Artificial intelligence for quantifying Crohn's-like lymphoid reaction and tumor-infiltrating lymphocytes in colorectal cancer

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ABSTRACT

Crohn's-like lymphoid reaction (CLR) and tumor-infiltrating lymphocytes (TILs) are crucial for the host antitumor immune response. We proposed an artificial intelligence (AI)-based model to quantify the density of TILs and CLR in immunohistochemical (IHC)-stained whole-slide images (WSIs) and further constructed the CLR-I (immune) score, a tissue level- and cell level-based immune factor, to predict the overall survival (OS) of patients with colorectal cancer (CRC). The TILs score and CLR score were obtained according to the related density. And the CLR-I score was calculated by summing two scores. The development (Hospital 1, N = 370) and validation (Hospital 2 & 3, N = 144) cohorts were used to evaluate the prognostic value of the CLR-I score. The C-index and integrated area under the curve were used to assess the discrimination ability. A higher CLR-I score was associated with a better prognosis, which was identified by multivariable analysis in the development (hazard ratio for score 3 vs score 0 = 0.22, 95% confidence interval 0.12–0.40, P < 0.001) and validation cohort (0.21, 0.05–0.78, P = 0.020). The AI-based CLR-I score outperforms the single predictor in predicting OS which is objective and more prone to be deployed in clinical practice.

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1. Introduction

Colorectal cancer (CRC), the third most common malignant neoplasm in the world, has a high mortality rate [1]. For stage II–III CRC patients, the current tumor-node-metastasis (TNM) tumor staging system has limited accuracy in treatment decision-making [2]. At present, many prognostic biomarkers have been proposed as supplements to the TNM staging system, especially immune-related predictors. For instance, tumor-infiltrating lymphocytes (TILs) have been confirmed to be positively correlated with prognosis in CRC patients [3–5].

The host lymphocyte response of patients has been regarded as an important reference for antitumor treatment [6,7]. CD3+ and CD8+ TILs are the most reported T cells that contribute to antitumor immune responses. The density and location of CD3+ and CD8+ T cells has been shown to be significantly correlated with the prognosis of CRC patients [8–10].

Similar to the lymphocyte infiltration at the advancing tumor edge, there is a lymphatic reaction around colorectal adenocarcinoma, characterized by lymphocyte aggregation (LA) and termed Crohn's-like lymphoid reaction (CLR). The CLR was confirmed to

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be positively correlated with T cells density [11–13]. At the early stage, CLR is mainly composed of T cells and mature dendritic cells. After maturation, CLR is mainly composed of B cells and dendritic cells. At present, two methods for CLR assessment are recognized and reproducible: one is to calculate the density of peritumoral LAs, and the other is to measure the maximum diameter of LAs [5,11,14].

With the development of artificial intelligence (AI), image-based prognostic biomarkers are easier to identify and quantify in whole-slide images (WSIs), which brings convenience to precision medicine. Galon et al. quantified $CD3^+$ and $CD8^+$ T cells in the center of the tumor and invasive margin to construct an immune score as a supplement to TNM stage, providing a reliable prognostic predictor for patients with stage I–III colon cancer [15]. However, they ignored the prognostic information contained in the highly organized lymphocytes. Lymphocytes infiltrating the stroma and cancer nest are a morphological expression of the host antitumor response at the cellular level [16–18]. Similarly, CLR could be regarded as a CRC-specific tertiary lymphoid structure, expressing the host antitumor response at the tissue level [19]. Moreover, in terms of immunological characteristics, the interactive effect between TILs and CLR has been confirmed [11]. Thus, we hypothesize that a comprehensive scoring system combining predictors from the cellular level (TILs) and tissue level (CLR) could provide a more accurate prediction of survival in stage II–III CRC patients. We called the scoring system the CLR-I (immune) score.

This study aims to quantify CLR and TILs in immunohistochemical (IHC)-stained WSIs using an AI-based method and assess the prognostic value of the CLR-I score for CRC patients.

2. Patients and methods

2.1. Patient cohort

This is a study approved by the Research Ethics Committee of Guangdong Provincial People's Hospital (Hospital 1), Yunnan Cancer Hospital (Hospital 2), and the Sixth Affiliated Hospital of Sun Yat-sen University (Hospital 3). The requirement for informed consent for this retrospective study was waived. These patients were diagnosed with CRC from March 2008 to May 2015 and underwent surgical resection for treatment. The inclusion and exclusion criteria are listed in the supplementary material. The clinicopathological data collected from the patient's medical records were as follows: sex, age, TNM stage, tumor location, tumor grade, microsatellite instability [MSI] status, and carcinoembryonic antigen (CEA, cutoff = 5 ng/mL, normal = 0–5 ng/mL). The outcome of interest in the study was overall survival (OS), defined as the time from randomization to death from any cause.

2.2. Tissue segmentation on IHC-stained WSIs

Paraffin-resected sections with tumor and invasive margins were stained with immunohistochemistry ($CD3^+$) and then scanned by digital whole-slide scanning (Aperio-AT2 and GT 450, Leica, USA) at $40\times$ magnification (resolution: 0.25–0.26 μm/pixel).

