Prognostic Value of Tumor-Infiltrating Lymphocytes in Schistosomiasis-Associated Colorectal Cancer

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Research Article

Keywords: tumor-infiltrating lymphocytes; colorectal cancer; schistosomiasis; prognosis

Posted Date: July 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-736856/v1

Abstract

Aim: To investigate the relationship between schistosomiasis and tumour infiltrating lymphocytes (TILs), and the prognostic value of TILs in schistosomal colorectal cancer (CRC).

Background: The association between TILs and CRC has long been suggested in the literature, but the association between TILs and schistosomiasis and the prognositic role of TILs in schistosomal CRC has never been reported previously.

Methods: Hematoxylin and eosin (H&E)-stained sections of 351 CRC tumours, which were completely resected, were evaluated for density of TILs in intratumoural (iTIL) and stromal compartments (sTIL). Its relationship with clinicopathological features, including schistosomiasis, and clinical outcomes were evaluated and the prognostic role of sTILs in schistosomal CRC was explored.

Results: Stromal TILs infiltration were correlated with smaller tumor size，less deeper pathological T stage, absence lymph node metastasis and less number of tumor budding (p＜0.05). However, there were no association between sTILs and shicstosomiasis. In the whole cohort, multivariate analysis identified gender, TNM Stage, *Schistosomiasis,* sTILs, lymph vascular invasion, lymph nodes positive for CRC were independent prognostic factors that associated with overall survival (OS) in CRC ($p < 0.05$). Patients were divided into two groups based on schistosomiasis infection status: colorectal cancer associated with schistosomiasis (CRC-NS set) and colorectal cancer without schistosomiasis (CRC-S set). In the CRC-NS set, multivariate analysis demonstrated that tumor budding, sTILs, lymph vascular invasion, lymph nodes positive for CRC were independent prognostic factors that associated with OS ($p < 0.05$). However, there were no association between sTILs and OS in CRC-S set (p＞0.05). Besides, sTILs were associated with favorable OS in CRC-NS patients but not in CRC-S patients, regardless of age.

Conclusion: Stromal TILs in the whole cohort and in the CRC-NS set were identified as an independent prognostic factor, but it was lack of prognostic role in schistosomal CRC. Stromal TILs was associated with less aggressive tumor features. Stromal TILs was associated with OS in CRC-NS patients but not in CRC-S patients, regardless of age.

Introduction

Schistosomiasis is an infectious disease that affects more than 230 million people worldwide, according to conservative estimates[1]. Qingpu District of Shanghai used to be one of the 10 areas with serious schistosomiasis epidemic in China[2]. Although China has achieved considerable success in combating this disease and the incidence and prevalence of schistosomiasis in China has dropped[3]. However, problems of treatment and outcome of a large number of late schistosomiasis patients left over from history are still remaining. In addition, multiple factors, such as an increase in the number and spread of oncomelania snails spread which affects schistosomiasis epidemics, thus there is still a risk of a rebound in the incidence in some areas[4]. In addition, schistosome eggs were occasionally found in pathological specimens of CRC under the microscope in our daily work.

Growing evidences have arisen in recent decades that inflammation is the cause of many malignant tumors[5, 6]. As the fourth most common cancer and the second leading cause of cancer deaths in the world[7], CRC represents an increasing number of cancers that correlated with inflammation[5, 8, 9]. Several studies have suggested that

long-term inflammation caused by chronic schistosomal infection is a key factor in the carcinogenic process of CRC[10]. It was reported that the sequestered eggs in the mucosa and submucosa incite a severe focal inflammatory reaction with cellular infiltration and consequent minute mucosal ulcerations, and microabscesses and granuloma formation. The continuous irritation produced by the egg nests eventually leads to fibrosis, mucosal hyperplasia, polyposis, and pseudopolyposis[11, 12]. However, all these studies were based on the analysis of data of clinicopathological characteristics, inflammation based prognostic systems have been rarely reported in the literature. Besides, our previous study showed that schistosomiasis was an independent poor prognosis for CRC patients[13], and clinicopathological characteristics were differences between patients with schistosomal and nonschistosomal CRC[13]. But the evidence that chronic inflammatory process occurring in schistosomiasis playing an important role in CRC progression is not providing.

The immune system is known to act against tumours and it has been postulated that TILs reflect a tumour related immune response[14–18]. TILs have been shown to provide prognostic and potentially predictive value in numerous literatures [19–21]. Ann C. Eriksen *et al* reported that low CD3 + and CD8 + TILs were associated with inferior prognosis of stage II colorectal cancer by immunohistochemistry [22]. Yamei Zhao *et al* combined the subtypes of TILs and the infiltrating sites with the anatomical sites of colorectal cancer to assess the association between each subset of TILs and the survival outcome by meta-analysis. Their results demonstrated that high-density TILs reflect favorable prognostic value in CRC[23]. However, the relationship between schistosomiasis and TILs, and the prognostic value of TILs in schistosomal colorectal cancer has never been reported. Accumulating evidence suggests that the extent of lymphocytic infiltration in tumor tissue can be assessed as a major parameter by evaluation of HE-stained tumor sections. K M Ropponen *et al* evaluated TILs in the centre and periphery of the tumours and around invasive carcinoma cells by HE-stained tumors sections and their data showed that TILs can provide important prognostic information in colorectal cancer to be used in evaluating for adjuvant therapy in different tumour stages[24]. In the present study, we undertook a study of sTILs to better determine the effect of schistosomiasis on CRC patient outcomes. And to evaluate the prognositc role of sTILs in schistosomal CRC.

