	1.559 (0.759-3.204)	0.227	1.538 (0.747-3.168)	0.243	1.116 (0.520-2.397)	0.778
Ethnicity								
Han	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Ethnic minority	1.743 (0.238-12.781)	0.585	1.619 (0.214-12.254)	0.641	1.603 (0.208-12.367)	0.651	2.428 (0.298-19.779)	0.407
Body mass index, kg/m^2								
Continuously	0.958 (0.865-1.061)	0.407	0.961 (0.868-1.065)	0.452	0.960 (0.862-1.069)	0.455	0.966 (0.863-1.081)	0.544
CEA, ng/ml								
Continuously	1.018 (1.002-1.034)	0.023[a]			1.020 (1.004-1.037)	0.013[a]	1.025 (1.008-1.043)	0.005[b]
Type								
Adenocarcinoma	1 [Reference]				1 [Reference]		1 [Reference]	
Other	5.462 (0.737-40.494)	0.097			4.608 (0.585-36.314)	0.147	5.747 (0.628-52.622)	0.122
Location								
Right colon	1 [Reference]				1 [Reference]		1 [Reference]	
Left colon	1.092 (0.467-2.554)	0.838			1.007 (0.401-2.528)	0.988	0.673 (0.238-1.907)	0.456
Rectal	1.253 (0.536-2.929)	0.603			1.308 (0.549-3.115)	0.544	1.740 (0.664-4.559)	0.26
Differentiation								
High	1 [Reference]						1 [Reference]	
Middle	2.427 (0.578-10.191)	0.226					1.526 (0.336-6.933)	0.584
Low	1.333 (0.188-9.471)	0.774					0.945 (0.118-7.576)	0.957
Stage								
I	1 [Reference]						1 [Reference]	
II	2.211 (0.478-10.233)	0.31					2.419 (0.468-12.518)	0.292
III	8.079 (1.893-34.471)	0.005[b]					7.778 (1.584-38.183)	0.012[a]
Vascular invasion								
No	1 [Reference]						1 [Reference]	
Yes	2.063 (0.994-4.281)	0.052					1.569 (0.663-3.716)	0.306

Table III. Continued.

Variable	Univariate model		Multivariate models					
			Model A		Model B		Model C	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Perineural invasion								
No	1 [Reference]						1 [Reference]	
Yes	2.177 (1.086-4.364)	0.028[a]					1.672 (0.777-3.600)	0.189
Ki67								
<60%	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
≥60%	2.356 (1.104-5.029)	0.027[a]	2.404 (1.124-5.141)	0.024[a]	2.556 (1.164-5.610)	0.019[a]	2.558 (1.079-6.064)	0.033[a]

[a]P<0.05, [b]P<0.01. HR, hazard ratio; CI, confidence interval.

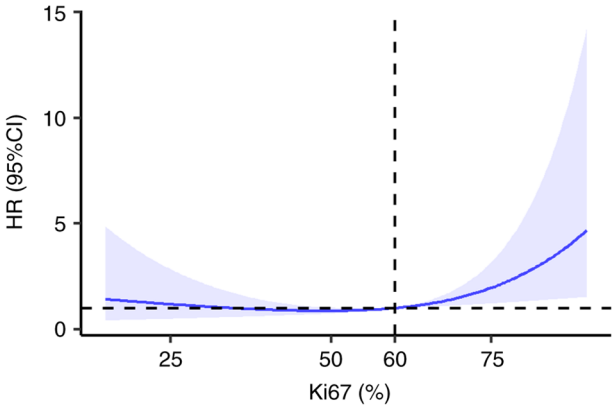

Figure 1. Association between Ki67 expression levels and the risk of death in patients with CRC. CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval.

