Comparison of non-schistosomal colorectal cancer and schistosomal colorectal cancer

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Research

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Abstract

**Aim:** The purpose of this study was to compare clinicopathological features of patients with non-schistosomal and schistosomal colorectal cancer to explore the effect of schistosomiasis on colorectal cancer (CRC) patients' clinical outcomes.

**Methods:** 351 cases of CRC were retrospectively analyzed in this study. Survival curves were constructed by using the Kaplan-Meier (K-M) method. Univariate and multivariate Cox proportional hazard regression models were performed to identify associations with outcome variables.

**Results:** Colorectal cancer patients with schistosomiasis (CRC-S) were significantly older (P=0.001) than the patients without schistosomiasis (CRC-NS). However, there were no significant differences between CRC-S and CRC-NS patients in other clinicopathological features. Schistosomiasis was associated with adverse overall survival (OS) upon K-M analysis (P=0.0277). By univariate and multivariate analysis, gender (P=0.003), TNM stage (P=0.001), schistosomiasis (P=0.025), lymphovascular invasion (P=0.030) and lymph nodes positive for CRC (P=0.001) were all independent predictors in the whole cohort. When patients were stratified according to clinical stage and lymph node metastasis state, schistosomiasis was also an independent predictors in patients with stage I tumors and in patients with lymph node metastasis, but not in patients with stage II tumors and in patients without lymph node metastasis.

**Conclusion:** Schistosomiasis was significantly correlated with OS and it was an independent prognostic factor for OS in the whole cohort. When patients were stratified according to clinical stage and lymph node metastasis state, schistosomiasis was still an independent unfavorably prognosis factor for OS in patients with stage I tumors or patients with lymph node metastasis.

Introduction

Growing evidences have emerged in recent decades that inflammation is the root of many malignant tumors[1, 2]. As the fourth most common cancer and the second leading cause of cancer deaths in the world[3], CRC represents a growing number of cancers that correlated with inflammation[1, 4, 5]. Schistosoma japonicum (S. japonicum) which is common in Southeast Asia[6], is regarded as a risk factor of CRC development[7]. Schistosomal infestation has been implicated in the aetiology of several human malignancies including bladder, liver, and CRC[8, 9]. The prevalent view is that the sequestered eggs in the mucosa and submucosa incite a severe inflammatory reaction with cellular infiltration and consequent granuloma formation. This in turn leads to mucosal ulceration, microabscess formation, polyposis, and neoplastic transformation[10]. But the causal relationship between S. japonicum and CRC still remained controversial[11]. Some case reports and descriptive studies from Africa and the Middle East raised the possibility of an association between S. japonicum infestation and induction of CRC[12-14]. Nonetheless, the pathological evidence supporting this conclusion is rather weak. While some research demonstrated that S. japonicum infestation was unrelated with CRC[15].

In the 1950s, schistosomiasis was epidemic at a large scale in regions along the Yangtze River and in more than 400 counties in South China[16]. Because of the effective prevention and cure measures taken in China in recent years, schistosomiasis has been eliminated in most epidemic regions. However, its spread is not yet completely controlled and schistosomiasis occurs every year in a small number of people in the epidemic regions of China[17]. Qingpu District of Shanghai used to be one of the 10 areas with serious schistosomiasis epidemic in China[18], and problems of treatment and outcome of a large number of late schistosomiasis patients left over from history are still remaining. Therefore, detailed knowledge about schistosomiasis is necessary to improve the accuracy of clinical prognosis prediction and will shed light on improving our ability to prevention and control of schistosomiasis.

In the present study, we made a retrospective analysis of schistosomiasis and clinicopathologic characteristics in 137 CRC-S patients and 214 CRC-NS patients to investigate the effect of schistosomiasis on CRC patients’ clinical outcomes.