Materials And Methods

Patients and samples

A total of 351 CRC patients were enrolled in this retrospective study. All patients had received curative resection without preoperative chemotherapy at Qingpu Branch of Zhongshan Hospital affiliated to Fudan University, from January 2008 to August 2016. And all patients 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 (□) patients with CRC as primary focus; (□) none of these patients had received any prior anti-tumor therapy; (□) patients were diagnosed as adenocarcinoma by pathology after resection of CRC. Exclusion criteria included (□) Tis tumours; (□) patients who lacked complete information; (□) patients with synchronous malignancy, such as liver cancer, lung cancer and ovarian cancer, were excluded; (□) 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

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 blinded to clinic data. The diagnosis of schistosomiasis was done by finding schistosome eggs in anywhere in the HE-stained slides. Patients with schistosomal CRC were identified by the presence of *Schistosoma* eggs in the intestinal tissues.

Assessment Of Tumor Budding

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[25]. To assess tumor budding in the 10-HPF method[26], 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 (×20) and the tumor budding count is reported. The evaluation of tumor budding was conducted by two pathologists who were blinded to clinic 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.

Histologic Evaluation Of Til

Evaluation of TILs were performed as previously described[27]. TILs were performed in H&E-stained FFPE sections, which were examined at ×10 and ×40 magnification fields using a conventional light microscope by two pathologists. The mean value was used for the analyses presented. Neither pathologist had any knowledge of the clinical information. Intratumoural infiltrating lymphocytes (iTIL) was defined as the percentage of mononuclear cells within the epithelium of the invasive tumour cell nests. Stromal infiltrating lymphocytes (sTIL) was defined as the percentage of tumour stroma containing infiltrating lymphocytes (area occupied by mononuclear cells in tumor stroma/total stromal area). TILs have evaluated stromal and intratumoral lymphocytes separately. Intratumoral TILs are defined as lymphocytes in tumor nests having cell-to-cell contact with no intervening stroma and directly interacting with carcinoma cells, while stromal TILs are located dispersed in the stroma between the carcinoma cells and do not directly contact carcinoma cells. Here, in our study, more than 2% of either stromal or intratumoural TIL were defined as high TIL.

Statistical analysis.

Data were analyzed using SPSS (version 20.0; IBM Corp.) and Graphpad 5.0. The association between sTILs 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. Every variable was analyzed using univariate analysis to identify all potentially important predictors and then variables with $P \leq 0.05$ in the univariate analysis were included in a multivariate analysis. Finally, multivariate Cox regression analysis was performed to identify predictive factors for OS. Clinically relevant variables that may have impacted outcomes, such as age, gender, TNM stage, lymph node metastasis, histological type and so on. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Study patients

Patient characteristics are summarized in Table 1. In the whole cohort, 39.0% (137 out of 351) were infected with schistosoma (Fig. 1). 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 (61%, 214 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 without lymph node metastasis were 60%. On the basis of the AJCC Staging Manual (eighth edition), there were very few highly differentiated cases in the follow-up data. Thus, highly differentiated and moderately differentiated cases were classified as "well differentiation", and classified poorly differentiated cases as "poor differentiation". 76% cases were well differentiated. As shown in Table 1, lymphovascular invasion, perineura invasion, lymph nodes positive for CRC and tumor budding were prone to appear in patients with stage III-IV tumors or patients with lymph node metastasis. More poorly differentiated tumors and deeper tumor invasion depth were also mostly observed in patients with late tumor stage or patients with lymph node metastasis. The distribution trend of other clinicopathologic features, such as colonic perforation, ulceration, histological type were similar within different subgroups.

Table 1
Clinicopathological characteristics of the CRC cohort

Characteristics	**All patients (N = 351)**	
	N	*%*
Age(<60ys)	83	*24.6*
Gender(Male)	214	*63.3*
Tumor location		
Rectum	94	*27.8*
Left colon	115	*34.0*
Right colon	142	*42.0*
Tumor size(<5cm)	174	*51.4*
Differentiation		
Well/moderately diff.	267	*78.9*
Poorly diff.	84	*24.9*
Lymph vascular invasion (positive)	122	*34.9*
Nervous invasion (positive)	31	*1.0*
Lymph nodes positive for CRC (>2)	42	*1.2*
Colonic perforation (Yes)	13	*0.4*
Tummor budding (≥ 5 cells)	219	*64.7*
Ulceration (Yes)	149	*44.0*
Histological type		
Adenocarcinoma	311	*92.0*
Mucinous/SRCC	40	*11.8*
Pathological T stage		
T1-2	80	*23.6*
T3-4	271	*77*
Lymph node metastasis		
No	207	*61.2*
Yes	144	*42.6*
TNM stage		
I	63	*18.6*

Characteristics	All patients (N = 351)	
II	119	*35.2*
III	132	*39.1*
IV	24	*7.1*
sTILs		
Poor	138	*40.8*
Rich	200	*59.2*
schistosomiasis	128	*37.9*

The relationship of sTILs and clinicopathological Features.

