The distinguishing Predictive Value of CD4, CD8, and C - reactive protein in the Prognosis of Schistosomal and Non-Schistosomal Colorectal Cancer

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Abstract

Background: The comparison of inflammation related factors between SCRC and non-schistosomal colorectal cancer (NSCRC) was not reported in previous literatures, and the inflammation based prognostic systems of SCRC were also unknown.

Aim: To explore the different roles of tumor infiltrating lymphocytes (TILs) and CRP in SCRC and in NSCRC, providing a possible predictive system to evaluate outcomes and to improve the risk stratification for CRC patients, especially for patients with schistosomiasis.

Methods: 351 CRC tumours were evaluated for density of CD4+, CD8+ T cells and CRP in intratumoural and stromal compartments by immunohistochemical using tissue microarray.

Results: There were no association between TILs and CRP and schistosomiasis. Multivariate analysis identified sCD4 ($p=0.038$), iCD8 ($p=0.003$), schistosomiasis ($p=0.045$) as independent prognostic factors for overall survival (OS) in the whole cohort; and sCD4 ($p=0.006$) and iCD8 ($p=0.020$) were independent prognostic factors for OS in the NSCRC and SCRC set, respectively. Besides, we found that there were no differences of TILs and CRP, which were distributed in different areas of tumor tissue, between CRC patients with and without schistosomiasis.

Conclusion: TILs in different tumor areas has distinguished predictive value in CRC patients with schistosomiasis and without schistosomiasis, reflecting variable tumor immunoenvironments in them.

Introduction

Schistosomiasis is a chronic parasitic disease caused by a trematode blood fluke of genus *Schistosoma*[1]. Schistosomiasis is a widespread endemic disease found in 74 countries [2] and over 250 million people are infected worldwide[3]. Schistosomiasis is now becoming a cause for concern in Europe, especially in southern Europe, because of climate change as well as infected travelers who return from endemic areas[4]. Qingpu District of Shanghai was once an endemic area of schistosomiasis, and the local people were deeply affected by it. There are still a large number of schistosomiasis-associated CRC patients left over from history. And schistosomiasis-associated CRC patients could be observed in our daily work occasionally.

It was suggested that schistosomiasis significantly correlated with increased colon cancer mortality in China[5]. Ming-Chai et al's study[6] revealed a similarity between chronic schistosomiasis and ulcerative colitis regarding predisposition to carcinoma in that pseudopolyps, ectopic regenerating glands, epithelial proliferation and ulceration are common in both of them. They suggested a strong relation between *Schistosoma japonicum* and CRC. A similar conclusion was drawn by Yu et al[7] from their studies on different types of schistosomal egg polyps. All these studies suggested that schistosomiasis is a risk factor for CRC. However, some suggested that if there is an increase in the risk of colorectal cancer, it is small [8, 9]. Thus, the relationship between schistosomiasis and inflammation and colorectal cancer were necessary to be unraveled. However, related work has rarely been reported previously.

It was known that tumor-infiltrating lymphocytes (TILs) reflect an active inflammatory tumor microenvironment. Several studies have been made to assess the prognostic significance of TILs in human cancers, and pronounced lymphocytic infiltration has been shown to be a prognostic parameter for better survival in CRC[10–12]. However, inflammation based prognostic systems for schistosomal colorectal cancer has never been reported in the literature. TILs are composed of various lymphocytes with diverse functions. CD4+, CD8+ are the most common lymphocytes. Of them, CD8+ T cells play a crucial role in protective immunity against many infectious pathogens and can eradicate malignant cells by releasing perforins and granzymes, which may contribute to tumour cell death[13]. CD4+ T cells secreted immunoregulatory cytokines such as IFN- γ and TNF that may induce cytolytic T cell responses in tumours. C-reactive protein (CRP), an acute phase reactant, primarily stimulates the innate immune system by facilitating phagocytosis, but also modulates adaptive immunity[14, 15]. Serum CRP has been shown to have prognostic value in CRC[16, 17]. However, the prognostic value of intratumor CRP remains unknown, especially in CRC patients with schistosomiasis.