The convolutional neural network (CNN) model VGG-19 was applied to classify the downsampled WSIs ($20\times$ magnification) into nine categories: tumor stroma, tumor epithelium, lymphocytes, mucus, normal mucosa, adipose tissue, debris, smooth muscle, and background (Fig. 1A). In this work, we used transfer learning in model training to maximize the use of labeled data and save computing resources. The high-performance VGG-19 model used to train the IHC dataset in the development cohort was derived from a previous work [20]. The details were as follows: the mini-batch size was set as 64, and the fixed learning rate

was $3 \times$ to train the model for ten epochs. This process has been described in previous work [20]. The WSIs segmentation were performed on the NVIDIA 3090 GPU desktop workstation.

2.3. Calculation of TILs score

As a subset of TILs, $CD3^+$ T cells in the tumor stroma have a strong positive correlation with the density of TILs. We calculated the $CD3^+$ T cells in the tumor stroma using the following steps. First, the stromal region ($20\times$ magnification) in the tissue segmentation map was extracted and resized to $40\times$ magnification, serving as the region of interest (ROI) for $CD3^+$ T cells counting. The image region within the ROI was tiled into 1024×1024 pixel^2 blocks. Furthermore, color deconvolution was used to separate the dyeing channel for 3,3-diaminobenzidine (DAB) channel images. Then, the Gaussian blur, superpixel segmentation, and watershed algorithm were used to segment nuclei in DAB channel images. The density of TILs was calculated as follows: the number of $CD3^+$ T cells divided by the area of tumor stroma (Fig. 1B). For each patient, TILs density was converted into percentiles according to the development cohort. The method for choosing cutoff values was based on the distribution of density and the reported methods [15,20]. The TILs density approximately obeyed a normal distribution in the development cohort (Supplementary Fig. S1A). The cutoff values were set at 33% and 66% to divide patients into three groups (TILs-low, TILs-intermediate, and TILs-high). For each case, TILs-low scored 0, TILs-intermediate scored 1, and TILs-high scored 2.

2.4. Calculation of the CLR score

The ROI for quantifying CLR density was the whole tumor area, including the tumor epithelium, stroma, and mucosa. We excluded the LAs with the following cases: LAs associated with mucosa, LAs near normal glands, and preexisting lymph nodes (Fig. 1C) [11,13]. The exclusion criteria were as follows: LAs with a dilated area intersecting with normal glands greater than 0.37 mm (the structure was a disk, and the radius was equal to 4) and LAs with an area <0.042 mm^2. Finally, the CLR density was calculated as the number of included LAs divided by the whole tumor area. The CLR density approximately obeyed a skewed distribution, and the concentration position was biased to the side with small values (Supplementary Fig. S1B). Referring to a previous method [5], the maximum selection rank statistic method was used to calculate the optimal stratification in the development cohort, and the cutoff value of CLR density was set at 0.121 follicles/mm^2 (Supplementary Fig. S2). For each case, a density between 0 and 0.121 follicles/mm^2 was defined as CLR-low and scored 0, and a density more than 0.121 follicles/mm^2 was defined as CLR-high and scored 1.

2.5. Calculation of the CLR-I score

To more accurately quantify patient's immunity, we constructed a comprehensive scoring system termed the CLR-I score, which was calculated by summing the TILs score (score 0, 1, 2) and CLR score (score 0, 1), ranging from 0 to 3 (Fig. 1D).

