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Research article

High peripheral neutrophil and monocyte count distinguishes renal cell carcinoma from renal angiomyolipoma and predicts poor prognosis of renal cell carcinoma

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ABSTRACT

Background: The presence of peripheral inflammatory cells has been linked to the prognosis of cancer. This study aims to investigate the distinct roles of absolute neutrophil count (ANC) and absolute monocyte count (AMC) in differentiating renal cell carcinoma (RCC) from renal angiomyolipoma (RAML), as well as their prognostic significance in RCC.

Methods: We conducted a comprehensive analysis of peripheral immune cell data, clinicopathological data, and tumor characteristics in patients diagnosed with RCC or RAML from January 2015 to December 2021. Receiver operating characteristic (ROC) curves, as well as univariate and multivariate analyses, were employed to assess the diagnostic utility of AMC and ANC in differentiating between RCC and RAML. Kaplan-Meier curve analysis was used to study the survival of RCC patients with different AMC and ANC. The prognostic value of AMC and ANC in RCC was investigated using COX univariate and multivariate analysis. The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases were used for bioinformatic correlation analysis.

Results: A total of 1120 eligible patients were included in the study. The mean preoperative AMC and ANC in patients with RCC were found to be significantly higher compared to those in patients with RAML ($P = 0.001$ and $P < 0.001$, respectively). High preoperative AMC and ANC significantly correlated with smoking history, tumor length, gross hematuria, and high T Stage, N stage, and pathological grade. In multivariate analyses, an ANC> 3.205 *10^9/L was identified to be independently associated with the presence of RCC (HR = 1.618, $P = 0.008$). High AMC and ANC were significantly associated with reduced OS and PFS ($P < 0.05$), and ANC may be an independent prognostic factor. Public database analysis showed that signature genes of tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) were highly expressed in ccRCC.

Conclusions: Elevated preoperative ANC and AMC can distinguish RCC from RAML and predict poor prognosis in patients with RCC. Furthermore, the signature genes of TAMs and TANs exhibit high expression levels in clear cell RCC.

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1. Introduction

Renal cell carcinoma (RCC), a malignancy of the renal parenchyma, is the most lethal among urinary cancers, impacting over 400,000 individuals globally annually. Its incidence has steadily increased in the past decade [1,2]. At present, partial or radical nephrectomy continues to be the established therapeutic approach for RCC, while immunotherapy has demonstrated efficacy in a subset of patients [3]. Regrettably, more than one-third of patients undergoing surgery for RCC experience recurrence and distant metastasis, while the comprehensive implementation of immunotherapy is hindered by the absence of biomarkers [4,5]. Furthermore, the differentiation between RCC and renal angiomyolipoma (RAML) in clinical practice poses a significant challenge, often resulting in delayed or excessive medical interventions and irreversible patient losses. Therefore, the identification of effective markers is essential for optimizing treatment strategies and predicting patient survival.

From a clinical perspective, the risk stratification and prognosis of RCC continue to heavily rely on the TNM stage and Fuhrman grade. Furthermore, certain tissue and molecular biomarkers, such as gankyrin, carbonic anhydrase IX (CAIX), and microRNA, have also been utilized for prognostic prediction in RCC [6–8]. Acquiring these markers, however, is a relatively laborious process, significantly constraining their overall applicability. And the preoperative differentiation between benign and malignant renal masses remains a well-recognized radiological diagnostic challenge. Studies on diagnostic methods for distinguishing RCC from RAML are rare and have mainly focused on imaging findings, such as contrast-enhanced CT, contrast-enhanced ultrasound, conventional MRI, and functional MRI techniques (diffusion-weighted imaging (DWI) and chemical-shift MRI) [9–11]. Even with the utilization of advanced radiographic techniques, confirmation typically necessitates a biopsy or surgical excision of the tissue [12]. Fortunately, we have identified a stable, reliable, and easily accessible absolute neutrophil count (ANC) and absolute monocyte count (AMC) as potential prognostic indicators for RCC. Additionally, these counts effectively address the challenge of distinguishing RCC from RAML.

Neutrophils and monocytes play crucial roles in the innate immune system, exhibiting close associations with the tumor

