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The prognostic and predictive value of tumor infiltrating Macrophage and Neutrophil in patient with clear cell renal cell carcinoma: Tumor infiltrating lymphocytes in renal cell carcinoma

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Abstract

Renal cell carcinoma (RCC) is the leading cancer affecting humans; however, the relationship between tumour-infiltrating lymphocytes (TILs) and patient prognosis in RCC is relatively unreported. This study aimed to investigate the relationships among factors (TIL, clinicopathological characteristics, and patient prognosis in RCC).

This retrospective study evaluated 533 patients with clear cell renal cell carcinoma (ccRCC) deposited in the the Cancer Genome Atlas between 2004 and 2015. We downloaded immune cell type absolute fraction data for ccRCC patients from the Cancer Immunome Atlas database. The CIBERSORT method was used to transform RNA-sequencing data into microarray data for the cancer genome atlas -ccRCC samples for which microarray and RNA-sequencing data were available on the the Cancer Immunome Atlas website.

The overall survival (OS) and disease free survival (DFS) analyses of ccRCC patients showed that M1 macrophages (OS, $P = .00000134$; DFS, $P = .00958$) and neutrophils (OS, $P = .00000723$; DFS, $P = .0255$) were significant. Age at diagnosis ($P < .0001$, c-index = 0.59), tumour stage ($P < .0001$, c-index = 0.667), stage ($P < .0001$, c-index = 0.729), neoplasm histological grade ($P < .0001$, c-index = 0.624), and haemoglobin level ($P < .0001$, c-index = 0.583) were independent predictors of OS. Similarly, the stage, haemoglobin level, and serum calcium level were independent predictors of DFS. There were significant correlations between the M1 macrophage fraction and tumour stage, stage, and neoplasm histological grade. Stage and neoplasm histological grade showed associations with the neutrophil fraction.

The correlations between TILs and prognosis and clinicopathological characteristics in ccRCC were demonstrated. The prognosis of ccRCC patients may differ according to the TIL fractions.

Abbreviations: ccRCC = clear cell renal cell cancer, DFS = disease free survival, HR = hazard ratio, OS = overall survival, TCGA = the cancer genome atlas, TILs = tumour-infiltrating lymphocytes.

Keywords: carcinoma, renal cell, database, lymphocytes, tumor-infiltrating, prognosis

1. Introduction

Renal cell carcinoma (RCC) is a common solid malignancy worldwide. The prognosis of patients with RCC is mainly dependent on the disease stage, and patients with disease at a high TNM stage possess a relatively poor prognosis. The identification of prognostic markers can offer useful prognostic details. The incidence of RCC is steadily increasing.[1] RCC is a heterogeneous disease, with the most frequent type being clear cell carcinoma (ccRCC) (75%).

RCC is an immunogenic solid cancer in humans, and pathological specimens contain large amounts of tumour-infiltrating lymphocytes (TILs).[2] RCC can impair host anti-tumour immunity.[3] Numerous cells and cytokines are involved in immune escape by cancer cells. Cancer cells express aberrant tumour-specific antigens but survive by avoiding immune detection through causing immunosuppression or deriving survival indicators from tumour-infiltrating immune cells.[4] TIL are an indicator of tumour inflammation, and it has been shown that TIL subsets perform essential functions in the development of many malignancies, including renal malignancies. CD8+ TILs are linked with poor overall survival (OS) and disease-free survival (DFS) in breast cancer.[5] In clinical stage I papillomavirus oropharyngeal cancer, a high TIL level is linked with better recurrence-free survival than a low TIL level.[6] Previous studies focused on the phenotypic and functional

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The datasets generated during and/or analyzed during the current study are publicly available.

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properties of CD4+/CD8+ tumour-infiltrating T lymphocytes (TILs), but the detailed relationships between TILs and clinicopathological characteristics of tumours and patient outcomes in clear cell renal cell cancer remain unknown.

In this study, we attempted to evaluate the relationships between the tumour-infiltrating lymphocyte (TIL) content and clinicopathological characteristics of tumours and patient outcomes, which have not yet been studied in clear cell renal cell cancer. The prognostic value of TILs was analysed in clear cell renal cell cancer.

2. Materials and methods

2.1. Study population

This retrospective study evaluated 533 patients with ccRCC deposited in the cancer genome atlas (TCGA) between 2004 and 2015. The eligibility criteria were pathologically confirmed ccRCC, available data for preoperative complete blood counts and available follow-up results. The exclusion criteria were an absence of TILs. The OS interval was calculated as the time from treatment until death or loss to follow-up. The DFS interval was calculated as the time from treatment until first disease progression. The ethical approval of this study is not necessary, because all data were downloaded from public database and these have been approved by patients.