2.6. Statistical analysis

The Kaplan-Meier (KM) survival curve was used for survival analysis. Chi-square tests and t tests were used to compare the demographic and clinicopathological characteristics of CRC patients between the two cohorts. Harrell's concordance index (C-index) with 1000 bootstrap replicates and the integrated area under the ROC curve (iAUC) were calculated to evaluate the discrimination ability of the CLR-I score, TILs score, and CLR score.
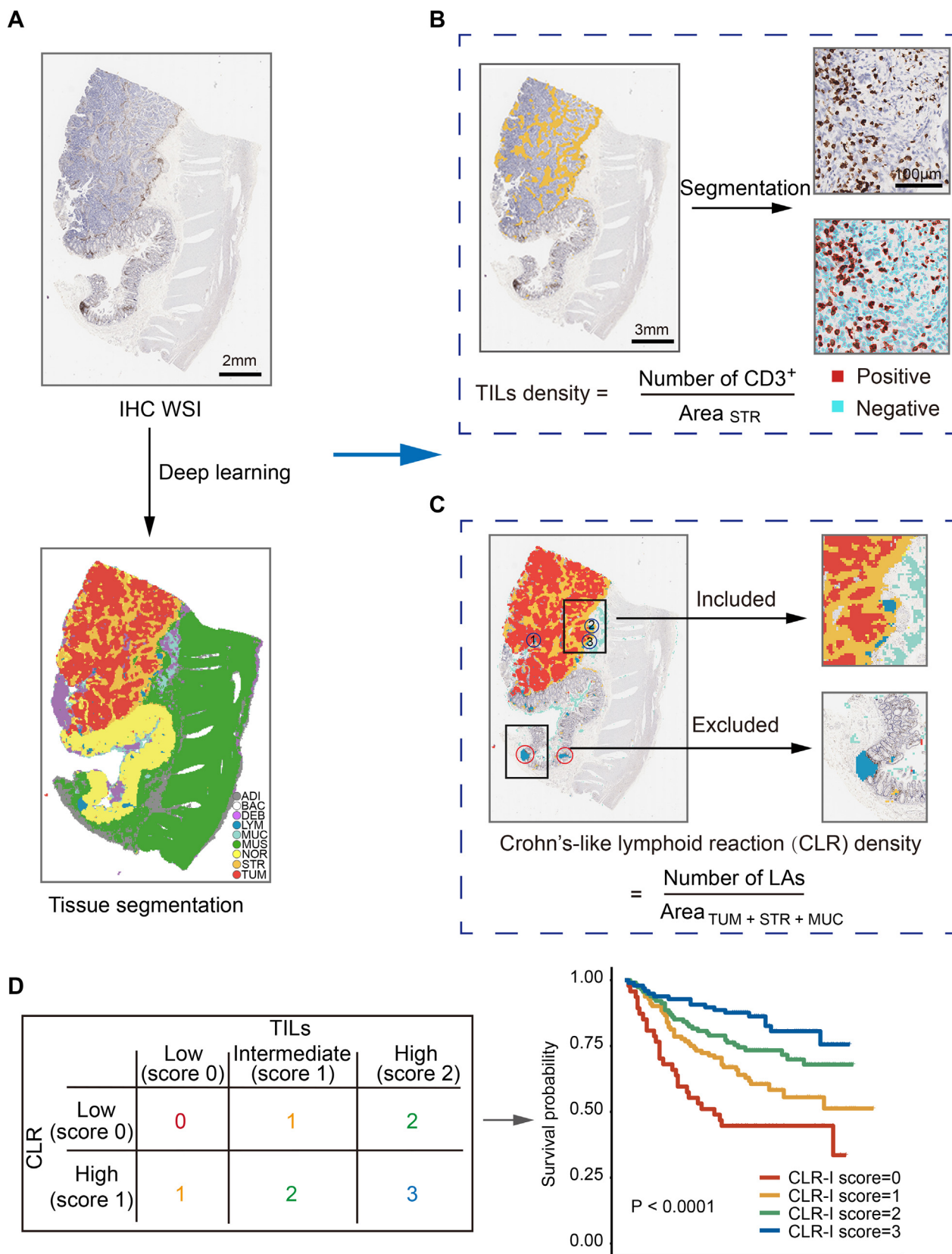
Fig. 1. Study workflow. (A) Convolutional neural network for nine-category tissue classification. (B) The nuclei in tumor stroma were automatically divided and counted, and the density of TILs was calculated as "the number of CD3⁺ divided by the area of tumor stroma". (C) Display diagram of included and excluded LAs. In the IHC-stained WSI, a total of 3 LAs related to CLR were included. The CLR density was calculated as "the number of LAs divided by the whole tumor area". (D) Calculation of the CLR-I score and the schematic diagram of Kaplan-Meier curve of the CLR-I score. TILs, tumor-infiltrating lymphocytes; LA, lymphocytes aggregation; IHC, immunohistochemical; WSI, whole-slide image; CLR, Crohn's-like lymphoid reaction; CLR-I, Crohn's-like lymphoid reaction-immune; ADI, adipose; BAC, background; DEB, debris; LYM, lymphocytes; MUC, mucus; MUS, muscle; NOR, normal mucosa; STR, stroma; TUM, tumor epithelium.

The principle for inclusion in multivariate Cox regression analysis was that indicators were statistically significant in the univariate analysis of the development cohort. R software (version 4.0.5) was used for statistical analysis. A two-sided significance level of 0.05 was used in the statistical significance report.

3. Results

3.1. Patients

A total of 514 patients with stage II–III CRC were included in the study, and they all had clinicopathological characteristics and follow-up information. Three hundred and seventy patients (220 males and 150 females) from Hospital 1 were regarded as the development cohort. The number of patients from Hospital 2 was 44, and the number of patients from Hospital 3 was 100. The latter two centers were combined into the validation cohort because of the small number of patients, with a total of 144 patients (82 males and 62 females). The median follow-up time was 69 months in the development cohort and 61 months in the validation cohort. The demographic characteristics and clinicopathological characteristics of this study sample are described in Table 1.

3.2. Prognostic significance of TILs score in the tumor stroma

In the development cohort, patient with a higher TILs score had a better survival outcome (hazard ratio [HR] for score 2 vs score 0 = 0.32, 95% confidence interval [CI] 0.19–0.52, P < 0.001; HR for score 1 vs score 0 = 0.65, 95% CI 0.44–0.97, P = 0.035), and the 5-year survival rates of the score 0–2 groups were 59.5%, 71.2%, and 86.6% (Fig. 2A). The results showed consistency in the validation cohort (HR for score 2 vs score 0 = 0.36, 95% CI 0.14–0.96, P = 0.041; HR for score 1 vs score 0 = 0.29, 95% CI 0.10–0.83, P = 0.022), and the 5-year survival rates of the three groups were 67.4%, 94.2%, and 87.1% (Fig. 2B).