Materials And Methods

Patients and samples

A total of 351 CRC patients were enrolled in this retrospective study. All patients had undergone primary surgical resection at Qingpu Branch of Zhongshan Hospital affiliated to Fudan University, from January 2008 to August 2016. All of operations followed the principle: adequate resection margins, en bloc high ligation of the inferior mesenteric artery (IMA) and lymphadenectomy. All circumferential margins were cleared. The number of positive lymph nodes and total number of retrieved lymph nodes were recorded. The inpatient medical records and pathological reports were reviewed and the patients were followed up by telephone. OS is defined as the interval from the surgical operation date to the last follow-up or death caused by CRC. Inclusion criteria included (i) patients with CRC as primary focus; (ii) none of these patients had received any prior anti-tumor therapy; (iii) patients were diagnosed as adenocarcinoma by pathology after resection of CRC. Exclusion criteria included (i) Tis tumours; (ii) patients who lacked complete information; (iii) patients with synchronous malignancy; (iv) patients with survival time less than one month.

Two expert pathologists reviewed HE-stained slides to determine the diagnosis and to restage the tumors according to the eighth edition of American Joint Committee on Cancer (AJCC).

Detection of schistosome ova and assessment of tumor budding

Schistosome ova were observed in all of original HE-stained formalin fixed paraffin-embedded (FFPE) sections (usually 4-6 slides), which were examined at ×10 and ×40 magnification fields using a conventional light microscope by two pathologists who were blind to clinic data. The diagnosis of schistosomiasis was done by finding schistosome eggs in HE-stained slides.
Tumor budding was defined as the presence of de-differentiated single cells or small clusters of up to 5 cells at the invasive front of CRC [19]. To assess tumor budding in the 10-HPF method [20], the invasive front is first scanned at low magnification (×4×10) to identify areas of highest budding density. Tumor buds are then counted under high magnification (×40) and the tumor budding count is reported. The evaluation of tumor budding was conducted by two pathologists who were blinded to clinical data. 5 tumor budding counts were used as breakthrough point. In brief, tumor bud counts greater than or equal to 5 were defined as high group, otherwise as low group.

**Statistical analysis**

The association between schistosomiasis and clinicopathological characteristics was evaluated by using the Chi square and Fisher's exact tests. K-M curves with log-rank tests were used to determine the prognostic significance for OS. Univariate and multivariate regression analyses were used to identify independent prognostic factors, and P < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection. Statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, IL).

**Results**

**Clinical characteristics in full cohort**

A total of 351 surgically resected FFPE primary CRC samples were included in the study. In the whole cohort, 39.0% (137 out of 351) were infected with schistosoma (Figure 1A). The clinical and pathologic features of the cohort are summarized in Table 1. In the whole cohort, age of patients at diagnosis ranged from 33 to 91 years (median, 69 years) and were predominantly male (60.2%, 212 out of 351). By anatomic site, 27% tumors were in the rectum, 33% in left colon and 40% in right colon. Lymph node metastasis were observed in 40% of patients and 46% of patients were at late stage disease. While patients ranged from 33 to 91 years (median, 69 years) and were predominantly male (60.2%, 212 out of 351). By anatomic site, 27% tumors were in the rectum, 33% in left colon and 40% in right colon. Lymph node metastasis were observed in 40% of patients and 46% of patients were at late stage disease. While patients