With these considerations, we evaluated CD4+, CD8+ and CRP in CRC by immunohistochemical and compared their different prognostic roles between CRC patients with and without schistosomiasis. These results may uncover the important role of CD4+, CD8+ and CRP in CRC and provide a possible predictive system to evaluate outcomes for patients with schistosomiasis.

Materials And Methods

Patients and sample selection.

In this retrospective study, 351 unselected CRC patients who received curative resection without preoperative chemotherapy at Qingpu Branch of Zhongshan Hospital affiliated to Fudan University, from January 2008 to August 2016 were included. Clinicopathological information of patients in this survey was collected from clinical records and pathology reports. The local Ethics Committee of Qingpu Branch of Zhongshan Hospital approved the protocol of this study, which was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The inclusion and exclusion criteria were as previously described [18].

Tissue microarray construction

Tissue microarrays (TMA) were constructed as previously described[19]. Hematoxylin and eosin (H&E)-stained slides from tissue blocks had been reviewed for adequacy of the representative areas of interest with a high density of tumour cells. The corresponding regions were marked on archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks. The representative areas (2 mm wide and 6 mm long) of the tumour were extracted and then vertically planted into

the recipient block one by one according to the corresponding location. The planting surface was aggregated on the aggregation instrument. An array was constructed with a maximum of 40 cores.

Immunohistochemical analysis

Three- to 5- μ m thick CRC tissues were consecutively cut, subsequently dewaxed and rehydrated through graded alcohols. Slides were immunohistochemically stained in Roche Ventana Benchmark XT automated slide stainer (Ventana Medical Systems, Roche, France) according to the manufacturer's instructions. Monoclonal and polyclonal anti-human antibodies were used for identification of CD4+ T cells (anti-CD4, 4B12, Dako), CD8+ T cells (anti-CD8, Ab4055, Abcam), CRP (Abcam, Cambridge, Massachusetts, Ab 32412, rabbit monoclonal Y284).

Pathological assessment of CD4 and CD8+ T cell density and C-reactive protein

The TMA slides were scanned using a scanner system (PRECICE 500B) at 40 $\times$ magnification. For CD4 and CD8, the densities of positively stained cells were evaluated on whole section slides using an image analysis system (Image J software, USA) (cells per square millimeter) (Fig. 1A-B). At least half of the core area was selected randomly, and the results of the calculated densities were extracted and put into an Excel file. Measurements were recorded as the mean number of positive cells per tissue unit in square millimetres as well as the number of positive cells among each 1-mm² tissue units.

The C-reactive protein (CRP) staining were located in the stromal cells and *tumoral cells* cytoplasm in a diffused manner (Fig. 3 A-C).

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 $\times$ and 40 $\times$ magnification fields using a conventional light microscope by two pathologists who were blinded to the clinical data. The diagnosis of schistosomiasis was done by finding schistosome eggs in HE-stained slides (Sup.Fig.1)

Tumor budding was defined as the presence of dedifferentiated single cells or small clusters of up to 5 cells at the invasive front of CRC[20]. The assessment of tumor budding was conducted as previously described[18]. Briefly, the 10-HPF method was used[21], the invasive front is first scanned at low magnification (4 $\times$ to 10 $\times$) to identify areas of highest budding density. Tumor buds are then counted under high magnification (40 $\times$), and the tumor budding count is reported. The evaluation of tumor budding was conducted by two pathologists who were blinded to the clinical data. Five tumor budding counts were used as breakthrough point. In brief, tumor bud counts greater than or equal to 5 were defined as the high group, otherwise as the low group.