2.2. Tumour infiltrating lymphocytes

The CIBERSORT method was used to transform RNA-sequencing data into microarray data for TCGA-ccRCC samples with microarray and RNA-sequencing data available at the Cancer Immunome Atlas, https://tcia.at/cellTypeFractions). We downloaded the immune cell type absolute fraction data for ccRCC from the Cancer Immunome Atlas database.[7]

2.3. Statistical analysis

Kaplan-Meier analyses with the log-rank test were performed to compare survival outcomes. Multivariate analysis was performed using a Cox proportional hazards regression model incorporating the variables with a P-value $< .05$ in univariate analyses. All statistical tests were two-sided, and differences were considered significant at $P < .05$. All analyses were performed using R software (version 3.5.1).

3. Results

Baseline patient characteristics are shown in Table 1. The median patient age at the time of diagnosis was 60 years old. In total, 188 of 533 patients (35.3%) were female, and 345 (64.7%) were male. A total of 17 of 533 (3.2%) patients underwent neoadjuvant therapy. TILs and haematologic parameters are reported as the mean and SD of each factor, and the factors were LDH (elevated: 12, normal: 73), serum calcium level (elevated: 10, normal: 150, low: 204), platelet count (elevated: 37, normal: 359, low: 46), WBC count (elevated: 164, normal: 267, low: 8), haemoglobin level (elevated: 5, normal: 185, low: 262), B cell fraction (0.01 ± 0.01), CD4 T cell fraction (0.03 ± 0.02), CD8 T cell fraction (0.03 ± 0.05), dendritic cell fraction (0.01 ± 0.02), M1 macrophage fraction (0.04 ± 0.03), M2 macrophage fraction (0.06 ± 0.03), neutrophil cell fraction (0.01 ± 0.01), and fraction (0.01 ± 0.01).

Table 1

Clinical and tumor infiltrating lymphocytes demograph of 533 ccRCC patients.

Characteristics	group	N	%
Diagnosis.Age,mean (SD)		60.63	12.14
Race.Category			
	ASIAN	8	1.5
	BLACK OR AFRICAN AMERICAN	56	10.5
	WHITE	463	86.9
	Missing	6	1.1
Ethnicity.Category			
	HISPANIC OR LATINO	26	4.9
	NOT HISPANIC OR LATINO	355	66.6
	Missing	152	28.5
Sex			
	Female	188	35.3
	Male	345	64.7
Neoadjuvant. Therapy			
	No	516	96.8
	Yes	17	3.2
Prior.Cancer.Diagnosis.Occurence			
	No	456	85.6
	Yes	77	14.4
Primary. Tumor. Laterality			
	Bilateral	1	0.2
	Left	251	47.1
	Right	281	52.7
Metastasis.Stage			
	M0	424	79.5
	M1	79	14.8
	MX	30	5.6
Neoplasm.Disease. Lymph.Node.Stage			
	N0	240	45
	N1	16	3
	NX	277	52
Neoplasm.Disease.Stage			
	Stage I	267	50.1
	Stage II	57	10.7
	Stage III	126	23.6
	Stage IV	83	15.6
Tumor.Stage			
	T1	21	3.9
	T1a	141	26.5
	T1b	111	20.8
	T2	55	10.3
	T2a	10	1.9
	T2b	4	0.8
	T3	5	0.9
	T3a	121	22.7
	T3b	52	9.8
	T3c	2	0.4
	T4	11	2.1
Neoplasm.Histologic.Grade			
	G1	14	2.6
	G2	229	43
	G3	206	38.6
	G4	76	14.3
	GX	5	0.9
	Missing	3	0.6
Hemoglobin.level			
	Elevated	5	0.9
	Normal	185	34.7
	Low	262	49.2
	Missing	81	15.2

Characteristics	group	N	%
Idh.level			
	Elevated	12	2.3
	Normal	73	13.7
	Missing	448	84.1
Serum.calcium.level			
	Elevated	10	1.9
	Normal	150	28.1
	Low	204	38.3
	Missing	169	31.7
Platelet.count			
	Elevated	37	6.9
	Normal	359	67.4
	Low	46	8.6
	Missing	91	17.1
WBC			
	Elevated	164	30.8
	Normal	267	50.1
	Low	8	1.5
	Missing	94	17.6
B.cells,mean (SD)		0.01	0.01
CD4.T.cells,mean (SD)		0.03	0.02
CD8.T.cells,mean (SD)		0.03	0.05
Dendritic.cells,mean (SD)		0.01	0.02
Macrophage.M1,mean (SD)		0.04	0.03
Macrophage.M2,mean (SD)		0.06	0.03
Monocyte,mean (SD)		0	0.01
Missingtural.killer.cells,mean (SD)		0.01	0.01
Neutrophil,mean (SD)		0.12	0.05
Regulatory.T.cells,mean (SD)		0.01	0.01
Uncharacterized.cells,mean (SD)		0.68	0.08
Disease.Free..Months.,mean (SD)		40.25	31.59
Overall.Survival..Months.,mean (SD)		44.26	32.28