| Characteristics       | All patients (N=351) | Patients with stage ≥5 cells (N=192) | Patients with stage T3-4 (N=159) | Patients with lymph node metastasis (N=144) | Patients without LNM (N=207) |
|-----------------------|----------------------|--------------------------------------|-----------------------------------|--------------------------------------------|----------------------------|
| Age (yes)             | 83                   | 46                                   | 24                                | 37                                         | 23                         |
| Gender (Male)         | 214                  | 118                                  | 61                                | 65                                         | 59                         |
| Tumor location        | Rectum               | 94                                   | 27                                | 50                                         | 25                         |
|                       | Left colon           | 115                                  | 33                                | 61                                         | 32                         |
|                       | Right colon          | 142                                  | 40                                | 81                                         | 42                         |
| Tumor size (cm)       | 174                  | 50                                   | 94                                | 50                                         | 80                         |
| Treatment different   | Well diff.           | 267                                  | 76                                | 165                                        | 86                         |
|                       | Poor diff.           | 84                                   | 24                                | 27                                         | 14                         |
| Lymphovascular invasion (positive) | 122                  | 35                                   | 46                                | 24                                         | 26                         |
| Nervous invasion (positive) | 31                   | 1.0                                  | 12                                | 6.0                                        | 6.0                        |
| Lymph nodes positive for CRC (≥2) | 42                   | 1.2                                  | 1.0                               | 6.0                                        | 8.0                        |
| Colon perforation (Yes) | 13                   | 0.4                                  | 8                                 | 4.0                                        | 9.0                        |
| Tumor budding (≥5 cells) | 219                  | 62                                   | 99                                | 52                                         | 120                        |
| Ulceration (Yes)      | 149                  | 42                                   | 79                                | 41                                         | 70                         |
| Histological type     | Adenocarcinoma       | 311                                  | 89                                | 173                                        | 90                         |
|                       | Mucinous/SRCC        | 40                                   | 11                                | 19                                         | 10                         |
| Pathological T stage  | T1-2                 | 80                                   | 23                                | 65                                         | 34                         |
|                       | T3-4                 | 271                                  | 77                                | 127                                        | 66                         |
| Lymph node metastasis | No                   | 207                                  | 69                                | 189                                        | 98                         |
|                       | Yes                  | 144                                  | 40                                | 3                                          | 2                          |
| TNM stage             | I-II                 | 190                                  | 54                                | ----                                        | 3                           |
|                       | III+ IV              | 161                                  | 46                                | ----                                        | 12                         |
| Schistosomiasis       | 137                  | 39                                   | 76                                | 40                                         | 61                         |

---: Data is not applicable; Abbreviation: N=Number

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| Schistosomiasis       | 137                  | 39                                   | 76                                | 40                                         | 61                         |

---: Data is not applicable; Abbreviation: N=Number

Survival analysis

The median follow-up time was 62.4 (125.13-144.4) months. During the follow up, there was 41.6% (146 out of 351) patients died. Mean and median time to OS was 62.54 and 62.85, respectively.

To investigate the association between schistosomiasis and clinical outcomes, we conducted K-M analysis according to schistosoma infection status. Result demonstrated that schistosoma infection was significantly associated with poor survival in total CRC patients (median survival time: 80.82 for CRC-S set; 119.20 for CRC-NS set. P=0.0277) (Figure 1B).

Further analysis was conducted to explore the effect of schistosoma infection on CRC patients with similar stage tumors. In stage I-II set (N=192), a K-M curve was plotted and found that schistosoma infection (40%) was uncorrelated with survival (P=0.5018) (Figure 2A). Nevertheless, in stage III+ set (N=159), K-M analysis showed significant correlation between schistosoma infection and OS (P=0.0260) (Figure 2B).
In patients with lymph node metastasis (N=144), schistosoma infection was observed in 39% (56 out of 144) CRC patients and associated with poor survival ($P=0.0249$) (Figure 2C). In contrast, there was no statistically significant difference observed in OS between CRC-S and CRC-NS patients without lymph node metastasis ($P=0.4005$) (Figure 2D).

**Univariate and multivariate analysis**

The Cox proportional hazards model was used to determine factors that may influence OS of CRC patients. In the whole cohort, by univariate analysis and multivariate analysis (Table 2), gender ($P=0.003$), TNM stage ($P=0.001$), schistosomiasis ($P=0.025$), lymphovascular invasion ($P=0.030$) and lymph nodes positive for CRC ($P=0.001$) were significantly independent predictors. Schistosomiasis was statistically significant associated with decreasing OS.

In late-stage (≥Ⅲ) CRC patients (Table 2), gender ($P=0.030$), pathological T stage ($P=0.12$), tumor differentiation ($P=0.016$), schistosoma infection ($P=0.008$) and lymph nodes positive for CRC ($P=0.004$) were significantly independent prognostic factor for OS. While in early-stage (<Ⅲ), lymph nodes positive for CRC ($P=0.007$) was the only independent prognostic factor for OS in multivariate analysis.