Statistical analysis

The associations between CD4 and CD8 and clinicopathologic features were analyzed using the chi-square (χ^2) test. Time-to-event outcomes were defined from date of initially curative resection to date of last follow-up. Univariate analysis was based on the Cox proportional hazards regression model. A multivariate Cox forward stepwise regression model was used to detect independent predictors of survival. The survival curves were compared using Kaplan-Meier method and log-rank test. All tests were two sided, and a *P*-value of less than 0.05 was considered as statistical significance. Data were analyzed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA).

Results

Study population

The clinicopathologic features of a total of 351 cases of CRC were summarized in Table 1. Briefly, the age of patients with schistosomiasis was dominantly older than that of patients without schistosomiasis. Unexpectedly, there was no significant difference in morphology between colorectal cancer associated with schistosomiasis and that of without schistosomiasis (Table 1). In the whole cohort, the median age was 69 years (range 33–91), with frequencies between women 39% (137 out of 351) and men 61% (214 out of 351). The location of the tumor in 27% of patients was rectum, in left colon was 33% and in right colon was 40%. On the basis of the AJCC Staging Manual (seventh edition), 76% cases were histologically graded as well to moderately differentiated, and 24% were poorly differentiated. The most predominant histological type was adenocarcinoma (311 patients), mucinous and signet ring cell carcinoma were 11% (34 patients). Vessel and nerve involvement were identified in 122 (35%) and 31 (1%) tumors, respectively. Lymph node metastasis was identified in 144 (40%) patients. Stage I-II cases accounted for 54% (190 out of 351), while stage III-IVa cases were 46% (161 out of 351), respectively. Schistosoma eggs could be observed under microscope in almost 39% cases.

Immunohistochemical Findings

CD4⁺T and CD8⁺T cell were observed both in cancer stroma and within cancer epithelium (i.e., intraepithelial). Representative pictures of lymphocyte infiltration are shown in Fig.1A–B. The distributions of CD4⁺T and CD8⁺T cell density are shown in Table 2 and Fig.2. The optimum cutoff value of CD4⁺T and CD8⁺T cell density were determined by X-tile program, which were 29 cell/mm² for intraepithelial CD4 (iCD4) (Table 2 and Sup Fig.2A-C) and 145 cell/mm² for stroma CD4 (sCD4) (Table 2 and Sup Fig.3A-C) and 77 cell/mm² for intraepithelial CD8 (iCD8) (Table 2 and Sup Fig.4A-C) and 645 cell/mm² for stroma CD8

(sCD8) (Table 2 and Sup Fig.5A-C). Patients were divided into 2 groups for further analysis based on their respective cutoff values. The C-reactive protein (CRP) positive staining were located in the stromal cells and *tum or al cells* cytoplasm in a diffused manner (Fig. 3A-C). The CRP positive staining was defined as positive, whereas negative staining was defined as negative.

Association of TILs density and CRP with clinicopathological features

The relationship between TILs density and patient demographics is listed in Table 3. No significant correlations were observed between iCD4+T cell density and clinical characteristics, such as age, gender, TNM stage, tumor differentiation and so on ($p \geq 0.05$). There was also no correlation between sCD4+T cell density and other clinical characteristics except for tumor budding ($p=0.031$), lymph node metastasis ($p=0.045$) and TNM stage ($p=0.001$). Furthermore, there were significant association between iCD8+T cell density and age ($p < 0.001$), tumor deposit ($p=0.032$). In addition, there were significant association between sCD8+T cell density and tumor deposit ($p=0.004$). However, there were no significant association between TILs in different tumor area and schistosomiasis ($p \geq 0.05$).

The association between sCRP and tCRP and clinical characteristic were listed in Supplementary Table 1. Results demonstrated that sCRP was inversely associated with tumor size ($p=0.020$) and colonic perforation ($p=0.001$). Besides, tCRP was also negatively associated with colonic perforation ($p=0.001$). Unexpectedly, there were no relationship between tCRP or sCRP and schistosomiasis as well as CD4+T cells and CD8+T cells.