TILs have evaluated stromal and intratumoral lymphocytes separately. Intratumoral lymphocytes are typically present in lower numbers and detected in fewer cases. The percentage of iTILs of 98% cases was about 1%. Moreover, iTILs are more heterogeneous and are difficult to observe on H&E-stained slides. Thus, the data of iTILs were abandoned in the following analysis. Representative TILs staining is shown in Fig. 2. sTILs ≥ 2% was defined as sTILs-rich, otherwise as sTILs-poor, and sTILs-rich were observed in 221 (63.0%) cases. The relationship between schistosomiasis and clinicopathological features was shown in Table 2. Except for age, there were no association between schistosomiasis and clinicopathological features, including sTILs. As shown in Table 2, higher sTILs infiltration were correlated with smaller tumor size,less deeper pathological T stage, absence lymph node metastasis and less number of tumor budding, which is a an important additional prognostic factor for patients with CRC[26, 28] (Table 2: $p<0.001$, $p<0.001$, $p<0.001$, $p = 0.050$, and $p = 0.046$). These results suggested that high sTILs infiltration correlated with less aggressive features of tumors. Whereas such correlations were not found between sTILs and age, ,TNM Stage, gender, schistosomiasis, tumor site, tumor differentiation, lymph vascular invasion, nervous invasion, histological type, bowel perforation, and so on (Table 2, $p>0.05$). In addition, sTILs was higher in CRC-NS patients (65% sTILs-rich) compared with that of CRC-NS patients (59% sTILs-rich) (Table 2).

Table 2
The association between clinicopathological characteristics and schistosomiasis and sTILs

Characteristic	All patients		*P*	sTILs		*P*
	CRC-NS (N = 214)	CRC-S (N = 137)		Poor (<2%) (N = 130)	Rich (≥ 2%) (N = 221)	
Age(<60ys)			<0.001			0.897
<60	78	5		30	53	
≥ 60	136	132		100	168	
Gender			0.467			0.07
Male	126	86		43	96	
Female	88	51		87	125	
Tumor site			0.274			0.112
Rectum	57	37		27	67	
Left colon	64	51		49	66	
Right colon	93	49		54	88	
Tumor size			0.985			<0.001
<5cm	106	68		49	125	
≥ 5cm	108	69		81	96	
Differentiation			0.570			*0.365*
Well/moderately diff.	165	102		95	172	
Poorly diff.	49	35		35	49	
Lymphvascula invasion			0.909			0.489
Negative	138	90		87	139	
Positive	76	47		43	82	
Nervous invasion			1.000			0.445
Negative	194	125		116	203	
Positive	20	12		14	18	
LNs positive for CRC			0.867			0.734
≤ 2	189	120		116	193	
>2	25	17		14	28	
Colonic perforation			0.966			0.774
No	206	132		126	212	

Characteristic	All patients		*P*	sTILs		*P*
	CRC-NS (N = 214)	CRC-S (N = 137)		Poor (<2%) (N = 130)	Rich (≥ 2%) (N = 221)	
Yes	8	5		4	9	
Tumor budding			0.652			<0.001
<5 cells	83	49		79	176	
≥ 5 cells	131	88		51	45	
Histological type			0.731			0.998
Adenocarcinoma	191	120		116	196	
Mucinous/SRCC	23	17		14	25	
Ulceration			0.740			0.656
No	125	77		77	125	
Yes	89	60		53	96	
Pathological T stage			0.562			<0.001
T1-2	51	29		17	66	
T3-4	163	108		113	155	
LNM			0.883			0.050
No	126	81		68	139	
Yes	88	56		62	82	
TNM stage			0.975			0.181
I	23	40		20	43	
II	47	72		47	74	
III	49	83		59	73	
IV	9	15		12	10	
sTILs			0.258			—
Poor	74	56		—	—	
Rich	140	81		—	—	
Schistosomiasis			—			0.258
Negative	—	—		74	140	
Positive	—	—		56	81	

Characteristic	All patients		*P*	sTILs		*P*
	CRC-NS (N = 214)	CRC-S (N = 137)		Poor (<2%) (N = 130)	Rich (≥ 2%) (N = 221)	

—:Data is not applicable; Abbreviation: sTILs = stromal tumour-infiltrating lymphocytes; CRC-NS = patients without schistosomiasis; CRC-S = patients with schistosomiasis; N = Number; LN = Lymph node. The association between schistosomiasis and clinicopathological characteristics was evaluated by using the Chi square and Fisher's exact tests.

Univariate And Multivariate Regression Analysis

In the whole cohort, univariate Cox regression analysis identified clinical factors statistically significantly associated with OS (Table 3) were age, gender, pathological T stage, lymph node metastasis, TNM stage, tumor differentiation, lymph vascular invasion, lymph nodes positive for CRC, tumor budding, *schistosomiasis*, and sTILs ($p < 0.05$). Variables demonstrating a significant effect on OS were included in the multivariate analysis. Gender, TNM Stage, *Schistosomiasis*, sTILs, lymph vascular invasion, lymph nodes positive for CRC ($p < 0.05$) were identified as independent prognostic factors that associated with OS (Table 3). Further analysis was conducted to explore the prognostic significance of sTILs in patients with and without *Schistosomiasis*. In the CRC-NS group, tumor budding, sTILs, lymph vascular invasion, and lymph nodes positive for CRC were independent prognostic factors for OS. However, merely TNM stage and lymph nodes positive for CRC were independent prognostic factors in the CRC-S group.