3.3. Prognostic significance of the CLR score

In the development cohort, 269 patients (56%) had a CLR score of 1; in the validation cohort, 122 patients (77.8%) had a CLR score of 1. Patients with a higher CLR score experienced a better prognosis (HR for score 1 vs score 0 = 0.55, 95% CI 0.38–0.79, P = 0.001) and a better 5-year survival rate (77.3% vs 58.4%; Fig. 2C) in the development cohort. A similar trend was observed in the validation cohort (HR = 0.47, 95% CI 0.23–0.99, P = 0.047; 5-year survival rate: 82.9% vs 67.5%; Fig. 2D).

3.4. The relationship between TILs and CLR

The density of CLR was correlated with TILs density. The group with a CLR score of 1 had a higher normalized TILs density (Supplementary Fig. S3A and B). In addition, as shown in Supplementary Fig. S3, in the comparison of CLR scores 0 and 1, TILs scores 1–2 accounted for a larger proportion in the development (70.6% vs 53.5%) and validation cohort (58% vs 34.4%).

3.5. Prognostic significance of the CLR-I score

The KM curves indicated that the patients with higher CLR-I scores had better OS in both cohorts (both P < 0.001; Fig. 3A and B). In univariate analysis, the CLR-I score was considered as a favorable predictor for OS (development cohort: HR for score 3 vs score 0 = 0.21, 95% CI 0.12–0.39, P < 0.001; validation cohort: 0.20, 0.05–0.77, 0.019; Table 2). The TNM stage and CLR-I score were included

in multivariate Cox regression to analyze survival according to the criteria, and they were the independent prognostic factors in CRC. In multivariate Cox regression analysis, a higher CLR-I score corresponded to a lower risk, prompting a better survival outcome for CRC patients in the development (HR for score 3 vs score 0 = 0.22, 95% CI 0.12–0.40, P < 0.001; Table 2) and validation cohort (0.21, 0.05–0.78, P = 0.020; Table 2).

3.6. The discrimination ability of the three scores

Moreover, we evaluated and compared the discrimination ability of the TILs score, CLR score, and CLR-I score. The bootstrap method was used to calculate the C-index distribution of the CLR-I score, TILs score, and CLR score in the development and validation cohort. In the development cohort, the CLR-I score outperformed TILs score (C-index 0.644 vs 0.626, P < 0.001 after Benjamini correction; iAUC 0.657 vs 0.642; Supplementary Table S1) or CLR score (C-index 0.644 vs 0.569, P < 0.001 after Benjamini correction; iAUC 0.657 vs 0.581; Supplementary Table S1). Similar results were observed in the validation cohort. The C-index and iAUC of the CLR-I score were both higher in the comparison of TILs score (C-index 0.646 vs 0.628, P < 0.001 after Benjamini correction; iAUC 0.683 vs 0.626; Supplementary Table S1) or CLR score (C-index 0.646 vs 0.568, P < 0.001 after Benjamini correction; iAUC 0.683 vs 0.639; Supplementary Table S1).
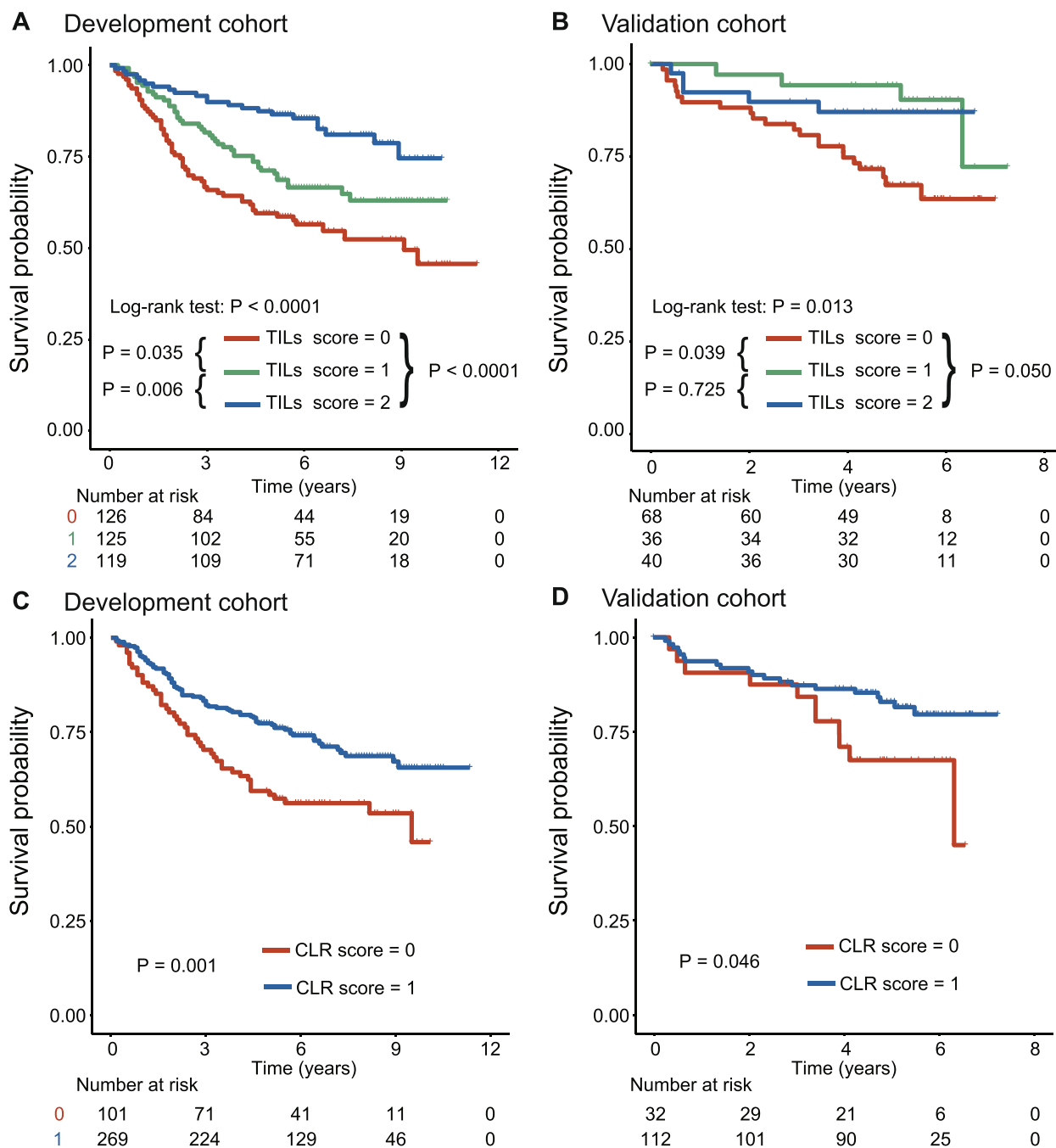
Table 1
The distributions of demographic and clinicopathologic characteristics of colorectal cancer patients in the two cohorts.