Univariate and multivariate regression analysis

In the whole cohort, univariate Cox regression analysis identified clinical factors statistically significantly associated with OS (Table 4) were age ($p=0.010$), gender ($p = 0.008$), pathological T stage ($p < 0.001$), Lymph node metastasis ($p < 0.001$), TNM stage ($p < 0.001$), tumor differentiation ($p < 0.001$), lymphovascular invasion ($p < 0.001$), tumor deposit ($p < 0.001$), tumor budding ($p < 0.001$), schistosomiasis ($p=0.044$), iCD4 ($P=0.015$), sCD4 ($p=0.002$), iCD8 ($p < 0.001$), whereas only gender ($p = 0.009$), pathology T stage ($p=0.035$), TNM stage ($p < 0.001$), sCD4 ($p = 0.045$), iCD8 ($p = 0.0089$), schistosomiasis ($p=0.045$) and tumor deposit were identified as independent risk factors for OS (Fig.4A).

In the NSCRC set, univariate Cox regression analysis identified clinical factors statistically significantly associated with OS (Table 4) were gender ($P=0.017$), pathological T stage ($p=0.001$), Lymph node metastasis ($p<0.001$), TNM stage ($p < 0.001$), tumor differentiation ($p = 0.003$), lymphovascular invasion ($p<0.001$), tumor deposit ($p < 0.001$), tumor budding ($p<0.001$), sCD4 ($p < 0.001$), and iCD8 ($p < 0.001$) were risk factors for OS. Variables demonstrating a significant effect on OS were included in the multivariate analysis. Gender ($p=0.023$), pathology T stage ($p=0.046$), sCD4 ($p=0.006$), tumor budding ($p=0.034$), and tumor deposit ($p < 0.0001$) were identified as independent prognostic factors that associated with OS in the this set (Fig.4B).

In SCRC set, univariate Cox regression analysis identified clinical factors statistically significantly associated with OS (Table 4) were lymph node metastasis ($p < 0.001$), TNM stage ($p < 0.001$), tumor deposit ($p < 0.001$), iCD8 ($p= 0.001$) and sCD8 ($p =0.040$) were risk factors for OS, whereas only Lymph node metastasis ($p < 0.001$), iCD8 ($p= 0.020$) and tumor deposit ($p < 0.001$) were identified as independent prognostic factors that associated with OS in this set (Fig.4C). Unexpectedly, there were no association between stromal CRP and tumoral CRP and OS in the whole cohort and subgroups.

Kaplan-Meier Analysis of OS

Kaplan-Meier analysis, which was based on the cutoff value of the density of CD4+T cells and CD8+T cells in different tumor areas, was conducted to assess the variable in overall survival (OS) among different groups. In the whole cohort, compared to the low-density group, patients in the sCD4 and iCD8 high-density group experienced significantly higher OS ($p=0.0020$, $p < 0.001$; Fig.5A and 5B). In the NSCRC set, patients in the sCD4 high-density group gained beneficial OS compared to patients in the low-density group ($p=0.0004$, Fig.5C). Furthermore, patients in the iCD8 high-density also possessed favorable survival compared to patients in the low-density ($p=0.0008$, Fig.5D). There was no association between sCRP or tCRP and OS in any subgroups.

Comparison of TILs density and positive stained CRP in CRC patients with and without schistosomiasis

We next compared the density of CD4+ and CD8+ T cells in CRC patients with or without schistosomiasis (data was not shown). The distribution of CD4+ T cells densities in stromal or tumoral cells between NSCRC and SCRC groups were no significant differences. Similarly, there were also no significant differences in the distribution of CD8+ T cells densities in stromal or tumoral cells between the NSCRC and SCRC group. The positivity rate of stromal CRP was 22% and 30% in the NSCRC set and SCRC set, respectively. Besides, the positivity rate of tumoral CRP was 14% and 15% in the NSCRC set and SCRC set, respectively.