3.1. Association of TILs with prognosis

After a mean follow-up time of 44 months (1st quartile, 3rd quartile, 18–63 months), 309 patients (58%) were disease-free and 126 patients (23.6%) had recurred or progressed. Of the 533 patients, 358 (67.2%) were alive, and 175 (32.8%) were dead (Table 1). Univariate and multivariate analyses of OS and DFS in ccRCC showed that M1 macrophages (OS, $P = .00000134$; DFS, $P = .00958$) and neutrophils (OS, $P = .00000723$; DFS, $P = .0255$) were significant (Table 2). Kaplan-Meier survival curve analysis showed that the DFS and OS of the renal cancer patients with a low M1 macrophage fraction (< 0.05) were significantly longer than those of the patients with a high M1 macrophage fraction (≥0.05) (OS, $P < .0001$; DFS, $P < .0001$) (Fig. 1A). The DFS and OS of the renal cancer patients with a high neutrophil fraction (≥0.13) were significantly longer than those of the patients with a low neutrophil fraction (< 0.13) (OS, $P < .0001$; DFS, $P < .0001$) (Fig. 1B).

3.2. Association of clinicopathological characteristics with prognosis

When the relationships between OS and clinicopathological variables were examined using univariate analysis, age at diagnosis ($P < .0001$, c-index = 0.59), tumour stage ($P < .0001$, c-index = 0.667), lymph node stage ($P < .0001$, c-index = 0.556), metastasis stage ($P < .0001$, c-index = 0.648), stage ($P < .0001$, c-index = 0.729), neoplasm histological grade ($P < .0001$, c-index = 0.624), type of neoadjuvant therapy administered prior to resection ($P = .027$, c-index = 0.512), haemoglobin level ($P < .0001$, c-index = 0.583), serum calcium level (high vs normal: $P < .0001$, c-index = 0.563), and platelet count ($P < .0001$, c-index = 0.592) were significant.

A multivariate analysis that controlled for all factors with a significant association detected in the univariate analysis ($P < .2$) revealed that age at diagnosis ($P < .0001$, c-index = 0.59), tumour stage ($P < .0001$, c-index = 0.667), stage ($P < .0001$, c-index = 0.729), neoplasm histological grade ($P < .0001$, c-index = 0.624), and haemoglobin level ($P < .0001$, c-index = 0.583) were independent predictors of OS.

Similarly, we found that stage, haemoglobin level, and serum calcium level were independent predictors of DFS (Tables 3 and 4).

Table 2

Univariable and multivariable survival analysis about tumor infiltrating lyphmocytes for ccRCC patients.

	OS				DFS			
	Univariable		Multivariable		Univariable		Multivariable	
Factors	*P* value	C-Index	HR(95%CI)	*P* value	*P* value	C-Index	HR(95%CI)	*P* value
B.cells	.4	–	–	–	.165	–	–	–
CD4.T.cells	2.00E-04	5.96E-01	9.465e-02 (2.498e-05,3.586e+02)	.575	.00221	0.591	3.113e-03 (4.000e-07,2.423e+01)	.20671
CD8.T.cells	.01	0.543	6.183e-01 (2.051e-02,1.864e+01)	.782	.475	–	–	–
Dendritic.cells	.1	–	–	–	.0211	0.57	8.443e-07 (4.299e-13,1.658e+00)	.05855
Macrophage.M1	2.00E-07	6.03E-01	2.369e+05 (1.567e+03,3.580e+07)	1.34E-06	.000219	0.603	5.915e+03 (8.283e+00,4.224e+06)	.00958
Macrophage.M2	.7	–	–	–	.712	–	–	–
Monocyte	.2	–	–	–	.615	–	–	–
NAtural.killer.cells	.03	0.565	6.973e-04 (3.298e-18,1.474e+11)	.666	.135	–	–	–
Neutrophil	2.00E-08	6.43E-01	2.619e-04 (7.135e-06,9.612e-03)	7.23E-06	.00241	0.597	1.151e-02 (2.288e-04,5.785e-01)	.0255
Regulatory.T.cells	.07	–	–	–	.136	–	–	–
Uncharacterized.cells	.2	–	–	–	.0591	–	–	–

ccRCC = clear cell renal cell cancer, CI = confidence interval, DFS = disease free survival, HR = hazard ratio, OS = overall survival.