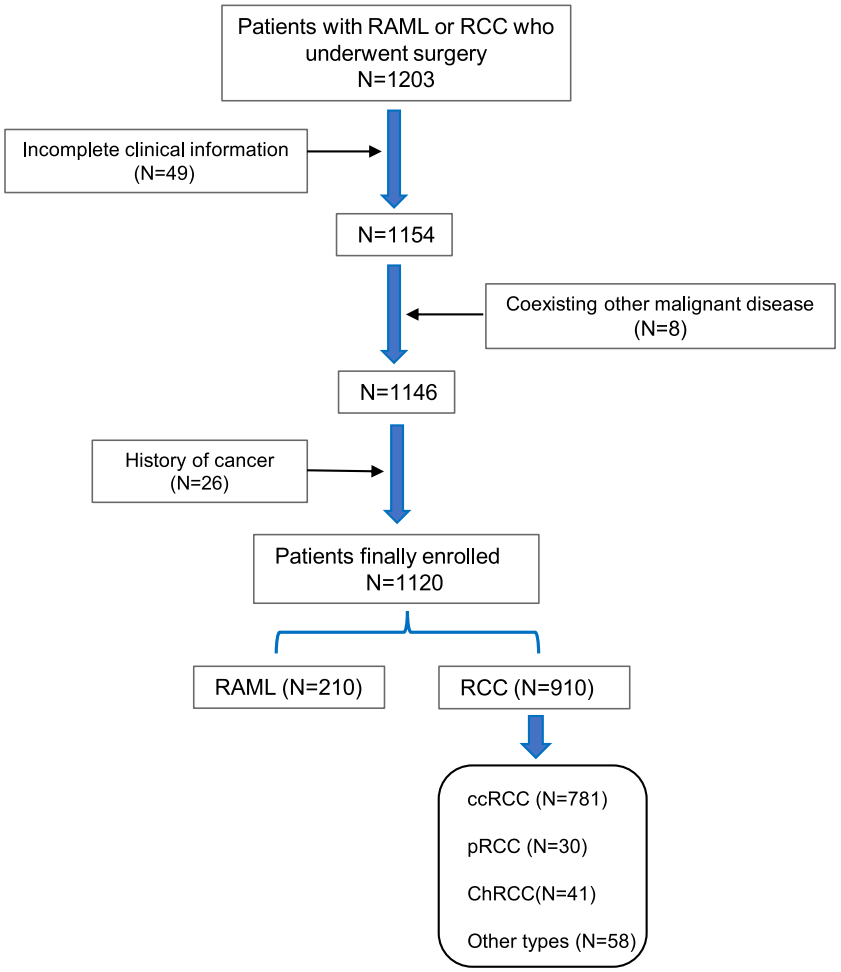


Fig. 1. Inclusion and exclusion criteria for screening 1120 patients with RAML or RCC who underwent surgery in this study. RAML, renal angiomyolipoma; RCC, renal cell carcinoma; ccRCC, clear cell renal cell carcinoma; pRCC, papillary renalcell carcinoma; ChRCC, chromophobe renal cell carcinoma.

microenvironment and serving as significant regulators of cancer initiation and advancement [13,14]. Previous research has consistently demonstrated a strong correlation between various components of the complete blood count, such as absolute lymphocyte count (ALC), ANC, AMC, absolute platelet count (APC), monocyte-lymphocyte ratio (MLR), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and the prognosis of certain types of cancer, elevated levels of circulating neutrophils and monocytes were significantly responsible for the poor prognosis of multiple cancers, such as hepatocellular carcinoma, gastric cancer, and lung cancer for example [15–19]. At present, the prognostic values of AMC and ANC in RCC have been the subject of limited study. We also found that the infiltration of neutrophils and monocytes was closely associated with the overexpression of the signature gene S100A9 of Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs). Furthermore, the overexpression of S100A9 has been demonstrated to serve as an indicator of unfavorable prognosis in RCC [20,21]. It is reasonable to speculate that increased ANC and AMC are associated with poor prognosis of RCC.

2. Materials and methods

2.1. Patients

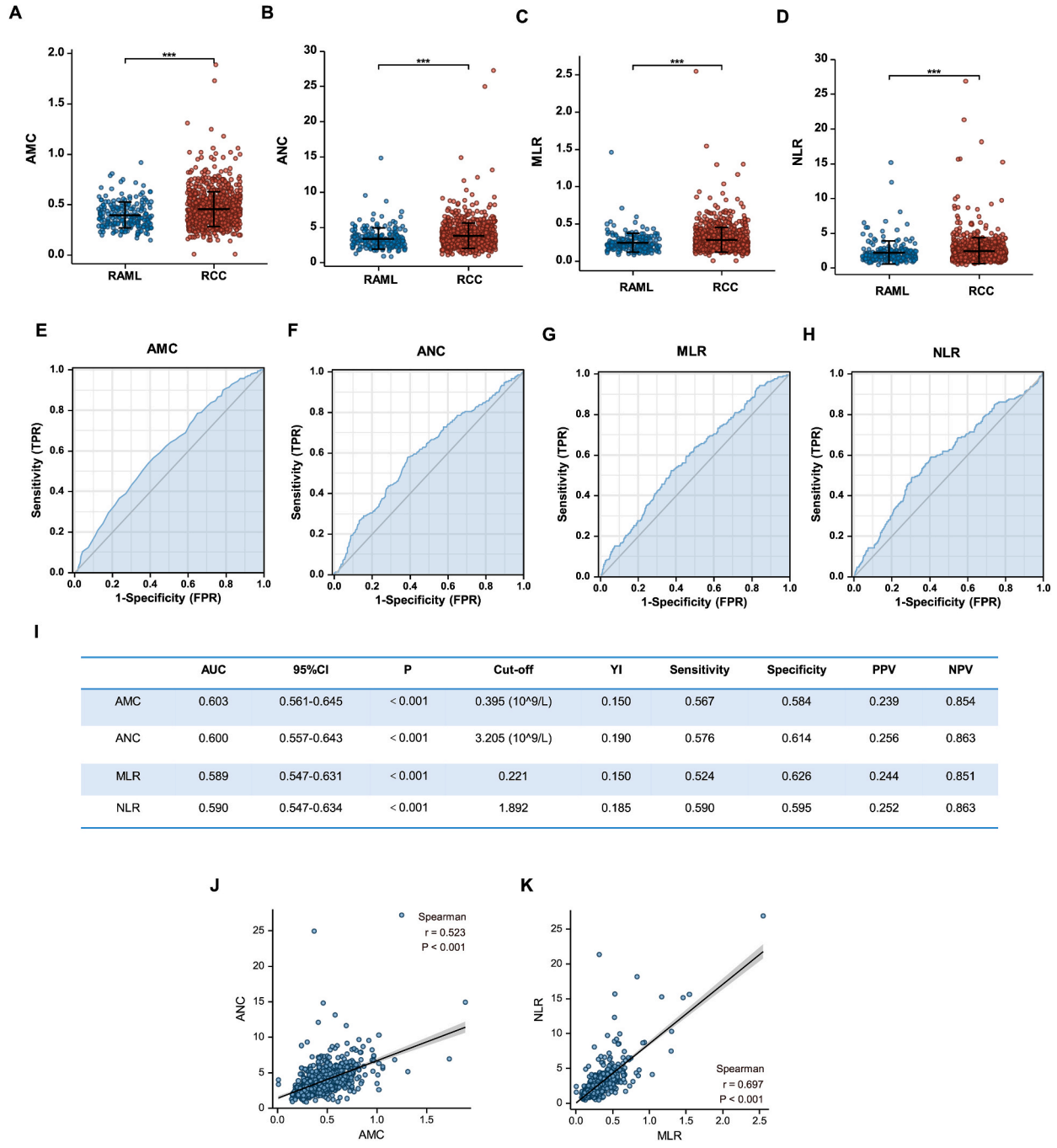
This retrospective study included 1203 patients pathologically diagnosed with RCC or RAML and underwent surgical treatment at the Department of Urology, Qilu Hospital of Shandong University, between January 2015 and December 2021. Taking the time of surgery as the starting point of observation, every patient was followed up regularly by telephone call until death or August 15, 2022. Patients who had received neoadjuvant treatment were not included in the study. According to the exclusion criteria, 1120 patients met the requirements (Fig. 1). Among the 1120 patients eventually included in the study, 210 and 910 had RAML and RCC,