Discussion

Schistosomiasis lead to inflammatory changes in CRC patients is well studied. However, direct evidences supporting inflammatory changes caused by inflammatory response after schistosomiasis infection is still lacking. Moreover, inflammation based prognostic systems for CRC were pronounced, but related predictors for schistosomal CRC have never been reported previously. In the present study, we found that CD4 + and CD8 + T cells distributed in different tumor areas were correlated with less aggressive tumor characteristics, but CRP distributed in different tumor areas were associated with more aggressive tumor features. In addition, results suggested that iCD4, sCD8, schistosomiasis, gender, pathological T stage, TNM stage and tumor deposit were

independent prognostic factors for OS in the whole cohort; In the NSCRC set, sCD4, tumor budding, gender, pathological T stage and tumor deposit were independent prognostic factors for OS; In the SCRC set, iCD8, lymph node metastasis and tumor deposit were independent prognostic factors for OS. Furthermore, we found that there were no differences of TILs densities and CRP positivity, which were distributed in different areas of tumor tissue, between CRC patients with and without schistosomiasis.

Our results demonstrated that iCD4 and sCD8 were associated with favorable OS for CRC patients. However, when patients were divided into NSCRC and SCRC set based on status of schistosomal infection, sCD4 and iCD8 were independent prognostic factor for NSCRC patients and for SCRC patients, respectively. These results remind us that TILs distributed in different tumor areas have distinguished biological behavior and function, and the immune microenvironment is different in NSCRC and SCRC patients. Meanwhile, the findings require us to stratify patients with schistosomiasis and might facilitate patient counseling and management. The findings also pave the way for customized chemotherapy in CRC patients with schistosomiasis and without schistosomiasis individually based on the infiltration of TILs, because CRC patients with high levels of TILs could have improved outcome with 5-fluorouracil-based chemotherapy.

CD8 + T cells are cytotoxic T lymphocytes that directly attack cancer cells and play a central role in anti-cancer immunity[22]. Previous studies revealed substantial evidence that the density of CD8 + TILs was associated with the favorable survival in patients with various types of cancer[22–24]. Consistent with previous reports, this study confirmed the usefulness of CD8 + T-cell densities as prognostic factors (Fig. 4 and Fig. 5). CD4 + T-cell density has been reported to be a unbeneficial prognostic factor in other types of cancers, e.g. lung, renal, prostate and breast cancer[25–28]. On the contrary, Taichi Kuwahara et al's study showed that CD + T cell densities were associated with favorable clinical outcomes[29]. This is consistent with our results. The reason for this discrepancy remains unclear, but it may be because the function of CD4 + T cells within the tumour microenvironment—i.e. in immune response activation or immunosuppression—may differ depending on the cancer type. Besides, we found that CRP was not associated with clinical outcome. This may be because CRP is a good predictor for clinical outcomes in the acute phase[30–32], but it is not suitable for chronic infection. It was well known that schistosoma egg deposited in the intestine provoke immune response and lead to chronic inflammation, and then lead to shisotomiasis. So it was speculated that the densities of TILs in NSCRC and SCRC patients was different. However, our results showed that the densities of TILs and CRP expression levels in the two sets were similar. As mentioned above, it may be because patients at diagnosis were not in the acute stage, but in the chronic stage. In addition, this may be explained that patients with schistosomiasis are obviously older than patients without schistosomiasis (Table 1), and the immune response of the elderly is weaker than that of the young.

Our retrospective study had several limitations. First, we do recognized the limitation of utilizing a TMA approach to assess expression of a biomarker that may only be locally present in samples, raising the possibility of false negatives, which could possibly change the significance of TILs in CRC. Second, because all results were generated at a single institution, the uniformity of the results may be low. Further work will be needed to validate the present results in a larger cohort of multicenters. Besides, validation studies may be performed on whole tissue slides to optimize the selection of regions most suitable for TILs assessment. Thirdly, the diagnosis of schistosomiasis was done by finding schistosome eggs in HE-stained slides. This will lead to positive cases miss and to generate data bias. We tried to use other methods to detect schistosomiasis related indicators, such as imaging data and serological tests. However, imaging data was not completed and serum could not be collected as the specimen used in this study was years ago. At present, more comprehensive materials related to schistosomiasis were collecting and will be used to validate results from this study.