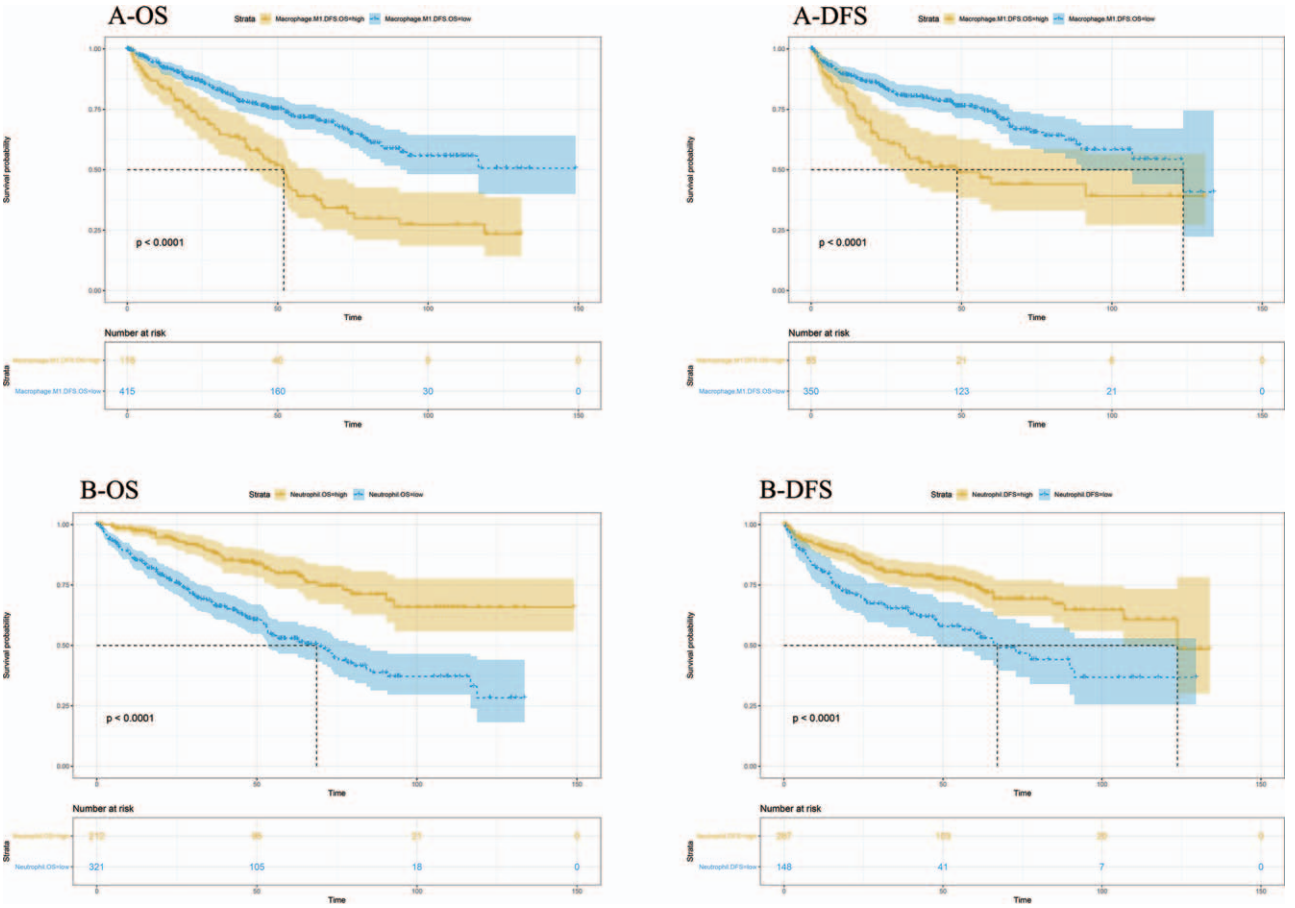

Figure 1. The Kaplan–Meier survival curve analysis. The DFS and OS of renal cancer patients with a low Macrophage-M1 Fraction (< 0.05) was significantly longer than those with a high Macrophage-M1 Fraction (≥0.05) (OS, $P < .0001$; DFS, $P < .0001$) (Fig. 1-A). The DFS and OS of renal cancer patients with a high Neutrophil Fraction (≥0.13) was significantly longer than those with a low Neutrophil Fraction (< 0.13) (OS, $P < .0001$; DFS, $P < .0001$) (Fig. 1-B).

3.3. Clinicopathological characteristics and association with TILs

There were significant positive correlations between the M1 macrophage fraction and tumour stage and neoplasm histological grade. Stage and neoplasm histological grade showed negative associations with the neutrophil fraction (Fig. 2A). There were significant correlations between the neutrophil fraction or M1 macrophage fraction and lymph node stage (Fig. 2B). The correlations of TILs with clinical characteristics are shown in Figure 3.

4. Discussion

Immune cell infiltration is a common feature of ccRCC, but the contribution of lymphocytes to prognosis and the associations between TILs and clinicopathological characteristics remain unclear. We identified significant prognostic and predictive values for TIL, specifically M1 macrophages and neutrophils, in patients with clear cell renal cell carcinoma. We found that there were significant correlations between the M1 macrophage fraction and tumour stage and neoplasm histological grade. Stage and neoplasm histological grade showed associations with the neutrophil fraction in ccRCC.

A dynamic and complex interaction exists between immune cells and tumour cells, and this interaction tightly controls tumour progression.[8] The presence of abundant TILs is associated with a favourable prognosis in various human solid tumours, including RCC. Tamma R et al.[9] indicate that bevacizumab-treated ccRCC samples present reduced microvascular density as well as a lower number of CD68-positive macrophages and tryptase-positive mast cells in comparison with the untreated patients. It may be due to a direct effect on angiogenic cytokines released by tumor cells and an indirect effect on the release of pro-angiogenic factors by TILs.