Table 1
Comparison of clinical characteristics between RAML and RCC in study patients.

Characteristics	RAML	RCC	P-Value
	N = 210	N = 910	
Age			**<0.001**
Mean ± SD	48.60 ± 12.83	55.29 ± 11.54	
Median (range)	50(14–75)	55(22–85)	
Tumor length(cm)			**0.020**
Mean ± SD	6.40 ± 5.51	4.77 ± 2.74	
Median (range)	4.85(0.5–30)	4(0.6–19)	
Gender			**<0.001**
Male	62(29.5 %)	603(66.3 %)	
Female	148(70.5 %)	307(33.7 %)	
Smoking history			**<0.001**
Yes	20(9.5 %)	673(74.0 %)	
No	190(90.5 %)	237(26.0 %)	
Tumor side			0.863
Left	108(51.4 %)	474(52.1 %)	
Right	102(48.6 %)	436(47.9 %)	
Gross hematuria			**<0.001**
Positive	6(2.9 %)	152(16.7 %)	
Negative	204(97.1 %)	758(83.3 %)	
Operation style			
RN	10(4.8 %)	518(56.9 %)	
PN	173(82.4 %)	373(41.0 %)	
Enucleation	26(12.4 %)	0(0.0 %)	
Puncture	1(0.4 %)	19((2.1 %)	
ANC (10^9/L)			**<0.001**
Mean ± SD	3.39 ± 1.50	3.82 ± 1.82	
AMC (10^9/L)			**0.001**
Mean ± SD	0.40 ± 0.13	0.45 ± 0.17	
ALC (10^9/L)			0.501
Mean ± SD	1.79 ± 0.63	1.74 ± 0.57	
PLT (10^9/L)			0.556
Mean ± SD	252.17 ± 73.48	251.63 ± 76.01	
NLR (10^9/L)			**<0.001**
Mean ± SD	2.19 ± 1.65	2.47 ± 1.86	
MLR (10^9/L)			**<0.001**
Mean ± SD	0.25 ± 0.13	0.29 ± 0.17	
PLR (10^9/L)			0.923
Mean ± SD	157.35 ± 71.24	159.22 ± 73.19	

RCC, renal cell carcinoma; RAML, renal angiomyolipoma; AMC, absolute monocyte count; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; PLT, platelet; NLR, neutrophil lymphocyte ratio; MLR,monocyte lymphocyte ratio; PLR, platelet lymphocyte ratio.SD, standard deviation.
$P < 0.05$ is considered as statistically significant.

respectively. Of the 910 patients with RCC, 781 were diagnosed as clear cell renal cell carcinoma (ccRCC), 30 as papillary renal cell carcinoma (pRCC), 41 as chromophobe renal cell carcinoma (ChRCC), and 58 as other types (including sarcomatoid renal cell carcinoma, renal carcinoid, squamous cell carcinoma, multilocular cystic renal neoplasm and unclearly types). And of the 910 RCC patients, 518 (56.9 %) patients underwent radical nephrectomy and 373 (41.0 %) underwent partial nephrectomy; of the 210 RAML patients, 10 (4.8 %) underwent radical nephrectomy, 173 (82.4 %) underwent partial nephrectomy, and 26 (12.4 %) underwent