In patients with lymph node metastasis (Table 2), gender ($P=0.026$), pathological T stage ($P=0.025$), schistosoma infection ($P=0.023$) and lymph nodes positive for CRC ($P=0.003$) were independently prognostic factors. In patients without lymph node metastasis (Table 2), TNM stage ($P=0.001$) and tumor budding ($P=0.014$) but not schistosoma infection were associated with OS in multivariate analysis. These results further proved that schistosoma infection may have different effects on CRC patients’ clinical outcomes, especially for patients with stage ≥Ⅲ tumor and patients with lymph node metastasis.

**Association of schistosomiasis with clinicopathological features**

The relationship between schistosomiasis and clinicopathological features was shown in Table 3. Patients with schistosomiasis were significantly older than the patients without schistosomiasis (median age: 74.0 years vs 64.0 years, $P=0.001$). Clinical stage of patients with schistosomiasis and without schistosomiasis were similar ($P=0.816$). In the total cohort, the male/female ratio was also higher in CRC-S set (1.67 vs 1.43). Besides, in patients with lymph node metastasis, there were significant association between male sex and female sex ($P=0.001$). There were no significant differences of other clinicopathological characteristics between CRC-NS and CRC-S set.

In order to further investigate the effect of schistosomiasis on particular CRC population, we divided the whole cohort into different groups according to their clinical stage or the state of lymph node metastasis and further subgrouped into CRC-S and CRC-NS set based on schistosomiasis. Except age, there were no correlation between other clinicopathological features and schistosomiasis when compared between CRC-NS and CRC-S sets in different groups (Table 3).

**Discussion**

At present, there is sufficient evidence to conclude that *S. haematobium* has a role in causing some types of bladder cancer[21-23] and hepatocellular carcinoma[6, 10]. There is limited evidence to suggest that *S. japonicum* leads to CRC.

Our study demonstrated that schistosomiasis was an independently unfavorable factor for OS ($P=0.0260$, Fig.1B; $P=0.025$, Table 2). These results indicated that schistosomiasis plays an important role in CRC progression and metastases. Shindo[24] reviewed 276 cases of large intestinal cancer with schistosomiasis and found significant differences between carcinoma with schistosomiasis and non-schistosomiasis associated carcinoma in symptoms, age, sex and histological findings, suggesting that schistosomiasis could induces the carcinoma. Ye et al.[25] reported that intestinal schistosomiasis was a risk factor for CRC and that the lesions caused by the disease might be considered precancerous. Liu et al.[26] reported that the history of colon schistosomiasis was a probable risk factor for the development of colorectal neoplasia, but only a few studies reported the clinicopathological characteristics and prognosis of patients with schistosomatis CRC. This might be explained as follows. Firstly, there is little relevant clinical data in the medical literature, limited to case reports, physicians know little about it[27, 28]. Secondly, cases of colonic schistosomiasis are rare leading to a small sample size and potential bias in data analysis. Previous reports[29, 30] showed that the development of CRC-S occurs in a younger age group unlike our findings. This might be explained by the following reasons. First, since effective prevention and control measures taken in China in 1983, the infection rate has decreased, which result in CRC-NS patients was in large quantity and relatively younger. Second, this disparity may be related to differences in hereditary factors and environmental carcinogens. Our results showed that there is also a male predominance(61%, Table 1) in the cohort, although there was no significant difference between CRC-NS and CRC-S patients (Table 3). The Qingshu district of Shanghai was previously predominantly rural and as more males were engaged in farm work, are likely to be at greater risk for exposure[31-33].

In the cohort, there were 22 (1.7%) patients were stage IV tumors, the survival time ranged from 1.25 months to 118 months. Although it is well known that stage IV tumors have a poor prognosis, we want to investigate the impact of schistosomia on CRC in the complete process.

Schistosomiasis was statistically significant for OS in the univariate analysis and was an independent prognosis factor in multivariate analysis in the whole cohort ($P=0.025$, HR=1.458, 95% CI=1.049-2.027). When patients were stratified based on clinical stage or state of lymph node metastasis, schistosomiasis was also a significantly independent predictor, except in patients with stage ≥Ⅲ tumor or without lymph node metastasis. Therefore, our observation indicates that schistosomiasis maybe a considerable risk for patients in different clinical stage, especially in late clinical stage. This conclusion may increase the debate that schistosomiasis is a weak risk of CRC[3, 34, 35].