Table 3
Univariate and multivariate Cox regression of clinicopathological for overall survival

Variable	All patients (N = 351)		CRC-NS (N = 214)		CRC-S (N = 137)	
	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)
Univariate analysis						
Age (<60ys)	0.010	1.770(1.149–2.726)	0.122	1.454(0.905–2.336)	0.232	21.827(0.139-3436.270)
Gender (male/female)	0.010	1.585(1.117–2.248)	0.017	1.780(1.110–2.853)	0.307	1.311(0.779–2.207)
Tumor size(5cm)	0.913	1.018(0.735–1.412)	0.591	0.886(0.569–1.378)	0.320	1.282(0.786–2.089)
Tumor site						
Rectum	—	Refer	—	Refer	—	
Left colon	0.908	1.025(0.676–1.553)	0.672	0.889 (0.515–1.534)	0.484	1.263(0.657–2.427)
Right colon	0.496	0.868 (0.579–1.303)	0.054	0.590 (0.344–1.010)	0.130	1.631(0.865–3.076)
Pathological T stage	<0.001	2.591(1.562–4.297)	<0.001	3.363(1.620–6.980)	0.087	1.851(0.915–3.747)
Lymph node metastasis	<0.001	2.783(1.999–3.875)	<0.001	2.447(1.573–3.807)	<0.001	3.552(2.141–5.894)
TNM stage	<0.001	3.197(2.271–4.501)	<0.001	2.764(1.752–4.358)	<0.001	4.219(2.497–7.128)
Differentiation	<0.001	1.878(1.326–2.659)	0.003	2.009(1.259–3.206)	0.054	1.668(0.991–2.809)
Lymph vascular invasion	<0.001	2.038(1.468–2.829)	<0.001	2.816(1.808–4.385)	0.275	1.321(0.801–2.180)
Nervous invasion	0.140	1.497(0.876–2.559)	0.319	1.424 (0.710–2.857)	0. 206	1.727(0.741–4.024)
LNs positive for CRC	<0.001	3.989(2.675–5.948)	<0.001	3.973(2.359–6.692)	<0.001	4.138(2.205–7.769)
Colonic perforation	0.817	0.889(0.329–2.403)	0.763	1.194(0.377–3.786)	0.500	0.506(0.070–3.657)
Tummor budding	<0.001	2.332(1.677–3.241)	<0.001	2.979(1.924–4.614)	0.055	1.646(0.989–2.740)
Schistosomiasis	0.048	1.390(1.002–1.927)	—	—	—	—

Variable	All patients (N = 351)		CRC-NS (N = 214)		CRC-S (N = 137)	
	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)
sTILs	<0.001	0.570(0.411–0.791)	0.002	0.499(0.321–0.776)	0.162	0.704(0.431–1.151)
Ulceration	0.624	0.920(0.660–1.282)	0.744	1.077(0.691–1.676)	0.212	0.725(0.437–1.201)
Histological type	0.703	1.096(0.684–1.758)	0.283	1.400(0.758–2.586)	0.467	0.760(0.362–1.594)
Multivariate analysis						
Gender	0.005	1.651(1.159–2.351)	—	—	—	
Pathological T stage	—	—	—	—	—	
TNM stage	<0.001	2.764(1.752–4.358)	—	—	<0.001	3.617(2.071–6.317)
Lymph node metastasis	—	—				
Tumor budding	—	—	0.034	1.694(1.039–2.761)		
Differentiation	—	—	—	—	—	
Schistosomiasis	0.032	1.435(1.031–1.998)	—	—	—	
sTILs	0.005	0.620(0.444–0.867)	0.009	0.539(0.339–0.855)		
Lymph vascular invasion	0.036	1.441(1.023–2.029)	0.002	2.108(1.313–3.384)	—	
LNs positive for CRC	<0.001	2.429(1.570–3.757)	0.002	2.404(1.366–4.232)	0.030	2.083(1.074–4.039)

—:Data is non-significant ; Abbreviation: CRC-NS = patients without schistosomiasis; CRC-S = patients with schistosomiasis; CI = confidence interval; HR = Hazard ratio; LN = Lymph node;

P < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection.

Survival Analysis

To investigate the prognostic value between sTILs and clinical outcomes, Kaplan-Meier analysis was conducted in the total cohort according to percentage of sTILs. Mean and median time to OS was 62.54 and 62.85(1.25–134.4) months, respectively. During the follow-up, there were 40% (141 out of 351) patients who died. Patients with sTILs-rich gain significant survival benefit compared with sTILs-poor (Fig. 3A; p = 0.007).

Given schistosomiasis was an independent risk factor in the univariate analysis, patients were further stratified by status of schistosomal infection. In the non-schistosomal group, higher sTILs were associated with favorable OS (Fig. 3B; p = 0.0017). But there were no significant association between sTIL and OS in the schistosomal group (Fig. 3C; p = 0.1593).

As mentioned above, there were no association between schistosomiasis and sTILs. This seems contrary to the expectation. Intriguingly, we found that patients in the CRC-S set were obviously older than patients in the CRC-NS set (Table.2), and median age of patients at diagnosis in the CRC-NS set were 64 years, but were 74 years in the CRC-S set. It is speculated that aging lead to immunosenescence[29]. Namely, the immune response is inert in the patients with schistosomiasis. Therefore, we further stratified patients based on age: patients younger than or equal to 65 years old and older than 65 year. Results demonstrated that sTILs associated with longer OS in the CRC-NS patients who were younger than or equal to 65 years old (Fig. 4A; p = 0.0436). However, there were no significant difference between sTILs and OS in CRC-S patients of this age group (Fig. 4B; p = 0.6962). Similarly, we found sTILs were associated with OS in the CRC-NS group (Fig. 4C; p = 0.0468), but not in CRC-S patients who were older than 65 years old (Fig. 4D; p = 0.2702).