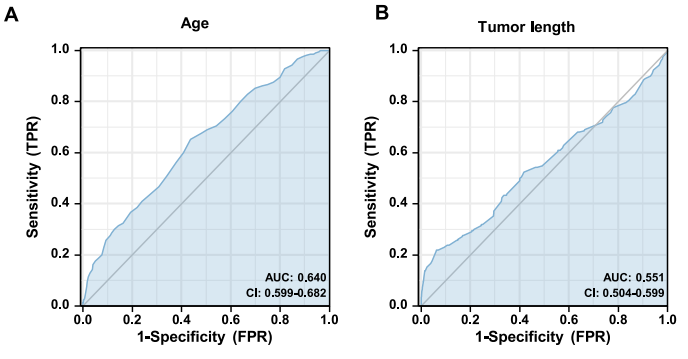
	AUC	95%CI	P	Cut-off	YI	Sensitivity	Specificity	PPV	NPV
AMC	0.603	0.561-0.645	< 0.001	0.395 (10^9/L)	0.150	0.567	0.584	0.239	0.854
ANC	0.600	0.557-0.643	< 0.001	3.205 (10^9/L)	0.190	0.576	0.614	0.256	0.863
MLR	0.589	0.547-0.631	< 0.001	0.221	0.150	0.524	0.626	0.244	0.851
NLR	0.590	0.547-0.634	< 0.001	1.892	0.185	0.590	0.595	0.252	0.863

Fig. 2. Levels of AMC, ANC, MLR, and NLR in RAML and RCC. (A) AMC; (B) ANC; (C) MLR level; (D) NLR level. Diagnostic value of (E) AMC, (F) ANC, (G) MLR, and (H) NLR for distinguishing RCC from RAML by ROC analysis. (I) The following table shows the data of ROC curves. (J) Line correlations between AMC and ANC. (K) Line correlations between MLR and NLR. RAML, renal angiomyolipoma; RCC, renal cell carcinoma; AMC, absolute monocyte count; ANC, absolute neutrophil count; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; ROC, receiver-operating characteristic; AUC, area under curve; YI, Youden Index; PPV, positive predictive value; NPV, negative predictive value; TRR, True Positive Rate; FPR, False Positive Rate; ***P < 0.001.

enucleation (Table 1).

2.2. Data collection

Clinical data, including patient age at diagnosis, smoking history, hematuria history, pre-treatment routine blood examination results, and tumor characteristics, were extracted from the electronic patient records of Qilu Hospital of Shandong University.



C

	AUC	95%CI	P	Cut-off	YI	Sensitivity	Specificity	PPV	NPV
Age	0.640	0.599-0.682	< 0.001	53.5 (year)	0.216	0.652	0.564	0.257	0.875
Tumor length	0.551	0.504-0.599	0.020	9.550 (cm)	0.158	0.219	0.938	0.451	0.839

D

Univariate analysis

Variables	HR	95%CI	P
Age (> 53.5 vs.≤53.5 year)	0.985	0.920-1.055	0.674
Gender (male vs.female)	4.689	3.382-6.501	< 0.001
Smoking history (ever vs.never)	3.345	2.062-5.428	< 0.001
Hematuria (yes vs.no)	6.818	2.972-15.641	< 0.001
Tumor length (≤9.55 vs. > 9.55 cm)	4.277	2.798-6.538	< 0.001
AMC (> 0.395 vs. ≤0.395 ×10^9/L)	1.832	1.353-2.481	< 0.001
ANC (> 3.205 vs. ≤3.205 ×10^9/L)	2.165	1.597-2.936	< 0.001

E

Multivariate analysis

Variables	HR	95%CI	P
Gender (male vs.female)	3.886	2.658-5.680	< 0.001
Smoking history (ever vs.never)	1.322	0.755-2.316	0.329
Hematuria (yes vs.no)	6.669	2.814-15.804	< 0.001
Tumor length (≤9.55 vs. > 9.55 cm)	6.239	3.788-10.275	< 0.001
AMC (> 0.395 vs. ≤0.395 ×10^9/L)	1.292	0.907-1.842	0.156
ANC (> 3.205 vs. ≤3.205 ×10^9/L)	1.618	1.131-2.315	0.008

Fig. 3. Diagnostic value of clinicopathological variables in distinguishing RCC from RAML. ROC curves for determination of cutoff value of (A) age and (B) tumor length regarding distinguishing RCC from RAML. (C) Data table for ROC curves. (D) Univariate and (E) multivariate analysis of preoperative variables on prediction of RCC. RAML, renal angiomyolipoma; RCC, renal cell carcinoma; AMC, absolute monocyte count; ANC, absolute neutrophil count; ROC, receiver-operating characteristic; AUC, area under curve; YI, Youden Index; PPV, positive predictive value; NPV, negative predictive value; TRR, True Positive Rate; FPR, False Positive Rate; HR, hazard ratio; CI, confidence interval.

Hematological parameters assessed prior to treatment comprised ALC, ANC, AMC, NLR, PLR, and MLR. Tumor histological grading was conducted using the WHO/ISUP grading system. Pathologic staging was determined based on the American Joint Committee on Cancer (AJCC) stage groupings I-IV.

2.3. Bioinformatic analysis of clinical data

Data from The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/tcga) and GSE15641 were used to extract RNA

Table 2
Correlations between preoperative AMC and clinicopathological parameters of all RCC patients.