Our study has several limitations. First, because it was performed at a single institution, the uniformity of the results may be low. Further work will be needed.
In summary, our observations support the pathogenetic role of schistosomiasis and shed light on the adverse effects of schistosomiasis on CRC patients.

Table 2. Univariate and multivariate Cox regression of clinicopathological for overall survival

| Variable                      | All patients | Patients with stage I-II disease | Patients with stage III-IV disease | Patients with LNM | Patients without LNM |
|-------------------------------|--------------|----------------------------------|-----------------------------------|-------------------|----------------------|
| Univariate analysis           |              |                                  |                                   |                   |                      |
| Age (60yrs)                   | 0.010        | 1.759(1.142-2.780)               | 0.009                             | 3.413(1.355-8.597) | 0.244                | 1.343(0.818-2.205)   |
| Gender (male/female)          | 0.008        | 1.602(1.129-2.271)               | 0.046                             | 1.897(1.010-3.364) | 0.018                | 1.679(1.093-2.533)   |
| Tumor size (5cm)              | 0.913        | 1.018(0.728-1.400)               | 0.735                             | 0.969(0.523-1.729) | 0.480                | 1.157(0.772-1.735)   |
| Tumor site                    |              |                                  |                                   |                   |                      |
| Rectum                        | 0.001        | 2.591(1.562-4.297)               | 0.633                             | 1.158(0.634-2.116) | 0.007                | 6.803(1.675-27.633)  |
| Right colon                   | 0.464        | 0.859(0.572-1.290)               | 0.860                             | 0.939(0.467-1.888) | 0.249                | 0.728(0.425-1.249)   |
| Pathological T stage          |              |                                  |                                   |                   |                      |
| Pathological T stage          | 0.001        | 2.802(2.012-3.902)               | 0.041                             | 4.410(1.063-18.289)| 0.558                | 0.828(0.441-1.556)   |
| Lymph node metastasis         |              |                                  |                                   |                   |                      |
| Lymph node metastasis         |              |                                  |                                   |                   |                      |
| TNM stage                     |              |                                  |                                   |                   |                      |
| Differentiation               |              |                                  |                                   |                   |                      |
| Nervous invasion              |              |                                  |                                   |                   |                      |
| Schistosomiasis               | 0.001        | 4.006(2.686-5.519)               | 0.007                             | 16.949(2.188-131.284)| 0.011                | 1.702(1.132-2.559)   |
| Colonic perforation           | 0.541        | 0.700(0.223-2.198)               | 0.024                             | 1.174(0.285-4.829) | 0.866                | 1.056(0.562-1.985)   |
| Tumor budding                 | 0.001        | 2.028(1.400-2.938)               | 0.043                             | 1.812(1.019-3.221) | 0.163                | 1.423(0.867-2.336)   |
| Schistosomiasis               | 0.044        | 1.399(1.000-1.940)               | 0.474                             | 1.225(0.703-2.132) | 0.011                | 1.699(1.128-2.560)   |
| Ulceration                    | 0.624        | 0.9205(0.660-1.282)              | 0.362                             | 0.766(0.431-1.360) | 0.971                | 1.008(0.670-1.514)   |
| Histological type             | 0.921        | 1.025(0.626-1.489)               | 0.797                             | 0.886(0.352-2.230) | 0.870                | 0.952(0.529-1.713)   |
| Multivariate analysis         |              |                                  |                                   |                   |                      |
| Gender                        | 0.003        | 1.676(1.178-2.384)               |                     |                   |                      |
| Pathological T stage          |              |                                  |                                   |                   |                      |
| Tumor metastasis              |              |                                  |                                   |                   |                      |
| Gender                        | 0.001        | 0.389(0.267-0.567)               |                     |                   |                      |
| Lymph node metastasis         |              |                                  |                                   |                   |                      |
| Tumor budding                 |              |                                  |                                   |                   |                      |
| Differentiation               |              |                                  |                                   |                   |                      |
| Schistosomiasis               | 0.025        | 1.458(1.049-2.027)               |                     |                   |                      |
| Lymphovascular invasion       | 0.030        | 1.461(1.036-2.069)               |                     |                   |                      |
| Lymph nodes positive for CRC  | 0.001        | 2.256(1.461-3.483)               | 0.007                             | 16.8587(2.176-130.580)| 0.004                | 1.911(1.230-2.969)   |