Conclusion

In present study, iCD4 + and sCD4 + T cell densities were independent beneficial prognostic factors for NSCRC patients and schistosomal-CRC (SCRC) patients, respectively. We believe that this study is the first to report the prognostic significance of TILs and CRP in schistosomiasis-associated CRC patients. Hence, the results remind us to stratified CRC patients based on the status of schistosomal infection.

Abbreviations

colorectal cancer=CR; Schistosomal colorectal cancer=SCRC; Schistosomal colorectal cancer=NSCRC; tumour infiltrating lymphocytes=TILs; C-reactive protein=CRP; Tissue microarray=TMA; intratumoral CD4=iCD4; stromal CD4=sCD4; intratumoral CD8=iCD8; stromal CD8=sCD8; Overall survival =OS; hematoxylin and eosin =H&E; Formalin fixed paraffin-embedded =FFPE;

Declarations

Acknowledgements:

None

Conflict of interest:

The authors declare that they have no competing interests.

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.

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Authors' contributions:

Wang WW and Zhai QL: project development, data analysis, manuscript writing/editing;

Jing HY: data collection, manuscript writing;

Bu DC, Liu J, Lu K, Liu JC, Xu YC, Zhu T, Hou YY and Yao JX : data analysis & collection, manuscript editing.

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Tables

Table 1

Clinicopathological characteristics of the CRC cohort

Characteristics	All patients (N=351)		Patients with schistosomiasis (N=137)		Patients without schistosomiasis (N=214)	
	N	%	N	%	N	%
Age(≤60ys)	268	76	132	96	136	64
Gender(Male)	214	61	86	63	126	59
Tumor location						
Rectum	94	27	37	27	57	27
Left colon	115	33	51	37	64	30
Right colon	142	40	49	36	93	43
Tumor size(≤5cm)	174	50	68	50	106	50
Differentiation						
Well to moderately diff.	267	76	102	74	165	77
Poor diff.	84	24	35	26	49	23
Lymphovascular invasion (Negative)	122	35	90	66	136	64
Nervous invasion (Negative)	31	1.0	125	91	194	91
Tumor deposit	42	1.2	120	88	189	88
Colonic perforation (Yes)	13	0.4	5	4	8	4
Tumour budding (≤5 cells)	219	62	99	72	156	73
Ulceration (No)	149	42	77	56	125	58
Histological type						
Adenocarcinoma	311	89	119	87	189	88
Mucinous/SRCC	40	11	18	13	25	12
Pathological T stage						
T1-2	80	23	29	21	51	24
T3-4	271	77	108	79	163	76
Lymph node metastasis						
No	207	60	81	59	126	59
Yes	144	40	56	41	88	41
TNM stage						
I+II	190	54	76	55	116	54
III+ IV	161	46	61	45	98	46
<i>schistosomiasis</i>	137	39	—	—	—	—

Table 2

Densities and cut-off values of the tumour infiltrating lymphocytes.

	Density (cell/mm ²)	Median value	Cutoff value	Low-density group(%)
iCD4	0-1857	74	29	31
sCD4	0-944	203	145	38
iCD8	0-2554	291	77	19
sCD8	0-2803	462	645	68

Notice: X-tile analysis of overall survival (OS) was performed using patients' data collected from the pathological system of the Qingpu District Center for Disease Control and Prevention to determine the optimal cut-off value for iCD4, sCD4, iCD8, sCD8 density. The optimal cut-off value for iCD4 density was 29.0, for sCD4 was 145.0, for iCD8 was 77 and for sCD8 was 645 cell/mm². Patients were divided into two groups: low-density group and high-density group based on the cutoff value of TILs.