	N(%)	AMC (mean ± SD)	P-Value
Patients	910		
Age[a]			0.076
≤55	459(50.4 %)	0.444 ± 0.169	
>55	451(49.6 %)	0.465 ± 0.178	
Gender			**<0.001**
Male	603(66.3 %)	0.478 ± 0.180	
Female	307(33.7 %)	0.409 ± 0.153	
Smoking history			**<0.001**
Yes	237(26.0 %)	0.499 ± 0.191	
No	673(74.0 %)	0.439 ± 0.165	
Tumor side			0.942
Left	474(52.1 %)	0.459 ± 0.188	
Right	436(47.9 %)	0.449 ± 0.158	
Gross hematuria			**0.012**
Positive	152(16.7 %)	0.493 ± 0.212	
Negative	758(83.3 %)	0.447 ± 0.165	
Tumor length[a]**(cm)**			**<0.001**
≤4	464(51.0 %)	0.430 ± 0.155	
>4	446(49.0 %)	0.480 ± 0.189	
ANC[a]**(10^9/L)**			**<0.001**
≤3.51	458(50.3 %)	0.383 ± 0.119	
>3.51	452(49.7 %)	0.527 ± 0.190	
ALC[a]**(10^9/L)**			**<0.001**
≤1.68	459(50.4 %)	0.432 ± 0.183	
>1.68	451(49.6 %)	0.478 ± 0.162	
PLT[a]**(10^9/L)**			**<0.001**
≤239.5	455(50.0 %)	0.429 ± 0.164	
>239.5	455(50.0 %)	0.480 ± 0.180	
NLR[a]			**<0.001**
≤2.08	458(50.3 %)	0.416 ± 0.135	
>2.08	452(49.7 %)	0.493 ± 0.199	
MLR[a]			**<0.001**
≤0.25	479(52.6 %)	0.378 ± 0.109	
>0.25	431(47.4 %)	0.540 ± 0.192	
PLR[a]			0.974
≤140.2	455(50.0 %)	0.451 ± 0.170	
>140.2	455(50.0 %)	0.458 ± 0.178	
T stage			**<0.001**
T1& T2	734(80.7 %)	0.439 ± 0.161	
T3& T4	176(19.3 %)	0.518 ± 0.209	
N stage			**<0.001**
N0	810(89.0 %)	0.443 ± 0.163	
N1	100(11.0 %)	0.547 ± 0.224	
Metastasis			**0.001**
M0	880(96.7 %)	0.451 ± 0.172	
M1	30(3.3 %)	0.556 ± 0.196	
Pathological stage			**<0.001**
I& II	679(74.6 %)	0.434 ± 0.153	
III& IV	231(25.4 %)	0.514 ± 0.215	
Histological grade			0.003
G1 & G2	550(60.4 %)	0.434 ± 0.148	
G3 & G4	218(24.0 %)	0.485 ± 0.197	
Missing data	142(15.6 %)		

Pathological stage is assessed in accordance with American Joint Committee on Cancer (AJCC) staging. Histological grade is assessed in accordance with WHO/ISUP classification. $p < 0.05$ is considered as statistically significant. RCC, renal cell carcinoma; AMC, absolute monocyte count; ALC,absolute lymphocyte count; ANC, Absolute neutrophil count; PLT,platelet NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; MLR,monocyte lymphocyte ratio.

[a] Continuous variables are expressed as median.