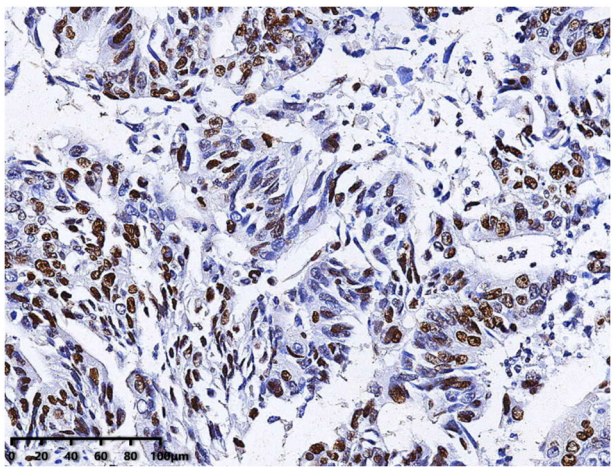

Figure 2. Immunohistochemistry for Ki67 testing in a representative CRC tissue showing labelling of ~60% of nuclei of CRC cell. Magnification, x200. CRC, colorectal cancer.

study, the RCS model was used to analyze the association between Ki67 levels and the HR of patient death, and the results of the present study determined the optimal cut-off value for Ki67 was 60%. Moreover, this level was verified using regression models, and the results demonstrated that Ki67 ≥60% is an independent risk factor for distant metastasis and death in patients with CRC (P<0.05). Notably, these results are comparable to those obtained by Weber *et al* (27). The Ki67 cut-off value may enable medical staff to accurately identify the risk of patient prognosis. However, potential confounding factors may affect the association between Ki67 and mortality, including comorbidities, patients receiving R0 resection, and frailty.

However, there are limitations to the present study. Notably, the statistically significant association between Ki67 >60% and mortality determined using multivariate Cox regression analysis may only be a result of the small sample size used in the present study. Thus, future studies will address this issue. In addition, immunohistochemical scoring is subject to an individual's experience. The objective evaluation of immunohistochemical analysis is key for future research, and

artificial intelligence may be a viable option (42,43). External verification was carried out using the limited samples in the present study; however, further verification of the accuracy of the cut-off value is required in future research. Moreover, selection biases may have occurred due to the samples being obtained from only one hospital, and some patients were lost in follow-up. The study design was also retrospective, meaning the integrity and authenticity of data records may affect the reliability of the results. In addition, certain prognostic factors could not be collected, such as comorbidities, history of R0 resection, and frailty. The detection of Ki67 expression in the tumor tissue of each patient was performed at different times, and detection conditions may have been inconsistent.

In conclusion, the results of the present study demonstrated that Ki67 expression may be used to predict the prognosis of patients with CRC, and the optimal cut-off value of Ki67 is 60%. This cut-off value may be used as a classification tool to guide the clinical management of patients with CRC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are not publicly available due to individual participants' privacy but are available from the corresponding author on reasonable request.