Discussion

Schistosomiasis is a common disease worldwide. In endemic areas, schistosomal infestation has been implicated in the aetiology of several human malignancies including bladder, liver, and CRC[30, 31]. Ye et al[32] reported that intestinal schistosomiasis was a risk factor for CRC and that the lesions caused by the disease might be considered precancerous. Our previous study demonstrated that schistosomiasis was an independent worse prognosis for OS in CRC patients[13]. In this study, we further confirmed that schistosomiasis was an independent unbeneficial prognosis factor for OS in CRC patients. However, sufficient evidence supports a causal relationship between schistosomal infestation and CRC has apparently low status within the canons of medicine and reports from the publishing world[33].

Specimens from patients with schistosomiasis showed associated inflammatory changes, pseudopolyps, and transitional mucosal changes of schistosomal granulomatous disease progressing to mucosal atypia and to carcinoma were reminiscent of colorectal carcinoma in patients with ulcerative colitis[34]. It was known that TILs reflects an active inflammatory tumor microenvironment. Accordingly, it was hypothesized that schistosomiasis infection could associated with vigorous sTILs infiltration. In this study, we found that sTILs was an independent favorable predictor factor for OS in the whole cohort. This was consistent with previous studies[35–37]. Stromal TILs also was an independent favorable predictor factor in CRC-NS patients, but was not in CRC-S patients. Furthermore, results demonstrated that sTILs was obviously higher in the CRC-NS set compared with of that in the CRC-S set. These results seems inconsistent with expectations and assumptions. It was reported that aging lead to immunosenescence[29]. We found that patients with schistosomiasis were obviously older than patients without schistosomiasis. We wonder whether immune response was inert in patients with schistosomiasis. Thus, patients were restratified by age, and results showed that sTILs associated with favorable OS in CRC-NS patients but not in CRC-S, regardless of age. This might be related to the complex immune response and immune microenvironment caused by schistosome infection and further work is needed to reveal the underlying

mechanism. Previous studies suggested that sTILs density at diagnosis were a prognostic factor for adjuvant and neoadjuvant therapy[38]. Our results suggested that patients with schistosomiasis should also be cautious when adopt radiotherapy and chemotherapy. Additionally, comparative analysis of sTILs infiltration with clinicopathologic features revealed that higher sTILs infiltration correlated with less aggressive features of tumors. This result is consistent with the literatures[24, 39]. These results further confirmed that sTILs played crucial role in the development and progression of CRC.

Given the functional heterogeneity of intratumoral lymphocytes, and negative immune regulators are present as part of a normal feedback loop reacting to an active and ongoing antitumor immune response, a focused evaluation of individual subsets may have limited value. Besides, the degree of lymphocytic infiltration assessed by simple evaluation of hematoxylin and eosin (H&E)- stained tumor sections has been shown to have predictive and prognostic value despite a lack of detailed information on the immune subpopulations of the infiltrate [38, 40–42]. In present study, we evaluated iTILs and sTILs, respectively, However, we found that iTILs are typically present in lower numbers and detected in fewer cases, they are more heterogeneous and are uneasy to observe on H&E-stained slides[38]. The percentage of iTILs of almost 98% cases in the total cohort was under 1%. Moreover, most current studies have found sTILs to be a superior and more reproducible parameter[43]. Thus, we focused on merely sTILs in the following analysis.

Several limitations associated with the present study warrant mentioning. Firstly, The diagnosis of schistosomiasis was done by finding schistosome eggs in HE-stained slides, lacking of other methods to prove schistosome infection. This may lead to the missing of the number of schistosomiasis positive cases. In order to reduce this limitation, schistosome ova were observed in all original HE-stained slides (usually 4–6 slides per case), which were obtained from original individual paraffin blocks and used for routine diagnosis. Besides, the medical records of the selected cases were checked carefully to further screen schistosome positive reports. Secondly, the working group states that evaluating sTIL as a continuous variable will allow for more accurate statistical analysis[38], but in practice, most pathologists will not report specific values. Simplicity is needed for a pathological methodology to be accepted widely. For this reason, sTILs were evaluated as a non-continuous variable in this study. Although, in the study, we found a positive correlation between sTILs and CRC outcomes, the precise functional roles of sTILs infiltration in CRC progression and its underlying molecular mechanisms remain obscure. Therefore, further analysis about the precise functional roles and underlying molecular mechanisms need to be investigated.

In conclusion, results demonstrated that there were no association between Schistosomiasis and stromal TILs, but higher stromal TILs showed a trend towards significance and improved survival. Further work to confirm whether there is significance for OS in a larger cohort in the Schistosoma group will be conducted.

Abbreviations

tumour infiltrating lymphocytes=TILs; colorectal cancer=CRC; hematoxylin and eosin =H&E；intratumour TILs=iTILS; stromal tumor infiltrating lymphocytes=sTILs; colorectal cancer patients with schistosomiasis =CRC-S; patients without schistosomiasis=CRC-NS; Overall survival =OS; inferior mesenteric artery =IMA; American Joint Committee on Cancer =AJCC; Kaplan-Meier=K-M; formalin fixed paraffin-embedded =FFPE;

Declarations

Ethics approval and consent to participate:

This study was approved by the medical ethics committee of Fudan University, 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.

Funding: This work is supported by China Shanghai Municipal Commission of Health and Family Planning(Grant No. 20194Y0162) and by The fourth round talent training plan of Qingpu District Health System (QKY2020-7)

Authors' contributions:

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

Acknowledgements: None

References

1. Vos T, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2163–96.
2. Yu SZ. [Schistosomiasis must be eradicated: a review of fighting schitosomiasis in Qingpu, Shanghai]. Zhonghua Liu Xing Bing Xue Za Zhi. 2016;37(7):1044–6.
3. Yang Y, et al. Integrated Control Strategy of Schistosomiasis in The People's Republic of China: Projects Involving Agriculture, Water Conservancy, Forestry, Sanitation and Environmental Modification. Adv Parasitol. 2016;92:237–68.
4. Li-Juan Z, et al. [Endemic status of schistosomiasis in People's Republic of China in 2016]. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi. 2017;29(6):669–77.
5. Mantovani A. Cancer: Inflaming metastasis. Nature. 2009;457(7225):36–7.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
7. Bray F, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
8. H SO, et al. Colorectal carcinoma associated with schistosomiasis: a possible causal relationship. World J Surg Oncol. 2010;8:68.