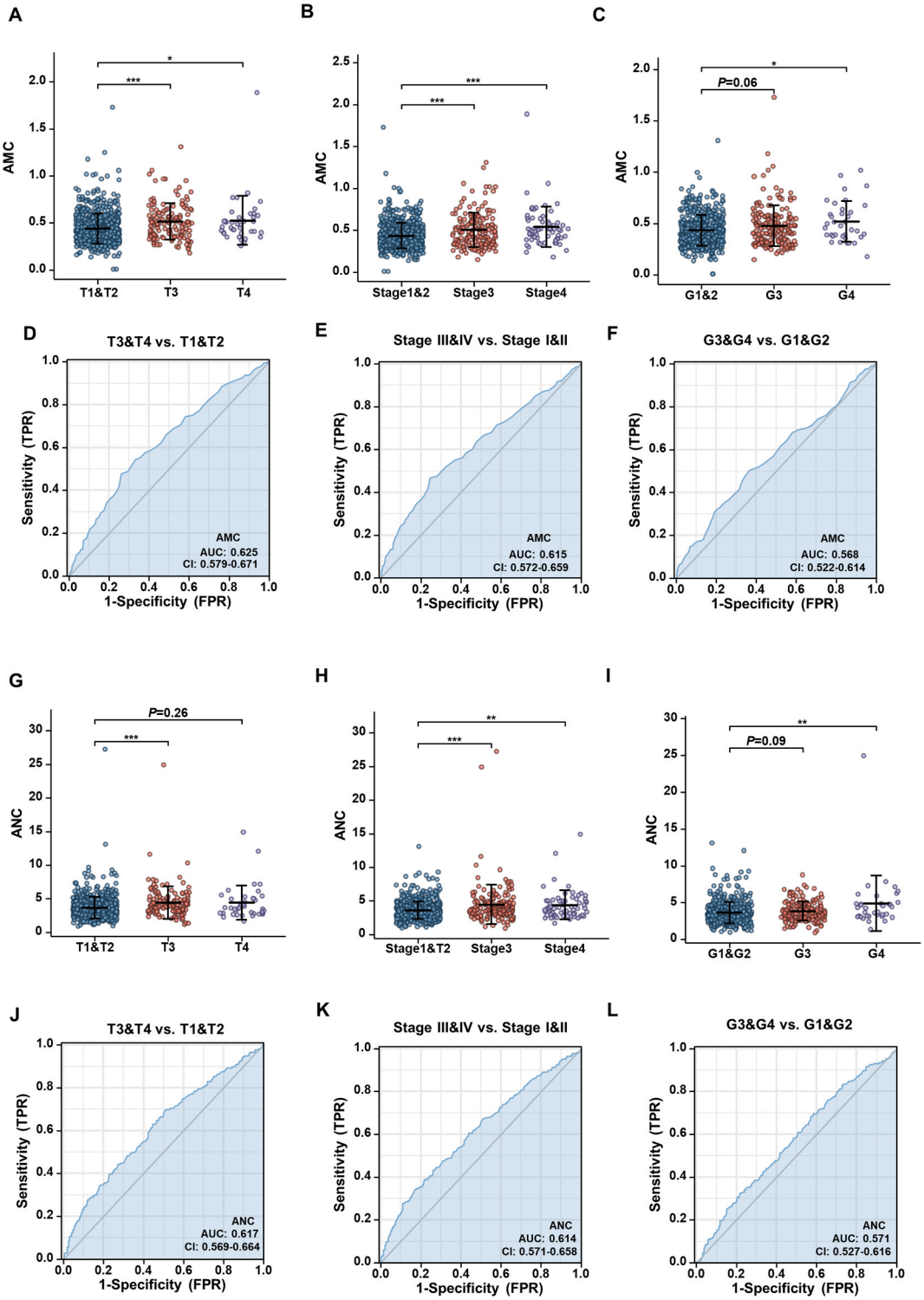


Fig. 4. AMC and ANC in RCC subgroups. Comparison of AMC levels among different (A) T stage, (B) pathologic stage, and (C) histologic grade. Diagnostic value of AMC for (D) high T stage, (E) high pathologic stage, and (F) high histologic grade. Comparison of ANC levels among different (G) T stage, (H) pathologic stage, and (I) histologic grade. Diagnostic value of ANC for (J) high T stage, (K) high pathologic stage, and (L) high histologic grade. RCC, renal cell carcinoma; AMC, absolute monocyte count; ANC, absolute neutrophil count; TRR, True Positive Rate; FPR, False Positive Rate; *P < 0.05, **P < 0.01, ***P < 0.001.

sequencing data and analyze the expression levels of TANs and TAMs signature genes in ccRCC. The ccRCC data set of the TCGA included 532 tumor samples and a dataset of 72 normal tissue samples, GSE15641 dataset included 32 tumor samples and 23 normal tissue samples.

2.4. Statistical analysis

The distributions of the continuous and categorical variables were expressed as the means and standard deviations (mean ± SD) and as the frequencies and percentages, respectively. The comparison of continuous variables between groups was conducted using the Mann–Whitney *U* test. Differences between categorical variables were assessed using the chi-square test. According to ROC curve analyses, the maximum Youden index (Youden index = sensitivity + specificity-1) was as the optimal threshold for differential diagnosis. The relationships between the variables were analyzed using Spearman's correlation test. The diagnostic value of the clinicopathological variables in distinguishing RCC from RAML was analyzed using univariate and multivariate logistic regression analyses. Hazard ratios (HR) and 95 % confidence intervals (CI) were calculated using a Cox regression model and the HR value of continuous variables was calculated with the lower values as the reference. Kaplan-Meier survival analysis was used to compare patients' overall survival (OS) and progression-free survival (PFS) in the different groups, and the significance was evaluated by Log-rank test. PFS was calculated as the time between surgery and the progression of the disease or death from RCC. OS was calculated from the date of surgery to death or the last follow-up date. The prognostic value of AMC and ANC in patients with RCC was further verified using univariate and multivariate analyses. Cox proportional hazard model was used for univariate and multivariate analyses. Statistical significance was set at $P < 0.05$. All statistical procedures were performed using Statistical Package for Social Sciences version 23.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Comparison of clinical parameters in renal angiomyolipoma and renal cell carcinoma

In the current investigation, a total of 210 patients diagnosed with renal angiomyolipoma (RAML) and 910 patients diagnosed with renal cell carcinoma (RCC) were included to assess the utility of absolute monocyte count (AMC), absolute neutrophil count (ANC), and other relevant parameters in differentiating between these two conditions; detailed data are presented in Table 1. Firstly, the AMC in patients with RCC was significantly higher compared to that in patients with RAML (Fig. 2A, $P = 0.001$), and the differences in ANC, MLR, and NLR between the two pathological types were consistent with AMC levels (Fig. 2B-D, $P < 0.001$). Furthermore, the age at onset of RCC is greater than that of RAML. Smoking history, gender, tumor length, and symptoms of hematuria also play pivotal roles in distinguishing between these two diseases. Subsequently, for the purpose of elucidating discriminative efficacy of above factors, ROC analysis was performed, and results indicated that areas under curve (AUC) of AMC, ANC, MLR, NLR were respectively 0.603 (95% CI:0.561–0.645), 0.600 (95%CI:0.557–0.643), 0.589 (95%CI:0.547–0.631), and 0.590 (95%CI:0.547–0.634) (Fig. 2E-H), and their cutoff values were 0.395*10^9/L, 3.205*10^9/L, 0.221, and 1.892, respectively (Fig. 2I $P < 0.001$). Furthermore, there was a positive correlation between ANC and AMC, as well as NLR and MLR (Fig. 2J-K $P < 0.001$). The AUC of age and tumor length were 0.640 (95% CI:0.599–0.682), 0.551 (95%CI:0.504–0.599) with cutoffs of 53.5 (year) and 9.55 (cm) (Fig. 3A-C $P < 0.001$, $P = 0.020$). According to the ROC curve analysis, the above optimal cut-off value was determined. Subsequently, AMC, ANC, age, tumor length and other continuous variables were categorized based on this optimal cut-off value. Their diagnostic efficacy for RCC was assessed through both univariate and multivariate logistic regression analysis. The findings suggest that ANC>3.205*10^9/L can serve as an independent indicator for distinguishing RCC and RAML. The results demonstrated significant significance in both univariate and multivariate analyses (Fig. 3D-E, HR = 2.165, $P < 0.001$; HR = 1.618, $P = 0.008$; respectively). Regrettably, while AMC showed a significant discriminatory effect in the univariate analysis (Fig. 3D HR = 1.832, $P < 0.001$), it did not maintain significance in the multivariate analysis (Fig. 3E HR = 1.292, $P = 0.156$).