— Data is non-significant; Abbreviation: CI = confidence interval; HR = Hazard ratio; P < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection.

to validate the present results. Second, patient selection bias is a possibility due to the nature of the retrospective study. Third, although we found a negative correlation between schistosomiasis and CRC outcomes, the precise functional roles of schistosomiasis in CRC progression and its underlying molecular mechanisms remain obscure. Chen et al. observed variable degree of colonic epithelial dysplasia in 60% of cases with S. japonicum colitis and regarded these changes as a transition on the way towards cancer development in schistosomal colonic disease[36]. A similar conclusion was drawn by Yu et al. from their studies on different types of schistosomal egg polyps[34]. All these results suggested the pro-tumor mechanisms of S. japonicum in tumor tissues. Therefore, further analysis about the functional roles of schistosoma infection and underlying molecular mechanisms need to be investigated. In addition, we were not sure if any of these patients suffered from familial cancer syndromes, such as Lynch syndrome. It was known that the proportion of patients with familial polyps and hereditary nonpolyposis CRC syndrome is higher in young patients (≤40 years old) [37, 38]. In our cohort, there were seven patients (0.02%) under 40 years old. However, work will continue to examine this possibility. Lastly, it was reported that schistosomiasis results from the host’s immune response to schistosome eggs and the granulomatous reaction evoked by the antigens they secrete[39], and the process of granulomas formation will be accompanied by chronic inflammation[40, 41], which may induces the development of tumor. However, we could not provide evidence in this study and detection of inflammatory markers will be conducted to strengthen the hypothesis in further work.

In summary, our observations support the pathogenetic role of schistosomiasis and shed light on the adverse effects of schistosomiasis on CRC patients.

Abbreviations
### Table 3. The association between clinicopathological characteristics and schistosomiasis in CRC cohort

| Characteristic                        | All patients | Stage I-II disease patients | Stage III disease patients | With lymph node metastasis patients | Without lymph node metastasis patients |
|---------------------------------------|--------------|-----------------------------|---------------------------|-------------------------------------|----------------------------------------|
|                                       | CRC-NS (N=214) | CRC-S (N=137)               | CRC-NS (N=116)            | CRC-S (N=76)                        | CRC-NS (N=98)                          | CRC-S (N=61)                          | CRC-NS (N=56)                          | CRC-S (N=126) |
|                                       | P            | P                           | P                         | P                                   |                                        | P                                      |                                        |              |
| **Age (≥60yrs)**                      |              |                             |                           |                                     |                                        |                                        |                                        |              |
| ≥60                                   | 0.001        | 0.001                       | 0.001                     | 0.001                               |                                        |                                        |                                        |              |
| <60                                   |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Gender                                |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Male                                  | 0.467        | 0.695                       | 0.520                     | 0.001                               |                                        |                                        |                                        |              |
| Female                                |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Tumor site                            |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Rectum                                | 0.274        | 0.750                       | 0.829                     | 0.806                               |                                        |                                        |                                        |              |
| Left colon                            | 0.450        | 0.63                        | 0.425                     | 0.35                                |                                        |                                        |                                        |              |
| Right colon                           | 0.420        | 0.62                        | 0.45                      | 0.26                                |                                        |                                        |                                        |              |
| Tumor size                            |              |                             |                           |                                     |                                        |                                        |                                        |              |
| <5cm                                  | 0.985        | 0.599                       | 0.581                     | 0.372                               |                                        |                                        |                                        |              |
| ≥5cm                                  |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Differentiation                       |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Well diff.                            | 0.909        | 0.732                       | 1.000                     | 1.000                               |                                        |                                        |                                        |              |
| Poor diff.                            | 0.909        | 0.732                       | 1.000                     | 1.000                               |                                        |                                        |                                        |              |
| Lymphovascular invasion               |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Negative                              | 0.001        | 0.001                       | 0.001                     | 0.001                               |                                        |                                        |                                        |              |
| Positive                              |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Nervous invasion                      |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Negative                              | 1.000        | 0.766                       | 1.000                     | 1.000                               |                                        |                                        |                                        |              |
| Positive                              |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Lymph node positive for CRC           |              |                             |                           |                                     |                                        |                                        |                                        |              |
| ≤2                                   | 0.867        | 0.998                       | 0.710                     | 1.000                               |                                        |                                        |                                        |              |
| >2                                   |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Colonic perforation                   |              |                             |                           |                                     |                                        |                                        |                                        |              |
| No                                   | 0.966        | 0.482                       | 0.373                     | 0.299                               |                                        |                                        |                                        |              |
| Yes                                  |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Tumor budding                         |              |                             |                           |                                     |                                        |                                        |                                        |              |
| ≥5 cells                             | 0.652        | 0.184                       | 0.454                     | 0.841                               |                                        |                                        |                                        |              |
| <5 cells                             |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Histological type                     |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Adenocarcinoma                        | 0.731        | 0.470                       | 0.590                     | 1.000                               |                                        |                                        |                                        |              |
| Macinous/SRCC                        |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Ulceration                            |              |                             |                           |                                     |                                        |                                        |                                        |              |
| No                                   | 0.740        | 0.881                       | 0.774                     | 0.495                               |                                        |                                        |                                        |              |
| Yes                                  |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Pathological T stage                  |              |                             |                           |                                     |                                        |                                        |                                        |              |
| I+ II                                 | 0.883        | 0.823                       | 0.641                     |                                     |                                        |                                        |                                        |              |
| T3-4                                 |              |                             |                           |                                     |                                        |                                        |                                        |              |
| Lymph node metastasis                 |              |                             |                           |                                     |                                        |                                        |                                        |              |
| No                                   | 0.816        | 0.816                       | 0.842                     |                                     |                                        |                                        |                                        |              |
| Yes                                  |              |                             |                           |                                     |                                        |                                        |                                        |              |
| ---                                   |              |                             |                           |                                     |                                        |                                        |                                        |              |