Authors' contributions

HTL, SY, YHH, NX, MZ, CJY, HLL, SK, ZHC and JF contributed to study conception and design. SY, YHH, and MZ were responsible for designing the methodology. SK and ZHC were responsible for material preparation. CJY and HLL were responsible for data collection. YHH and NX were responsible for data analysis. HTL and SY were responsible for the original draft preparation. JF was responsible for review and editing. All authors have read and approved the final manuscript. HTL and YHH confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Ethical approval was waived by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global patterns and trends in colorectal cancer incidence and mortality. Gut 66: 683-691, 2017.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
4. Lortet-Tieulent J, Georges D, Bray F and Vaccarella S: Profiling global cancer incidence and mortality by socioeconomic development. Int J Cancer 147: 3029-3036, 2020.
5. Yamamoto T, Kawada K and Obama K: Inflammation-related biomarkers for the prediction of prognosis in colorectal cancer patients. Int J Mol Sci 22: 8002, 2021.
6. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM and Wallace MB: Colorectal cancer. Lancet 394: 1467-1480, 2019.
7. Lech G, Słotwiński R, Słodkowski M and Krasnodębski IW: Colorectal cancer tumour markers and biomarkers: Recent therapeutic advances. World J Gastroenterol 22: 1745-1755, 2016.
8. Walker J and Quirke P: Prognosis and response to therapy in colorectal cancer. Eur J Cancer 38: 880-886, 2002.
9. Mahar AL, Compton C, Halabi S, Hess KR, Weiser MR and Groome PA: Personalizing prognosis in colorectal cancer: A systematic review of the quality and nature of clinical prognostic tools for survival outcomes. J Surg Oncol 116: 969-982, 2017.
10. Gerdes J, Schwab U, Lemke H and Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31: 13-20, 1983.
11. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U and Stein H: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133: 1710-1715, 1984.
12. Li LT, Jiang G, Chen Q and Zheng JN: Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 11: 1566-1572, 2015.
13. Menon SS, Guruvayoorappan C, Sakthivel KM and Rasmi RR: Ki-67 protein as a tumour proliferation marker. Clin Chim Acta 491: 39-45, 2019.
14. Fodor IK, Hutchins GG, Espiritu C, Quirke P and Jubb AM: Prognostic and predictive significance of proliferation in 867 colorectal cancers. J Clin Pathol 65: 989-995, 2012.
15. Li P, Xiao ZT, Braciak TA, Ou QJ, Chen G and Oduncu FS: Association between Ki67 index and clinicopathological features in colorectal cancer. Oncol Res Treat 39: 696-702, 2016.
16. Luo ZW, Zhu MG, Zhang ZQ, Ye FJ, Huang WH and Luo XZ: Increased expression of Ki-67 is a poor prognostic marker for colorectal cancer patients: A meta analysis. BMC Cancer 19: 123, 2019.
17. Ma YL, Peng JY, Zhang P, Liu WJ, Huang L and Qin HL: Immunohistochemical analysis revealed CD34 and Ki67 protein expression as significant prognostic factors in colorectal cancer. Med Oncol 27: 304-309, 2010.
18. Iatropoulos MJ and Williams GM: Proliferation markers. Exp Toxicol Pathol 48: 175-181, 1996.
19. Tong G, Zhang G, Liu J, Zheng Z, Chen Y, Niu P and Xu X: Cut-off of 25% for Ki67 expression is a good classification tool for prognosis in colorectal cancer in the AJCC8 stratification. Oncol Rep 43: 1187-1198, 2020.
20. Miller I, Min M, Yang C, Tian C, Gookin S, Carter D and Spencer SL: Ki67 is a graded rather than a binary marker of proliferation versus quiescence. Cell Rep 24: 1105-1112.e5, 2018.
21. Salminen E, Palmu S, Vahlberg T, Roberts PJ and Söderström KO: Increased proliferation activity measured by immunoreactive Ki67 is associated with survival improvement in rectal/recto sigmoid cancer. World J Gastroenterol 11: 3245-3249, 2005.
22. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, *et al*: Assessment of Ki67 in breast cancer: Recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst 103: 1656-1664, 2011.
23. Sun X and Kaufman PD: Ki-67: More than a proliferation marker. Chromosoma 127: 175-186, 2018.