3.2. Associations between preoperative AMC level and clinicopathological variables in patients with RCC

The comprehensive presentation of clinicopathological variables can be found in Table 2. Statistically significant differences in AMC levels were observed within certain subgroups; for example, patients with a higher T stage RCC exhibited elevated AMC levels compared to those with a lower T stage RCC ($P < 0.001$), and patients with node metastasis or distant metastasis also possessed elevated AMC levels ($P < 0.001$). The preoperative AMC in male patients was significantly elevated compared to female patients, and individuals with a history of smoking or hematuria exhibited higher AMC levels than those without. High levels of AMC, as demonstrated in Table 2, are indicative of late pathologic stage and high histologic grade (Fig. 4A-C) and ROC analysis further supported this hypothesis (Fig. 4D-F).

3.3. Associations between preoperative ANC level and clinicopathological variables in patients with RCC

After conducting an analysis of the associations between clinicopathological variables of RCC and AMC levels, the significance of ANC within the RCC variables was further investigated and summarized in Table 3. In line with AMC, the findings indicated that individuals with a higher T stage of RCC exhibited increased ANC levels in comparison to those with a lower T stage ($P < 0.001$), and patients with node metastasis exhibited higher levels of ANC compared to those without node metastasis ($P < 0.001$). However, in

comparison to patients without distant metastasis, those with distant metastasis exhibited elevated neutrophil counts; but the difference did not reach statistical significance. Furthermore, our analysis revealed that patients with a history of smoking and gross hematuria demonstrated higher levels of ANC. The preoperative ANC accumulation is also indicative of advanced pathologic stage and high histologic grade (Fig. 4G-I), which was confirmed by ROC analysis (Fig. 4J-L)

Table 3
Correlations between preoperative ANC and clinicopathological parameters of all RCC patients.

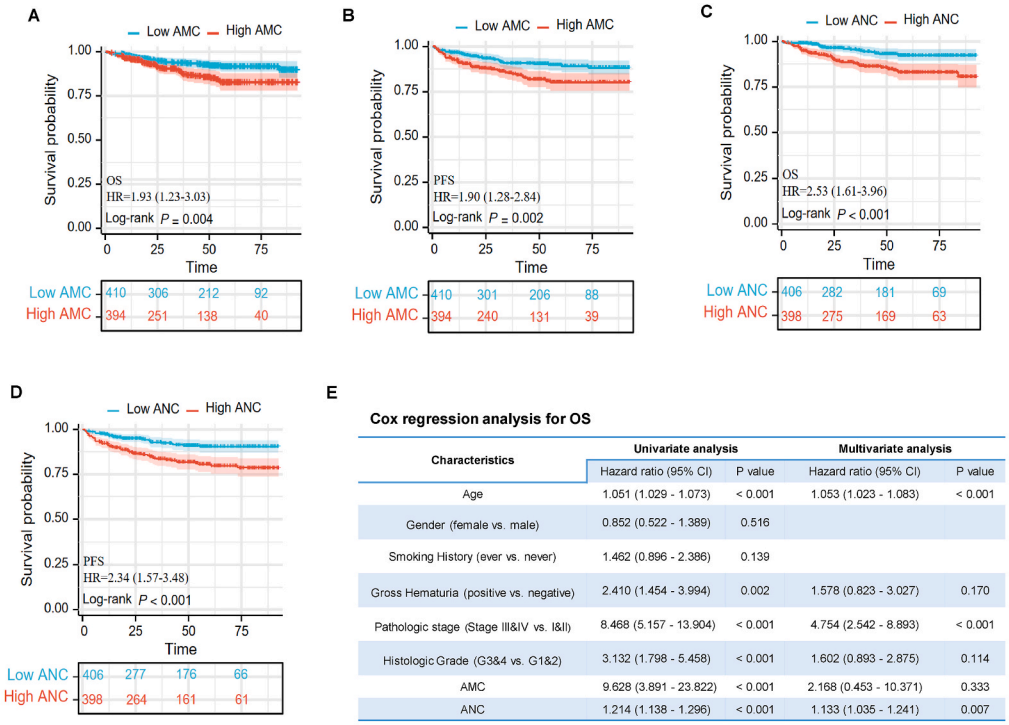
Characteristics	N(%)	ANC (mean ± SD)	P-Value
Patients	910(100 %)		
Age[a]			0.561
≤55	459(50.4 %)	3.84 ± 1.78	
>55	451(49.6 %)	3.81 ± 1.85	
Gender			**<0.001**
Male	603(66.3 %)	3.99 ± 1.94	
Female	307(33.7 %)	3.49 ± 1.50	
Smoking history			**<0.001**
Yes	237(26.0 %)	4.22 ± 2.15	
No	673(74.0 %)	3.68 ± 1.67	
Tumor side			0.654
Left	474(52.1 %)	3.82 ± 1.81	
Right	436(47.9 %)	3.82 ± 1.83	
Tumor length[a]**(cm)**			**<0.001**
≤4	464(51.0 %)	3.50 ± 1.27	
>4	446(49.0 %)	4.16 ± 2.20	
Gross hematuria			**<0.001**
Positive	152(16.7 %)	4.46 ± 3.07	
Negative	758(83.3 %)	3.70 ± 1.41	
AMC[a]**(10^9/L)**			**<0.001**
≤0.42	461(50.7 %)	3.27 ± 1.50	
>0.42	449(49.3 %)	4.40 ± 1.93	
ALC[a]**(10^9/L)**			**0.005**
≤1.68	459(50.4 %)	3.78 ± 2.18	
>1.68	451(49.6 %)	3.86 ± 1.36	
PLT[a]**(10^9/L)**			**<0.001**
≤239.5	455(50.0 %)	3.52 ± 1.45	
>239.5	455(50.0 %)	4.13 ± 2.08	
NLR[a]			**<0.001**
≤2.08	455(50.0 %)	3.03 ± 0.87	
>2.08	455(50.0 %)	4.62 ± 2.14	
MLR[a]			**<0.001**
≤0.25	459(50.4 %)	3.39 ± 1.15	
>0.25	451(49.6 %)	4.27 ± 2.22	
PLR[a]			**0.008**
≤140.20	455(50.0 %)	3.63 ± 1.35	
>140.20	455(50.0 %)	4.02 ± 2.17	
T stage			**<0.001**
T1 and T2	734(80.7 %)	3.68 ± 1.60	
T3 and T4	176(19.3 %)	4.43 ± 2.45	
N stage			**<0.001**
N0	810(89.0 %)	3.69 ± 1.45	
N1	100(11.0 %)	4.89 ± 3.44	
Metastasis			0.066
M0	880(96.7 %)	3.81 ± 1.83	
M1	30(3.3 %)	4.20 ± 1.41	
Pathological stage			**<0.001**
I& II	679(74.6 %)	3.61 ± 1.31	
III& IV	231(25.4 %)	4.45 ± 2.74	
Histological grade			**0.002**
G1 & G2	550(60.4 %)	3.65 ± 1.44	
G3 & G4	218(24.0 %)	4.00 ± 1.96	
Missing data	142(15.6 %)		