---: Data is not applicable; Abbreviation: N=Number. The association between schistosomiasis and clinicopathological characteristics was evaluated using Fisher's exact tests.

colorectal cancer=CRC; colorectal cancer patients with schistosomiasis =CRC-S; patients without schistosomiasis=CRC-NS; Schistosoma japonicum=S. japonicum; American Joint Committee on Cancer =AJCC; formalin fixed paraffin-embedded (FFPE); Overall survival =OS;

### Declarations

**Ethics approval and consent to participate:**

This study was approved by the medical ethics committee of Fudan University (Ethical approval number 2019-017), in accordance with the Helsinki Declaration of 1975. Prior written informed consent was obtained from all patients.

**Consent for publication:** Written informed consent was obtained from each participant.

**Availability of data and materials:**

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

**Conflict of interest:** The authors declare that they have no competing interests.

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Authors' contributions

Weixia Wang contributed data analysis, manuscript editing, article revision and data supplement. Kui Lu and Limei Wang assessed all the dyeing slices. Hongyan Jing contributed to the research design, data analysis, and manuscript writing. Weiyu Pan, Sinian Huang, Yanchao Xu, Dacheng Bu, Meihong Cheng, Jing Liu, Jicun Liu, Weidong Shen, Yingyi Zhang and Junxia Yao contributed to the data collection and perform experiments. Ting Zhu contributed to the data analysis and manuscript editing. All authors read and approved the final manuscript.

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Figures

Figure 1

A. Typical sample of schistosomiasis-associated colorectal cancer, the red arrows indicate schistosome ova (HE, ×100); B. Kaplan-Meier analysis of overall survival in whole CRC cohort according to schistosoma infection status. P value was calculated by log-rank test.

Figure 2

Kaplan-Meier analysis of overall survival in stratified CRC. A. Patients with stage I-II tumors (N=192, P=0.5018); B. Patients with stage III-IV tumors (N=159, P=0.0260); C. Patients with lymph node metastasis (N=144, P=0.0249); and D. patients without lymph node metastasis (N=207, P=0.4005); P value was
calculated by log-rank test.