9. Chen MG. Assessment of morbidity due to Schistosoma japonicum infection in China. Infect Dis Poverty. 2014;3(1):6.
10. Herman AM, et al. Colorectal cancer in a patient with intestinal schistosomiasis: a case report from Kilimanjaro Christian Medical Center Northern Zone Tanzania. World J Surg Oncol. 2017;15(1):146.
11. Gryseels B. Schistosomiasis. Infect Dis Clin North Am. 2012;26(2):383–97.
12. Willingham AR, et al. Schistosoma japonicum in the pig: the host-parasite relationship as influenced by the intensity and duration of experimental infection. Am J Trop Med Hyg. 1998;58(2):248–56.
13. Wang W, et al. Comparison of non-schistosomal colorectal cancer and schistosomal colorectal cancer. World J Surg Oncol. 2020;18(1):149.
14. Jass JR. Lymphocytic infiltration and survival in rectal cancer. J Clin Pathol. 1986;39(6):585–9.
15. Svennevig JL, et al. Lymphoid infiltration and prognosis in colorectal carcinoma. Br J Cancer. 1984;49(3):375–7.
16. Aaltomaa S, et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer. 1992;28A:4–5): p. 859 – 64.
17. Furukawa T, et al. T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. Cancer. 1985;56(11):2651–6.
18. Tsujitani S, et al. Langerhans cells and prognosis in patients with gastric carcinoma. Cancer. 1987;59(3):501–5.
19. Aaltomaa S, et al. Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer. 1992;28A:4–5): p. 859 – 64.
20. Alexe G, et al. High expression of lymphocyte-associated genes in node-negative HER2 + breast cancers correlates with lower recurrence rates. Cancer Res. 2007;67(22):10669–76.
21. Denkert C, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105–13.
22. Eriksen AC, et al. The Prognostic Value of Tumor-Infiltrating lymphocytes in Stage II Colon Cancer. A Nationwide Population-Based Study. Transl Oncol. 2018;11(4):979–87.
23. Zhao Y, et al. The prognostic value of tumor-infiltrating lymphocytes in colorectal cancer differs by anatomical subsite: a systematic review and meta-analysis. World J Surg Oncol. 2019;17(1):85.
24. Ropponen KM, et al. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol. 1997;182(3):318–24.
25. Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer–ready for diagnostic practice? Hum Pathol. 2016;47(1):4–19.
26. Horcic M, et al. Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol. 2013;44(5):697–705.
27. Salgado R, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71.
28. Koelzer VH, Zlobec I, Lugli A. Tumor budding in colorectal cancer–ready for diagnostic practice? Hum Pathol. 2016;47(1):4–19.
29. Kovaiou RD, Herndler-Brandstetter D, Grubeck-Loebenstein B. Age-related changes in immunity: implications for vaccination in the elderly. Expert Rev Mol Med. 2007;9(3):1–17.

30. Yosry A. Schistosomiasis and neoplasia. Contrib Microbiol. 2006;13:81–100.
31. Ishii A, et al. Parasite infection and cancer: with special emphasis on Schistosoma japonicum infections (Trematoda). A review. Mutat Res. 1994;305(2):273–81.
32. Ye C, et al. Endoscopic characteristics and causes of misdiagnosis of intestinal schistosomiasis. Mol Med Rep. 2013;8(4):1089–93.
33. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1–241.
34. Gryseels B. Schistosomiasis. Infect Dis Clin North Am. 2012;26(2):383–97.
35. Denkert C, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105–13.
36. Loi S, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31(7):860–7.
37. Loi S, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol. 2014;25(8):1544–50.
38. Salgado R, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71.
39. Mlecnik B, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol. 2011;29(6):610–8.
40. Loi S, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31(7):860–7.
41. Curigliano G, Perez EA. Immunoscoring breast cancer: TILs remember what they target. Ann Oncol. 2014;25(8):1455–6.
42. Jun SY, et al. Prognostic Significance of Stromal and Intraepithelial Tumor-Infiltrating Lymphocytes in Small Intestinal Adenocarcinoma. Am J Clin Pathol. 2020;153(1):105–18.
43. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst. 2009;101(21):1446–52.

Figures

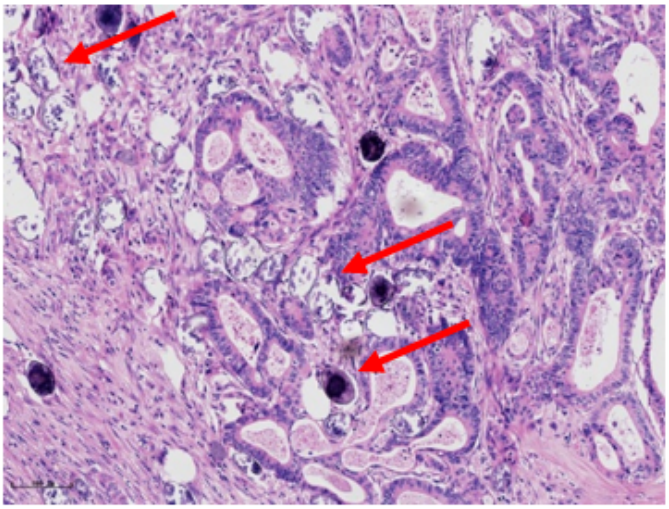

Figure 1

Typical sample of schistosomiasis-associated colorectal cancer, the red arrows indicate schistosome ova (HE, ×100).