It is known that malignancy is usually not truly a local disease but rather a systemic disease. Consequently, it is believed that an individual's defence system performs a significant function in malignancy development. Therefore, studies have proven that many inflammatory molecule-based rating systems are useful. These systems are predictors of malignancy prognosis and therapeutic impact.[10–15] For example, lymphocytes are essential immune cells in both humoural and cellular antitumour immune reactions, and they restrict the proliferation and metastasis of tumour cells.[16] Decreased lymphocyte counts are related to general suppression of the immune system in patients with malignancy and tend to be related to a relatively poor prognosis.[15,17] Gigante M et al.[19] Analyze low serum levels of αKlotho were independent adverse prognostic factors for CSS (hazard ratio [HR] = 2.11; $P = .03$) and PFS (HR = 2.18; $P = .03$). These results indicate that a decreased αKlotho expression is

Table 3

Univariable and multivariable survival analysis about clinical factors for ccRCC patients (OS).

	OS				
	Univariable			Multivariable	
Factors	HR(95%CI)	*P* value	C-Index	HR(95%CI)	*P* value
Diagnosis.Age	1.029 (1.016,1.042)	8.48E-06	5.90E-01	1.0297 (1.007089,1.053)	.009783
Sex	0.9496 (0.698,1.292)	.742			
Ethnicity.Category		.00856	0.54	4.0689 (0.813163,20.360)	.087597
NOT HISPANIC OR LATINO VS HISPANIC OR LATINO	4.647 (1.478,14.61)				
Race.Category					
WHITE	ref			ref	
BLACK OR AFRICAN AMERICAN	0.8848 (0.47949,1.633)	.695			
Primary.Tumor.Laterality	7.101e-01 (0.5271,0.9566)	.0243	0.539	0.8672 (0.544495,1.381)	.54853
Prior.Cancer.Diagnosis.Occurence	1.189 (0.7729,1.83)	.43			
Tumor.Stage		9.60E-14	6.67E-01	0.5066 (0.219945,1.167)	.110216
<T3 VS ≥T3	3.152 (2.33,4.264)				
Neoplasm.Disease.Lymph.Node			5.56E-01		
N0	ref			ref	
N1	3.5034 (1.8634,6.587)	9.94E-05		0.7712 (0.174978,3.399)	.731354
NX	0.8294 (0.6104,1.127)	2.32E-01		0.8211 (0.507700,1.328)	.421734
Metastasis.Stage			6.48E-01		
M0	ref			ref	
M1	4.3730 (3.2061,5.965)	<2e-16		0.1237 (0.008527,1.794)	.125598
MX	0.9321 (0.2948,2.946)	9.05E-01		0.1402 (0.010157,1.936)	.142427
Neoplasm.Disease.Stage			7.29E-01		
stage I	ref			ref	
stage II	1.188 (0.6408,2.203)	5.84E-01		0.7958 (0.307961,2.056)	.637
stage III	2.636 (1.7616,3.944)	2.43E-06		2.8863 (0.964307,8.639)	.058094
stage IV	6.505 (4.4546,9.498)	<2e-16		28.2628 (1.702232,469.257)	.019751
Neoplasm.Histologic.Grade		1.37E-07	6.24E-01		.052646
G1/2 VS G3/4	2.209 (1.645,2.967)			1.7621 (0.993544,3.125)	
Neoadjuvant.Therapy		.0207	0.512		.62526
YES VS NO	2.126 (1.122,4.027)			0.6889 (0.154382,3.074)	
Hemoglobin.level			5.83E-01		
Normal	ref			ref	
Low	2.268 (1.591,3.232)	5.99E-06		1.8912 (1.064135,3.361)	.029867
High	5.999 (1.852,19.429)	2.81E-03		8.0666 (1.772736,36.706)	.006923
Idh.level		.529			
Normal VS High	0.7102 (0.2447,2.062)				
Serum.calcium.level			5.63E-01		
Normal	ref			ref	
Low	0.7975 (0.563,1.130)	2.03E-01		0.8941 (0.525870,1.520)	.67924
High	3.8647 (1.908,7.827)	1.74E-04		1.3532 (0.523101,3.500)	.53283
Platelet.count			5.92E-01		
Normal	ref			ref	
Low	1.649 (1.022,2.661)	4.05E-02		1.2808 (0.665490,2.465)	.458799
High	3.762 (2.491,5.682)	3.01E-10		1.3250 (0.629098,2.791)	.459007
WBC					
Normal	ref				
Low	1.7977 (0.7323,4.413)	.201			
High	0.7651 (0.5399,1.084)	.132			

ccRCC = clear cell renal cell cancer, CI = confidence interval, DFS = disease free survival, HR = hazard ratio, OS = overall survival, ref = reference.