Demographic and tumor characteristics	Development cohort (N = 370)	Validation cohort (N = 144)	P
Age, year	63.18 (11.99)	61.01 (12.50)	0.070*
Sex			0.674#
Male	220 (59.5)	82 (56.9)	
Female	150 (40.5)	62 (43.1)	
Location			<0.001#
Colon	211 (57.0)	44 (30.6)	
Rectum	159 (43.0)	100 (69.4)	
pT. stage			0.622#
1	2 (0.5)	1 (0.7)	
2	15 (4.1)	4 (2.8)	
3	317 (85.7)	119 (82.6)	
4	36 (9.7)	20 (13.9)	
pN. stage			0.244#
0	165 (44.6)	70 (48.6)	
1	125 (33.8)	54 (37.5)	
2	79 (21.4)	20 (13.9)	
Not available	1 (0.3)		
TNM			0.505#
II	165 (44.6)	70 (48.6)	
III	205 (55.4)	74 (51.4)	
Tumor grade			<0.001#
High	305 (82.4)	129 (89.6)	
Low	45 (12.2)	5 (3.5)	
Other	20 (5.4)	3 (2.1)	
Not available		7 (4.9)	
CEA			0.007#
<5	193 (52.2)	90 (62.5)	
≥5	147 (39.7)	52 (36.1)	
Not available	30 (8.1)	2 (1.4)	
MSI status			<0.001#
MSI	27 (7.3)	0 (0)	
MSS	227 (61.4)	0 (0)	
Not available	116 (31.4)	144 (100)	

Abbreviations: TNM, tumor-node-metastasis; CEA, carcinoembryonic antigen; MSI, microsatellite instability; MSS, microsatellite stability.
Notes: Number (%) is used for categorical variables, and mean (SD) is used for continuous variables; P value was performed by *t*-test or Chi-square test where appropriate (* *t*-test; # Chi-square test); The threshold value for CEA was 5 ng/mL, and 0–5 ng/mL was regarded as normal.

Fig. 2. The Kaplan-Meier analysis for the TILs and CLR in the development and validation cohort. (A, B) Kaplan-Meier curves of overall survival for three groups (TILs score = 0, 1, 2). (C, D) Kaplan-Meier curves of overall survival for two groups (CLR score = 0,1). TILs, tumor-infiltrating lymphocytes; CLR, Crohn's-like lymphoid reaction

3.7. The relationship between the CLR-I score and MSI

In addition, we analyzed the association between MSI and the CLR-I score in patients (N = 254) with available MSI and microsatellite stability (MSS) information. We found that a higher CLR-I score was associated with a higher proportion of MSI: the proportion of MSI for scores 0 to 3 was 6.9%, 3.66%, 13.9%, and 16.9%, respectively (P = 0.039; Fig. 3C). In addition, the trend was more obvious after combining the low scores (0–1) into the low-score group and high scores (2–3) into the high-score group (MSI proportion: 4.50% vs 15.4%; P < 0.01; Fig. 3D).