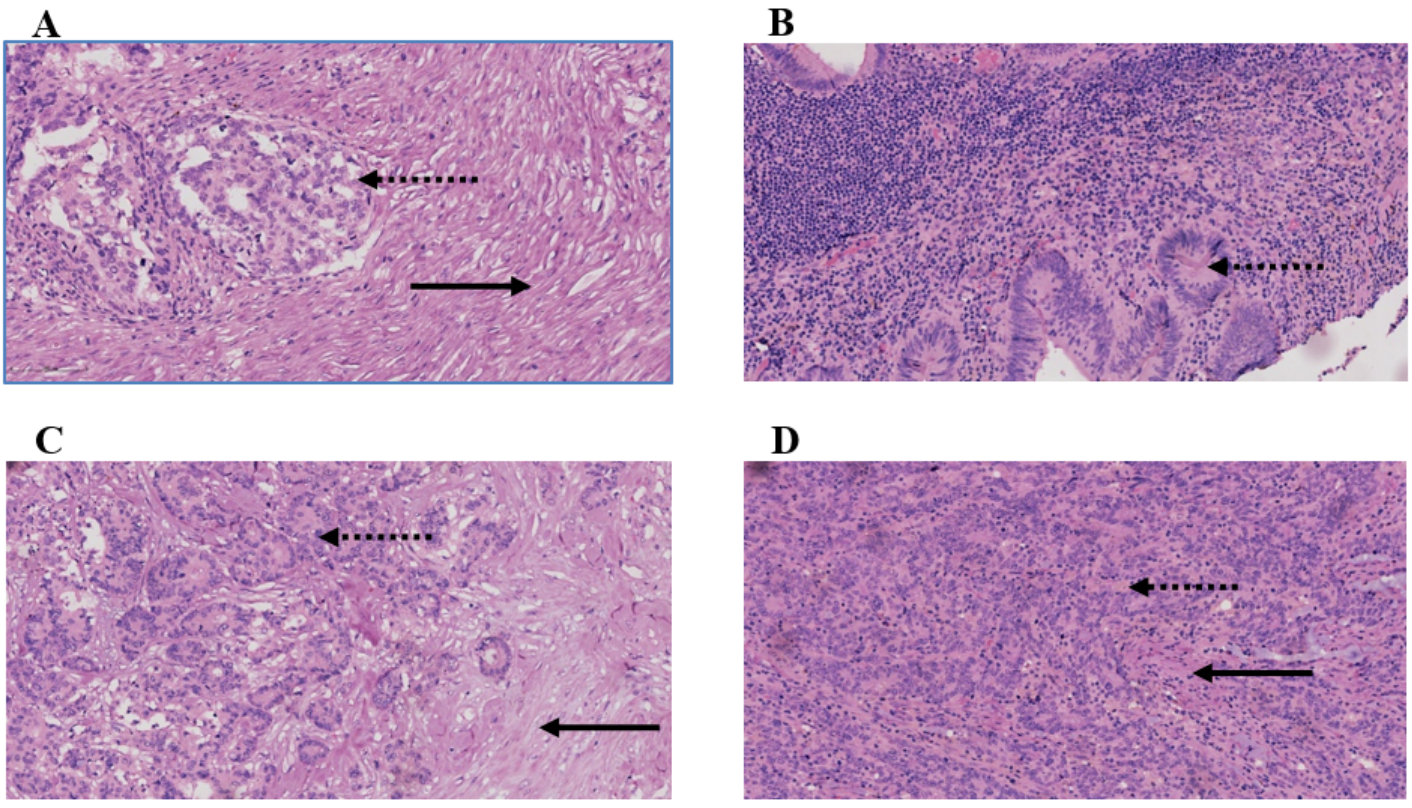

Figure 2

Representative views of TILs in CRC. Examples of low sTILs (A) and high sTILs (B) in stromal compartments (HE, 20×), low iTILs (C) and high iTILs (D) in intraepithelial compartments (HE, 20×), dashed arrows and solid arrows indicate tumoural and stromal area.