24. Scholzen T and Gerdes J: The Ki-67 protein: From the known and the unknown. J Cell Physiol 182: 311-322, 2000.
25. Graziano F and Cascinu S: Prognostic molecular markers for planning adjuvant chemotherapy trials in Dukes' B colorectal cancer patients: How much evidence is enough? Ann Oncol 14: 1026-1038, 2003.
26. Torén W, Ansari D and Andersson R: Immunohistochemical investigation of prognostic biomarkers in resected colorectal liver metastases: A systematic review and meta-analysis. Cancer Cell Int 18: 217, 2018.
27. Weber JC, Nakano H, Bachellier P, Oussoultzoglou E, Inoue K, Shimura H, Wolf P, Chenard-Neu MP and Jaeck D: Is a proliferation index of cancer cells a reliable prognostic factor after hepatectomy in patients with colorectal liver metastases? Am J Surg 182: 81-88, 2001.
28. Wang L, Liu Z, Fisher KW, Ren F, Lv J, Davidson DD, Baldridge LA, Du X and Cheng L: Prognostic value of programmed death ligand 1, p53, and Ki-67 in patients with advanced-stage colorectal cancer. Hum Pathol 71: 20-29, 2018.
29. Ishida H, Miwa H, Tatsuta M, Masutani S, Imamura H, Shimizu J, Ezumi K, Kato H, Kawasaki T, Furukawa H and Kawakami H: Ki-67 and CEA expression as prognostic markers in Dukes' C colorectal cancer. Cancer Lett 207: 109-115, 2004.
30. Dawson H, Koelzer VH, Karamitopoulou E, Economou M, Hammer C, Muller DE, Lugli A and Zlobec I: The apoptotic and proliferation rate of tumour budding cells in colorectal cancer outlines a heterogeneous population of cells with various impacts on clinical outcome. Histopathology 64: 577-584, 2014.
31. Fernández-Cebrián JM, Santos MN, Kuborn PV, de Lama MP, Martín-Cavanna J, Martínez PP, Escudero BF and Fernández MR: Can the clinical outcome in stage II colon carcinomas be predicted by determination of molecular marker expression? Clin Transl Oncol 9: 663-670, 2007.
32. Wu XS, Xi HQ and Chen L: Lgr5 is a potential marker of colorectal carcinoma stem cells that correlates with patient survival. World J Surg Oncol 10: 244, 2012.
33. Melling N, Kowitz CM, Simon R, Bokemeyer C, Terracciano L, Sauter G, Izbicki JR and Marx AH: High Ki67 expression is an independent good prognostic marker in colorectal cancer. J Clin Pathol 69: 209-214, 2016.
34. Wei DM, Chen WJ, Meng RM, Zhao N, Zhang XY, Liao DY and Chen G: Augmented expression of Ki-67 is correlated with clinicopathological characteristics and prognosis for lung cancer patients: An up-dated systematic review and meta-analysis with 108 studies and 14,732 patients. Respir Res 19: 150, 2018.
35. Nielsen TO, Leung SCY, Rimm DL, Dodson A, Acs B, Badve S, Denkert C, Ellis MJ, Fineberg S, Flowers M, *et al*: Assessment of Ki67 in breast cancer: Updated recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst 113: 808-819, 2021.
36. Ajani JA, Wang X, Izzo JG, Crane CH, Eng C, Skibber JM, Das P and Rashid A: Molecular biomarkers correlate with disease-free survival in patients with anal canal carcinoma treated with chemoradiation. Dig Dis Sci 55: 1098-1105, 2010.
37. Nash GM, Gimbel M, Shia J, Nathanson DR, Ndubuisi MI, Zeng ZS, Kemeny N and Paty PB: KRAS mutation correlates with accelerated metastatic progression in patients with colorectal liver metastases. Ann Surg Oncol 17: 572-578, 2010.
38. Shin IY, Sung NY, Lee YS, Kwon TS, Si Y, Lee YS, Oh ST and Lee IK: The expression of multiple proteins as prognostic factors in colorectal cancer: Cathepsin D, p53, COX-2, epidermal growth factor receptor, C-erbB-2, and Ki-67. Gut Liver 8: 13-23, 2014.
39. Ivanecz A, Kavalar R, Palfy M, Pivec V, Sremec M, Horvat M and Potrč S: Can we improve the clinical risk score? The prognostic value of p53, Ki-67 and thymidylate synthase in patients undergoing radical resection of colorectal liver metastases. HPB (Oxford) 16: 235-242, 2014.
40. Seigneurin D and Guillaud P: Ki-67 antigen, a cell cycle and tumor growth marker. Pathol Biol (Paris) 39: 1020-1028, 1991 (In French).
41. Desquilbet L and Mariotti F: Dose-response analyses using restricted cubic spline functions in public health research. Stat Med 29: 1037-1057, 2010.
42. Ramesh AN, Kambhampati C, Monson JR and Drew PJ: Artificial intelligence in medicine. Ann R Coll Surg Engl 86: 334-338, 2004.
43. Hamet P and Tremblay J: Artificial intelligence in medicine. Metabolism 69s: S36-S40, 2017.