3.8. Predictive value of the CLR-I score

We also analyzed the survival of patients with treatment information in stage II CRC. Due to the small number of CRC patients in stage II with a CLR-I score of 0 and a CLR-I score of 1 (Supplementary Fig. S4), we combined the groups with a CLR-I score of 0–1 into a group and performed the same operation in the groups with a CLR-I score of 2–3. We found that in the lower CLR-I score (0–1) group, patients would benefit from adjuvant chemotherapy. The 5-year survival rates of the adjuvant chemotherapy group and the surgery-only group were 82.9% and 67.5%, respectively
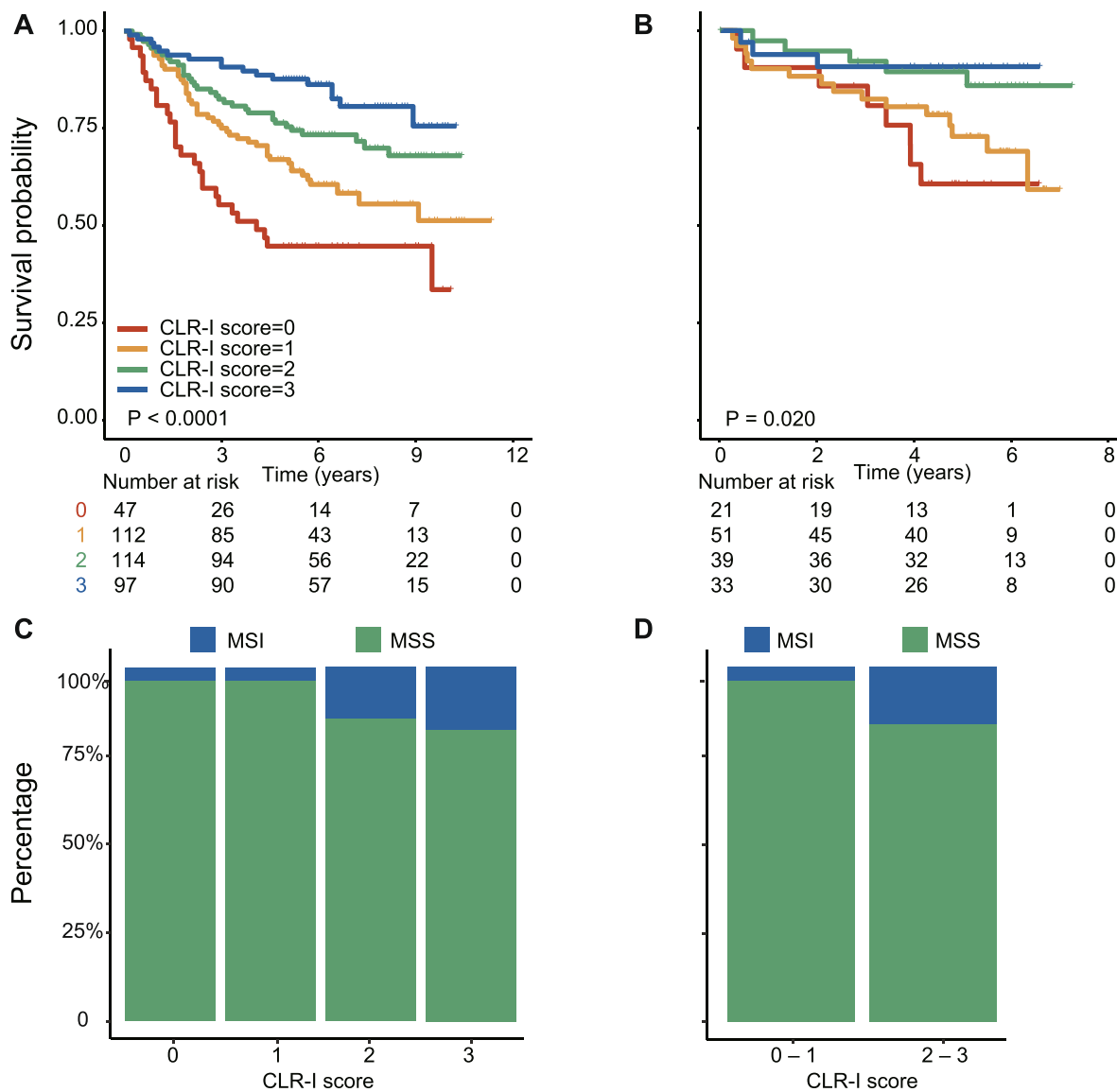
Fig. 3. Kaplan-Meier analysis in the development and validation cohort. (A, B) Kaplan-Meier analysis for CLR-I score (range from 0 to 3). (C, D) The bar chart reflected the proportion of MSI in the CLR-I score. CLR-I, Crohn's-like lymphoid reaction-immune; MSI, microsatellite instability.