correlated with RCC progression, and suggest a key role of declining αKlotho in the onset of cancer metastasis. Papale M et al[20] indicate that urinary Raf Kinase Inhibitor Protein encompasses both the unphosphorylated and the phosphorylated form and that their combined evaluation can help in the diagnosis and prognosis of ccRCC. Gigante M et al[19] analyze low serum levels of αKlotho were independent adverse prognostic factors for CSS (HR = 2.11; $P = .03$) and PFS (HR = 2.18; $P = 0.03$). Lucarelli G et al. show age, the presence of visceral metastases, and high levels of CA 15-3 are independent adverse prognostic factors for CSS in renal cell carcinoma and elevated IDO1 activity, the kynurenines, Glucose-6-phosphate isomerase and KYN-to-tryptophan ratio are a prognostic impact on PFS and CSS. They find that silencing of NDUFA4L2 affects cell viability, increases mitochondrial mass, and induces ROS generation in hypoxia.[21–25]

Furthermore, neutrophils are actually recognized to play important roles in host defence and invading microorganism elimination. Neutrophils that migrate via signalling by cytokines generated by tumour cells are referred to as tumour-associated

Table 4

Univariable and multivariable survival analysis about clinical factors for ccRCC patients (DFS).

	DFS				
	Univariable			Multivariable	
Factors	**HR(95%CI)**	***P* value**	**C-Index**	**HR(95%CI)**	***P* value**
Diagnosis.Age	1.009 (0.9941,1.024)	2.36E-01	5.37E-01	1.0070 (0.98129,1.0334)	.595808
Sex	1.418 (0.9592,2.095)	.08			
Ethnicity.Category		.17			
NOT HISPANIC OR LATINO VS HISPANIC OR LATINO	1.784 (0.7806,4.077)				
Race.Category					
WHITE	ref				
BLACK OR AFRICAN AMERICAN	7.833e-01 (0.3815,1.608)	.506			
Primary.Tumor.Laterality	6.316e-01 (0.4446,0.8973)	.0103	0.571	0.5943 (0.36257,0.9742)	.039061
Prior.Cancer.Diagnosis.Occurence	1.427 (0.8178,2.492)	.211			
Tumor.Stage		<2e-16	7.08E-01	1.0781 (0.41901,2.7740)	.87604
<T3 VS ≥T3	4.599 (3.198,6.616)				
Neoplasm.Disease.Lymph.Node			5.79E-01		
N0	ref			ref	
N1	4.7844 (2.3522,9.732)	1.55E-05		1.2219 (0.34645,4.3093)	.755338
NX	0.7358 (0.5113,1.059)	9.84E-02		0.6436 (0.37447,1.1062)	.110796
Metastasis.Stage			6.91E-01		
M0	ref			ref	
M1	8.6873 (6.0029,12.572)	<2e-16		0.6856 (0.13959,3.3675)	.642091
MX	0.7214 (0.1763,2.952)	6.50E-01		0.3853 (0.07324,2.0266)	.260141
Neoplasm.Disease.Stage			8.07E-01		
stage I	ref			ref	
stage II	2.124 (1.035,4.359)	4.00E-02		0.8537 (0.29607,2.4614)	.769639
stage III	4.639 (2.782,7.736)	4.08E-09		2.6087 (0.83074,8.1921)	.100519
stage IV	19.032 (11.564,31.322)	< 2e-16		6.5471 (1.10151, 38.9148)	.038802
Neoplasm.Histologic.Grade		7.70E-08	6.46E-01		.419348
G1/2 VS G3/4	2.494 (1.787,3.481)			1.2485 (0.72852,2.1396)	
Neoadjuvant. Therapy		.0846			
YES VS NO	2.06 (0.906,4.683)				
Hemoglobin.level			5.78E-01		
Normal	ref			ref	
Low	1.833 (1.2408,2.709)	2.34E-03		0.6997 (0.39978,1.2246)	.211113
High	5.087 (0.692,37.389)	1.10E-01		54.9203 (5.24182,575.4189)	.000831
Idh.level		.943			
Normal VS High	0.9564 (0.2841,3.22)				
Serum.calcium.level			5.71E-01		
Normal	ref			ref	
Low	0.7789 (0.5071,1.197)	2.54E-01		1.0832 (0.63108,1.8592)	.771917
High	6.9476 (2.4455,19.738)	2.74E-04		3.3388 (1.02101,10.9182)	.046113
Platelet.count			5.55E-01		
Normal	ref			ref	
Low	1.038 (0.5224,2.061)	9.16E-01		1.3644 (0.58993,3.1556)	.710638
High	3.321 (1.9167,5.754)	1.87E-05		1.1443 (0.56132,2.3329)	.467653
WBC					
Normal	ref				
Low	1.7145 (0.5393,5.451)	.361			
High	0.6797 (0.4526,1.021)	.0627			

ccRCC = clear cell renal cell cancer, CI = confidence interval, DFS = disease free survival, HR = hazard ratio, OS = overall survival, ref = reference.