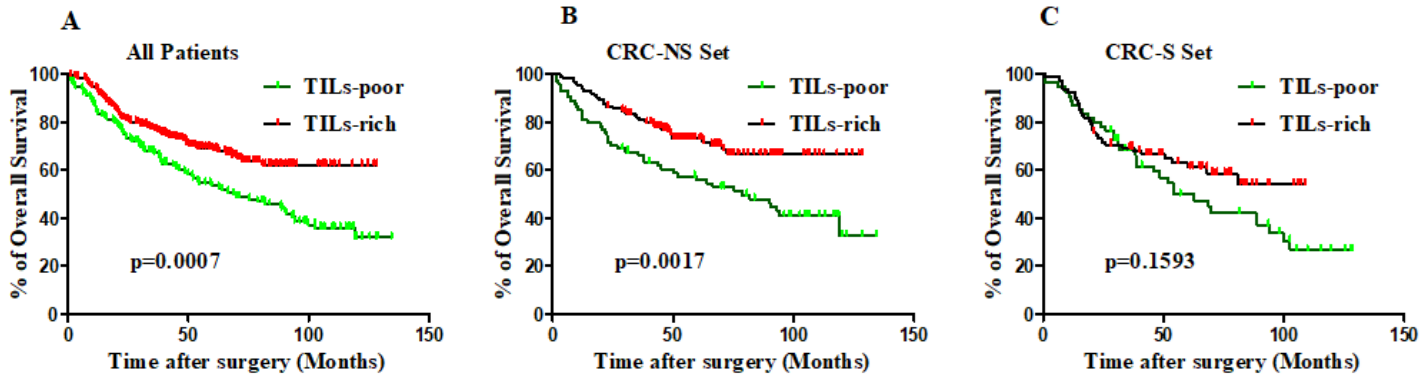

Figure 3

Kaplan-Meier curves of OS revealing prognostic significance of sTILs in CRC. Among 351 patients, a significantly better prognosis was observed in patients with high sTILs (> 2%) in the whole cohort and in the CRC-NS set (p = 0.0007 and 0.0017) (A and B). In patients with schistosomiasis, sTIL (>2%) was not associated with OS (p = 0.1593) (C).

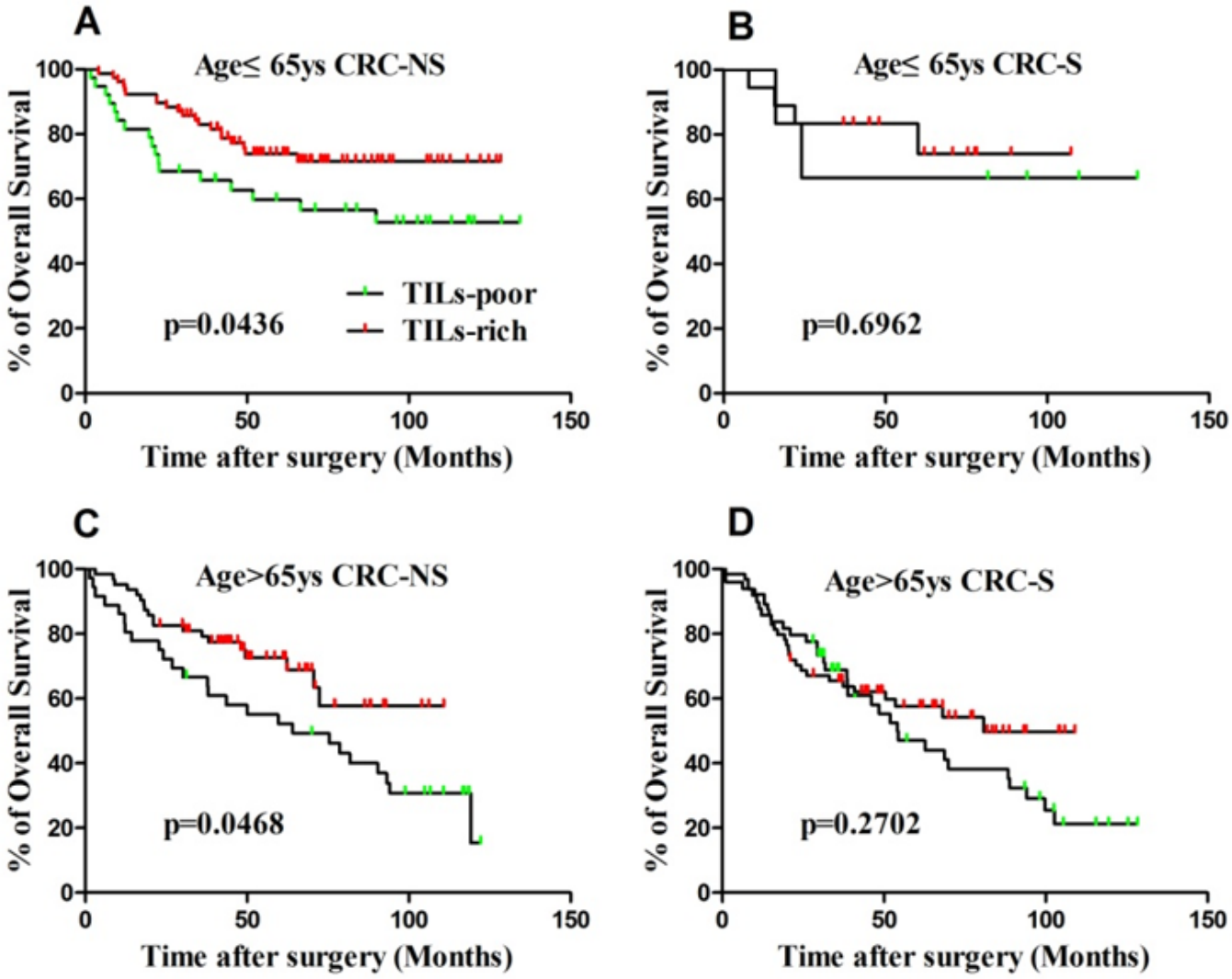

Figure 4

Kaplan-Meier curves of OS revealing prognostic significance of sTILs in CRC patients with different age group. 351 patients were stratified by age: patients younger than or equal to 65 years old and older than 65 years old. Stromal TILs associated with longer OS in the CRC-NS patients who were younger than or equal to 65 years old (A) (p=0.0436). However, there were no significant difference between sTILs and OS in the CRC-S patients of this age group (p=0.6962) (B); Similarly, sTILs were associated with OS in CRC-NS group (p=0.0468) (C), but not in CRC-S patients who were older than 65 years old (p=0.2702) (D).