Table 3

The association between clinicopathological characteristics and sTILs

Characteristic	iCD4		<i>P</i> value	sCD4		<i>P</i> value	iCD8		<i>P</i> value	sCD8	
	Low (N=110)	High (N=241)		Low (N=135)	High (N=216)		Low (N=65)	High (N=286)		Low (N=238)	High (N=113)
Age(≥60ys)			0.223			0.520			0.001		
<60	21	62		29	54		5	78		55	28
≥60	89	179		106	162		60	208		188	85
Gender			0.198			0.574			0.095		
Male	72	140		79	133		45	167		148	68
Female	38	101		56	83		20	119		95	45
Tumor site			0.676			0.773			0.442		
Rectum	29	65		34	60		16	78		66	30
Left colon	33	82		45	70		18	97		75	41
Right colon	48	94		56	86		31	111		102	42
Tumor size			0.818			0.913			0.496		
<5cm	56	118		67	107		29	145		115	63
≥5cm	54	123		68	108		36	141		128	50
Differentiation			0.998			0.699			0.337		
Well diff.	84	183		100	167		47	220		182	88
Poor diff.	25	58		35	49		18	66		61	25
Lymphovascular invasion			0.091			0.169			0.316		
Negative	63	163		80	146		39	187		158	70
Positive	47	78		55	70		26	99		85	43
Nervous invasion			0.693			0.706			0.998		
Negative	99	220		124	195		59	260		219	104
Positive	11	21		11	21		6	26		24	9
Tumor deposit			0.375			0.181			0.032		
≤2	94	215		115	194		52	257		206	106
>2	16	26		20	22		13	29		37	7
Colonic perforation			0.555			0.259			0.715		
No	105	233		128	210		62	276		233	110
Yes	5	8		7	6		3	10		10	3
Ulceration			0.163			0.739			0.998		
No	57	145		80	122		37	165		141	65
Yes	53	96		55	94		28	121		102	48
Pathological T stage			0.891			0.432			0.871		
I+ II	24	56		27	53		14	66		50	32
III	86	185		108	163		51	220		193	81
Lymph node metastasis			0.198			0.045			0.677		
No	59	148		70	137		37	170		140	68

	Yes	51	93	65	79	28	116	103	45
TNM stage				0.16		0.001		0.275	
	I+II	54	138	58	134	32	160	129	65
	III+ IV	56	103	77	82	33	126	114	48
Tumor budding				0.636		0.031		0.888	
	≤5 cells	64	146	73	137	39	171	139	72
	≥5 cells	46	95	62	79	26	115	104	41
Histological type				0.591		0.863		0.831	
	Adenocarcinoma	94	214	117	191	57	251	208	104
	Mucinous/SRCC	16	27	18	25	8	35	35	9
Schistosomiasis				0.639		0.144		0.998	
	Negative	65	149	75	139	40	174	147	67
	Positive	45	92	59	78	26	111	96	41

—:Data is not applicable; Abbreviation:N =Number; LN= Lymph node; iCD4=intratumoral CD4; sCD4=stromal CD4; iCD8=intratumoral CD8; sCD8=stromal CD8

association between schistosomiasis and clinicopathological characteristics was evaluated by using the Chi square and Fisher's exact tests.

Table 4

Univariate and multivariate Cox regression of clinicopathological for overall survival