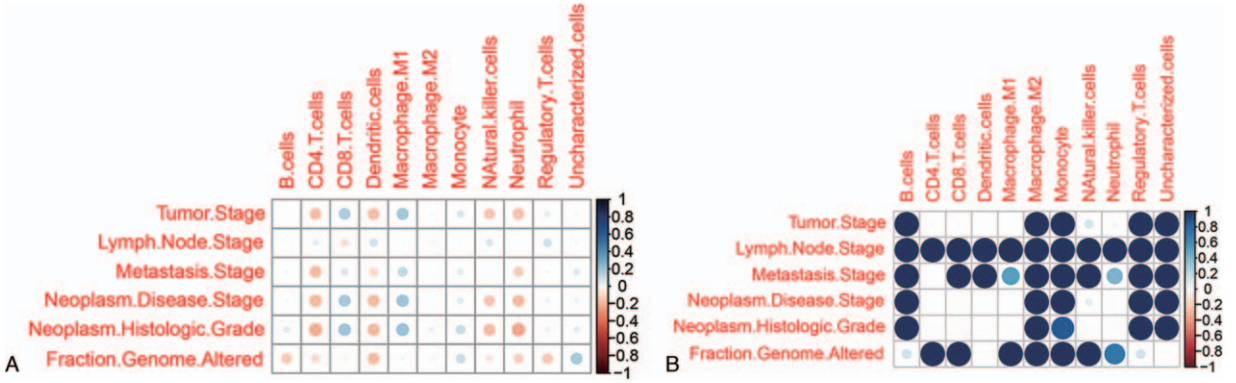

Figure 2. The significant correlation between TILs and clinical demograph. There is the significant positive correlation between Macrophage-M1 Fraction and Tumor-Stage, Neoplasm-Histologic-Grade. The Stage, Neoplasm-Histologic-Grade showed negative association with Neutrophil Fraction in the Fig. 2-A. There was the statistical correlation (*P* value) between Neutrophil Fraction, Macrophage-M1 Fraction and Lymph-Node-Stage in the Fig. 2-B.