(P = 0.022; Fig. 4A). However, in the higher CLR-I score (2–3) group, the survival rates of only surgery and adjuvant chemotherapy were not statistically significant (P = 0.650; Fig. 4B).

4. Discussion

The study explored an AI-based fully automatic method to quantify TILs and CLR density in the WSIs of CRC patients. Furthermore, we assessed the prognostic value of the CLR-I score, which was derived from TILs and CLR density. The higher CLR-I score was associated with a favorable prognosis, independent of the TNM stage and other clinical factors, and showed a better discrimination ability than the single biomarker in predicting prognosis.

It is not unexpected to find that TILs and CLR are correlated with prognosis, which has been confirmed in many studies [5,12,13,21,22], including this study. The type, location, and density of immune cells are important factors affecting host immunity [23]. At present, the prognostic value of TILs in the primary tumor center and invasive margin has been supported by mounting evidence in solid tumors [24–27]. However, it is difficult to evaluate TILs at the edge of some tumor margins with infiltrative growth patterns [28], while stromal tumor-infiltrating lymphocytes (sTILs) are easier to be quantified. We automatically quantified the density of sTILs and constructed an immune score named TILs score. The high score of TILs was inclined to suggest a favorable prognosis [20].

As transmural lymphoid aggregates, CLR is easier to be quantified than TILs. In our study, a fully automated method was used to quantify and score CLR density. We found that a CRC patient with high density of CLR indeed has correlation with a better 5-year survival rate (development cohort: 77.3% vs 58.4%; validation cohort: 82.9% vs 67.5%), which was consistent with previous research result [5].

We constructed a useful immune score with the combination of cellular level (TILs) and tissue level (CLR) predictors which termed CLR-I score. A patient with a higher CLR-I score had a higher 5-year survival rate (score 3 vs score 0: 87.6% vs 44.7%). In the evaluation of the accuracy of predicting survival, the CLR-I score showed a better discrimination ability than the single predictor, which may be related to the interaction between TILs and CLR.

Table 2
Univariate and multivariate Cox risk regression analyses for overall survival.

| | Univariate analysis | | | | Multivariate analysis | | | |
| | Development cohort | | Validation cohort | | Development cohort | | Validation cohort | |
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
Age	1.03 (1.01–1.04)	<0.001*	1.02 (0.99–1.05)	0.200	1.03 (1.00–1.05)	0.023*		
Sex								
Female	Ref		Ref					
Male	1.09 (0.76–1.57)	0.600	1.20 (0.58–2.47)	0.600				
Location								
Colon	Ref		Ref					
Rectum	1.11 (0.78–1.58)	0.600	1.09 (0.50–2.38)	0.800				
TNM stage								
II	Ref		Ref		Ref		Ref	
III	2.80 (1.87–4.19)	<0.001*	2.04 (0.98–4.26)	0.058	2.78 (1.86–4.16)	<0.001*	1.94 (0.93–4.06)	0.079
Tumor grade								
High	Ref		Ref					
Low	1.28 (0.77–2.15)	0.300	6.37 (1.89–21.5)	0.003*				
Other	0.83 (0.34–2.04)	0.700						
CLR-I score								
0	Ref		Ref		Ref		Ref	
1	0.59 (0.36–0.94)	0.028*	0.67 (0.28–1.59)	0.400	0.59 (0.37–0.96)	0.032*	0.67 (0.28–1.58)	0.400
2	0.37 (0.22–0.62)	<0.001*	0.26 (0.09–0.81)	0.020*	0.38 (0.23–0.63)	<0.001*	0.28 (0.09–0.86)	0.026*
3	0.21 (0.12–0.39)	<0.001*	0.20 (0.05–0.77)	0.019*	0.22 (0.12–0.40)	<0.001*	0.21 (0.05–0.78)	0.020*