Variable	All patients		NSCRC		SCRC	
	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)
Univariate analysis						
iCD4	0.015	0.660(0.472-0.923)	0.076	0.662(0.419-1.044)	0.123	0.676(0.411-1.112)
sCD4	0.002	0.602(0.435-0.834)	0.001	0.452(0.292-0.701)	0.750	0.924(0.566-1.507)
iCD8	0.001	0.445(0.310-0.638)	0.002	0.459(0.283-0.745)	0.001	0.412(0.239-0.711)
sCD8	0.176	0.776(0.538-1.120)	0.057	0.612(0.369-1.015)	0.040	0.538(0.297-0.972)
sCRP	0.761	1.060(0.728-1.545)	0.478	1.231(0.685-2.210)	0.640	1.168(0.610-2.234)
tCRP	0.263	0.754(0.460-1.236)	0.796	0.906(0.430-1.908)	0.301	0.615(0.244-1.547)
Age (≥60ys)	0.010	1.759(1.142-2.708)	0.122	1.454(0.905-2.336)	0.232	21.827(0.139-3436.270)
Gender (male/female)	0.008	1.602(1.129-2.271)	0.017	0.562(0.350-0.901)	0.307	1.311(0.779-2.207)
Tumor size(5cm)	0.913	1.018(0.728-1.400)	0.591	0.886(0.569-1.378)	0.320	1.282(0.786-2.089)
Tumor site						
Rectum		Refer		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	0.464	0.859 (0.572-1.290)	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	2.802(2.012-3.902)	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	1.889(1.334-2.674)	0.003	2.009(1.259-3.206)	0.054	1.668(0.991-2.809)
Lymphovascular invasion	0.001	3.251(1.987-5.318)	0.001	2.816 (1.808-4385)	0.275	1.321 (0.801-2.180)
Nervous invasion	0.140	1.497(0.876-2.559)	0.391	1.424 (0.710-2.857)	0.206	1.727 (0.741-4.024)
Tumor deposit	0.001	4.006(2.686-5.973)	0.001	3.973(2.359-6.692)	0.001	4.138(2.205-7.769)
Colonic perforation	0.541	0.700(0.223-2.198)	0.763	1.194(0.377-3.786)	0.500	0.506(0.070-3.657)
Tummor budding	0.001	2.028(1.400-2.938)	0.001	2.824(1.813-4.400)	0.237	1.354(0.819-2.238)
<i>Schistosomiasis</i>	0.044	1.399(1.009-1.940)	—	—	—	—
Ulceration	0.624	0.9205(0.660-1.282)	0.744	1.077(0.691-1.676)	0.971	1.008(0.670-1.514)
Histological type	0.921	1.025(0.626-1.680)	0.283	1.400 (0.758-2.586)	0.467	0.760(0.362-1.594)

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

iCD4=intratumoral CD4; sCD4=stromal CD4; iCD8=intratumoral CD8; sCD8=stromal CD8;P < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection.

Figures

Figure 1

Immunohistochemical (IHC) staining of representative CD4 expression and CD8 expression positivity in different tumor areas. **A.** CD4 expression positivity (100×) in the intratumoral area (iCD4) (inside the circle) and stromal area (sCD4) (outside the circle). **B.** CD8 expression positivity (200×) in the intratumoral area (iCD8) (inside the circle) and stromal area (sCD8) (outside the circle).

Figure 2

Heterogeneity of tumor-infiltrating immune cells. The density of iCD4+ (A), sCD4+ (B), iCD8+ (C), sCD8+ (E) cells differed significantly according to tumor location (results were examined by using the unpaired *t*-test).

Figure 3

Immunohistochemical (IHC) staining pattern for C-reactive protein (CRP). **A.** a negative immunohistochemical staining pattern (100×); **B.** Positive IHC staining pattern for tumoral CRP(200×). **C.** Positive IHC staining pattern for stromal CRP(100×).

Figure 4

Prognostic analysis in CRC patients. **A.** Multivariate Cox regression analysis performed in the whole cohort; **B.** Multivariate Cox regression analysis performed in NSCRC patients, the value hazard ratio (HR) were log10 transformed; **C.** Multivariate Cox regression analysis performed in SCRC patients, the value hazard ratio were log2 transformed.

Figure 5

Kaplan-Meier curves for overall survival (OS) of colorectal cancer (CRC) patients with low or high TILs density. A. Stromal CD4+ T cell density in the whole cohort; B. Intratumoral CD8+ T cell density in the whole cohort; C. Stromal CD4+ T cell density in CRC patients without schistosomiasis (NSCRC); D. Intratumoral CD8+ T cell density in CRC patients with schistosomiasis (SCRC).

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