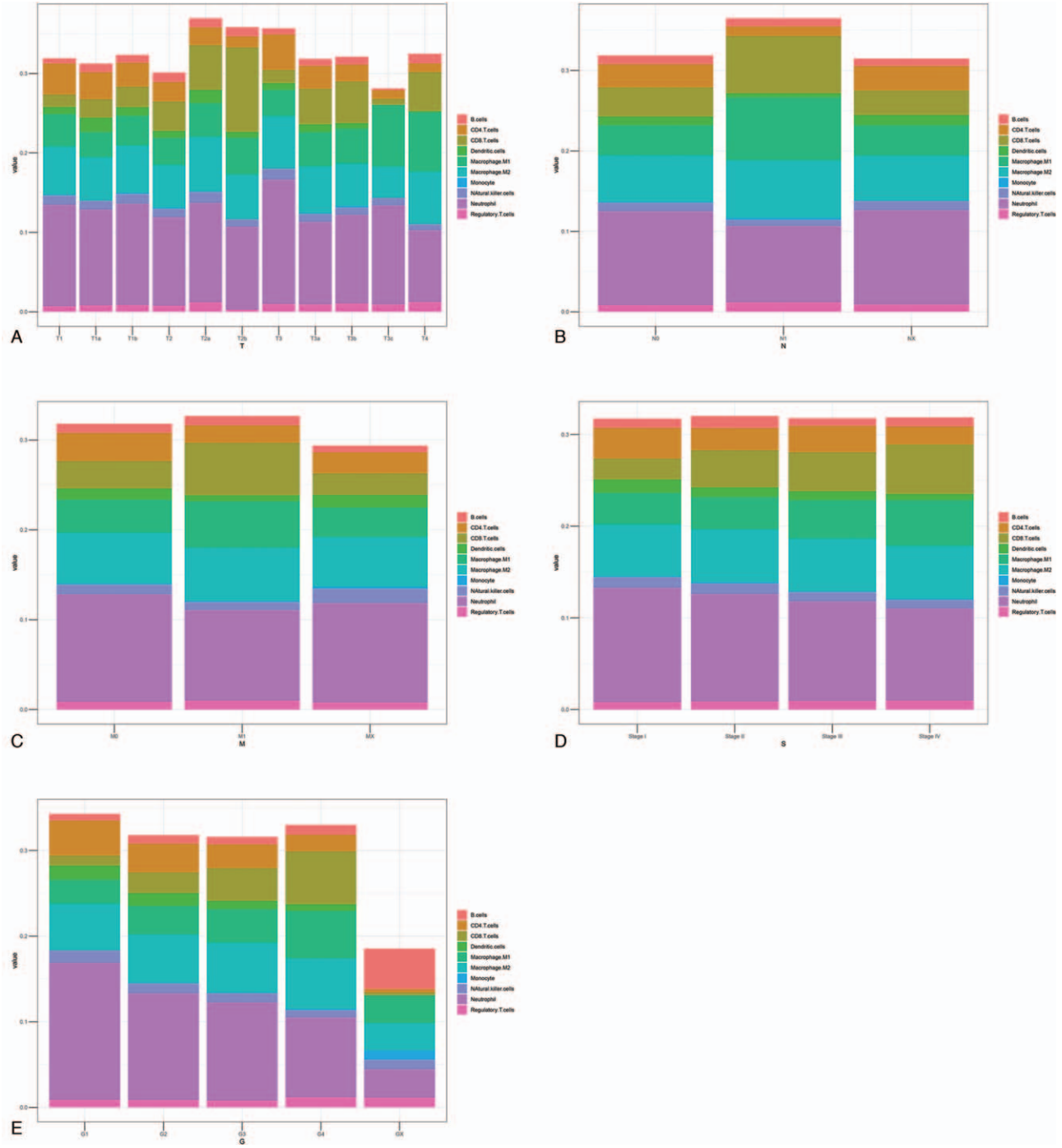

Figure 3. The distribution of TILs in the clinical characteristics.

neutrophils, which have been shown to contribute to tumour progression through curbing antitumour immunity by acting on local inflammation, angiogenesis, and lymphangiogenesis and are gradually becoming recognized for performing an essential task in cancer biology. As with all other leukocytes, neutrophils move towards cancer cells under the influence of particular chemokines, cytokines, and cellular adhesion molecules, and the tumour microenvironment has been proven to be responsible for the recruitment of neutrophils to tumour sites. Neutrophils take part in growth and inflammation and therefore constitute an important portion of the tumour-infiltrating inflammatory cells associated with tumour cell proliferation in many tumour types. Neutrophilia is definitely triggered by the para-neoplastic process of primary tumours and occasionally by the secretion of granulocyte colony-stimulating factor from bone marrow granulocytic cells because of the strong interaction between malignant cells and bone.[18,26]

Monocytes differentiate into tumour-associated macrophages in tumour tissue and cause cancer progression via the generation of numerous cytokines and growth factors that contribute to

angiogenesis and immunosuppressive reactions.[27] Platelets are actually associated with a poor cancer prognosis because they lead to platelet-derived growth factor, vascular endothelial growth factor, and platelet factor 4, which can result in cancerous growth, cell proliferation or invasion into other cells.[28,29] The latest research on tumour-associated macrophages in RCC indicates that a number of CD 68+ macrophages have shortened survival, an accumulation of M2 macrophages expressing the scavenger receptor CD 163 occurs and interferon regulatory factor 4 is expressed in ccRCC.[30] Several other authors have investigated the intratumoural stability of polarized tumour-associated macrophages and demonstrated that specifically CD 206/mannose receptor-positive macrophages (M2 type) are actually associated with decreased survival in RCC patients.[31] Macrophages can polarize into a variety of different phenotypes depending on environmental stimuli. The extremes of this range include the M1 phenotype, which is connected with active microbial killing.[32,33] Tumour-associated macrophages and tumour cells also produce IL-10, which effectively blunts the anti-tumour response mediated by cytotoxic T cells. Conversely, CD68-expressing macrophages are ubiquitous. There are various articles on the promotion of distant cancer cell metastasis by tumour-associated macrophages.

In this study, among the various TIL parameters, M1 macrophages and neutrophils were proven to be independent predictors of OS and DFS in renal cancer patients. These results suggest that the effects of the human immune response on cancer progression may differ. We showed that the presence of TILs, especially M1 macrophages and neutrophils, is associated with patient prognosis. The accumulation of tumour-infiltrating M1 macrophages increased and that of neutrophils decreased from G1 to G4. Non-metastatic tumours tended to have increased numbers of M1 macrophages and reduced numbers of neutrophils. Of note, node-positive tumours tended to have more TILs than node-negative tumours, which was not in line with observations in breast cancer and melanoma.[34,35]

Circulating lymphocytes affect TIL recruitment and may be associated with the immune response in the tumour. Tumour-associated macrophages also cause monocytes to enter tumour tissue, secrete multiple cytokines, and induce an immune response that causes tumour growth. Therefore, in this study, we investigated the relationships between clinicopathological characteristics and TILs to verify this association. There were significant positive correlations between the M1 macrophage fraction and tumour stage and neoplasm histological grade. Stage and neoplasm histological grade showed negative associations with the neutrophil fraction. Since there are few studies addressing these associations, more research is needed to clarify these relationships.

5. Conclusion

We have demonstrated the correlations between TILs and patient prognosis and clinicopathological characteristics in ccRCC. The prognosis of ccRCC patients may differ depending on the TIL fraction. The haemoglobin level was an independent predictor of OS. Similarly, the stage, haemoglobin level, and serum calcium level were independent predictors of DFS. There were significant positive correlations between the M1 macrophage fraction and tumour stage, stage, and neoplasm histological grade. Stage and neoplasm histological grade showed negative associations with the neutrophil fraction.

Therefore, it may be necessary to adopt a different approach in future immune-related studies of this disease.

Author contributions

Data curation: Weiqiang Xu.
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Project administration: Chao Guan and Mingli Gu.
Software: Xu Jiang.
Supervision: Chao Guan and Mingli Gu.
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Writing – review & editing: Weiqiang Xu and Xu Jiang.

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