Notes: *P < 0.05.
Abbreviations: HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis; CLR-I score, Crohn's-like lymphoid reaction-immune score.
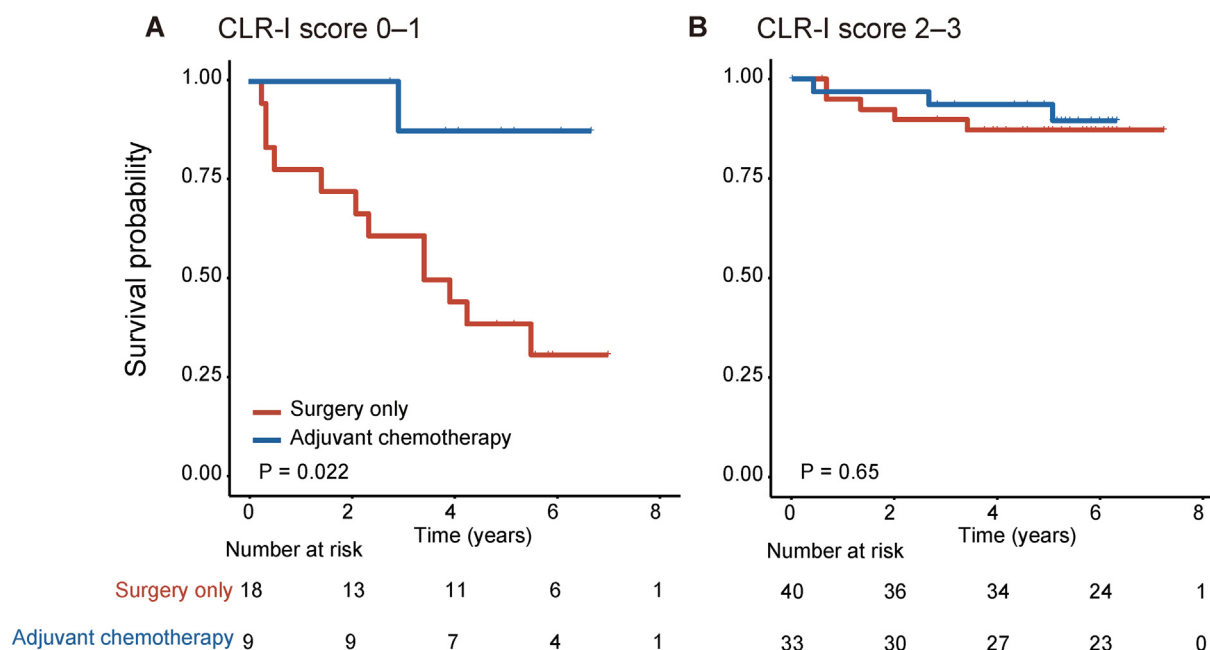

Fig. 4. Kaplan-Meier analysis for adjuvant chemotherapy in the two integrated CLR-I score groups. (A) Kaplan-Meier analysis for group of CLR-I score 0–1. (B) Kaplan-Meier analysis for group of CLR-I score 2–3. CLR-I, Crohn's-like lymphoid reaction-immune.

MSI, an important prognostic risk parameter of CRC immunotherapy, is present in approximately 15% of CRCs [29–31]. We found that the group with a higher CLR-I score had a higher proportion of MSI patients (score 3 vs 0: 16.9% vs 6.9%), which suggests that the CLR-I score has a function to discriminate patients with MSI features. This may stem from the correlation between MSI and immunity. Many studies, including our previous study, have confirmed the correlation between high CLR density and MSI [5,32]. In addition, as reported, MSI is usually characterized by strong lymphocyte infiltration [33,34].

We also studied the response of CRC patients with different immune scores to adjuvant chemotherapy. The American Society of Clinical Oncology recommended that patients with a small number of lymph nodes could be considered insufficiently staged to receive adjuvant chemotherapy [35]. A recent study showed that poor prognosis was associated with a low rate of TILs in stage II CRC patients who did not receive adjuvant therapy [36], which is consistent with our result. We found that a patient with a low CLR-I score was more likely to benefit from adjuvant chemotherapy, while a patient with a high CLR-I score obtained a longer survival time through an immune response. Due to the individual differences in the therapeutic efficacy of adjuvant chemotherapy in stage II patients, risk stratification of patients to avoid unnecessary treatment is particularly important [37]. The CLR-I score con-

structed by CLR and TILs can identify stage II CRC patients who will benefit from adjuvant chemotherapy and may guide clinicians to make individualized treatments.

Our research is retrospective, and the number of samples is limited. At present, the results obtained still need further prospective research and extensive verification.

In conclusion, we proposed an AI-based automatic pipeline to quantify the density of TILs and CLR in the stroma and constructed an immune score termed CLR-I to simplify the process of verifying prognostic value. It was found that the CLR-I score had a better discrimination ability than the single predictor (TILs score and CLR score) in predicting the survival of CRC patients. The CLR-I score based on AI is a simple and repeatable method that has the value of transforming into routine clinical application.

Ethics approval and consent to participate

This retrospective study was approved by Guangdong Provincial People's Hospital, Yunnan Cancer Hospital, and the Sixth Affiliated Hospital of Sun Yat-sen University. The informed consent was waived due to the confidential data. Moreover, this study was consistent with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

ZYL funded the project. KZ, ZYL conceived the research content and design. ZYL, LYH, KZ, supervised the studies. YXZ, SY, HFY, YRY, ZHL, LW acquired the data. SQY, KZ, YJL performed the experiments. YX, SQY, performed the statistical analysis. YX wrote the manuscript and made figures and tables. KZ, SY, polished the manuscript. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Yao Xu: Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Shangqing Yang:** Software, Validation, Formal analysis. **Yaxi Zhu:** Resources. **Su Yao:** Resources. **Yajun Li:** Data curation. **Huifen Ye:** Data curation. **Yunrui Ye:** Data curation. **Zhenhui Li:** Data curation. **Lin Wu:** Data curation. **Ke Zhao:** Methodology, Investigation, Writing – review & editing. **Liyu Huang:** Supervision. **Zaiyi Liu:** Conceptualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2022.09.039.

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