Pathological stage is assessed in accordance with American Joint Committee on Cancer (AJCC) staging. Histological grade is assessed in accordance with WHO/ISUP classification. $p < 0.05$ is considered as statistically significant. RCC, renal cell carcinoma; AMC, absolute monocyte count; ALC,absolute lymphocyte count; ANC, Absolute neutrophil count; PLT,platelet NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio; MLR,monocyte lymphocyte ratio.

[a] Continuous variables are expressed as median.

3.4. Increasingly preoperative AMC and ANC levels indicated a poorer prognosis of RCC

Kaplan-Meier curves were constructed to examine the association between preoperative AMC and ANC levels and RCC prognosis. The AMC and ANC levels were categorized into high and low groups based on the median, with overall survival (OS) and progression-free survival (PFS) used as prognostic indicators. Curves revealed that patients with lower AMC level had more preferable OS (Fig. 5A, HR = 1.93,95%CI:1.23–3.03, P = 0.004) and PFS (Fig. 5B, HR = 1.90,95%CI:1.28–2.84, P = 0.002), similarly, patients with lower ANC level had more preferable OS (Fig. 5C, HR = 2.53,95%CI:1.61–3.96, P < 0.001) and PFS (Fig. 5D, HR = 2.34, 95%CI:1.57–3.48, P < 0.001). We conducted univariate and multivariate Cox regression analyses, considering factors such as patient age, sex, smoking history, gross hematuria, pathologic stage, and histologic grade to examine the independent prognostic significance of AMC and ANC. The findings indicated that ANC emerged as a significant independent predictor of prognosis in RCC patients in the final multivariate analysis (OS: HR = 1.133, P = 0.007; PFS: HR = 1.141, P = 0.021). In univariate analysis, AMC had the highest hazard ratio (OS: HR = 9.628, P < 0.001; PFS: HR = 7.901, P < 0.001), whereas the HR of AMC remained elevated in multivariate analysis but did not reach



E

Cox regression analysis for OS

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	1.051 (1.029 - 1.073)	< 0.001	1.053 (1.023 - 1.083)	< 0.001
Gender (female vs. male)	0.852 (0.522 - 1.389)	0.516		
Smoking History (ever vs. never)	1.462 (0.896 - 2.386)	0.139		
Gross Hematuria (positive vs. negative)	2.410 (1.454 - 3.994)	0.002	1.578 (0.823 - 3.027)	0.170
Pathologic stage (Stage III&IV vs. I&II)	8.468 (5.157 - 13.904)	< 0.001	4.754 (2.542 - 8.893)	< 0.001
Histologic Grade (G3&4 vs. G1&2)	3.132 (1.798 - 5.458)	< 0.001	1.602 (0.893 - 2.875)	0.114
AMC	9.628 (3.891 - 23.822)	< 0.001	2.168 (0.453 - 10.371)	0.333
ANC	1.214 (1.138 - 1.296)	< 0.001	1.133 (1.035 - 1.241)	0.007

F

Cox regression analysis for PFS

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	1.040 (1.021 - 1.059)	< 0.001	1.044 (1.019 - 1.069)	< 0.001
Gender (female vs. male)	0.964 (0.631 - 1.472)	0.865		
Smoking History (ever vs. never)	1.268 (0.813 - 1.977)	0.304		
Gross Hematuria (positive vs. negative)	3.233 (2.119 - 4.931)	< 0.001	2.194 (1.301 - 3.702)	0.003
Pathologic stage (Stage III&IV vs. I&II)	7.680 (5.011 - 11.770)	< 0.001	4.015 (2.394 - 6.734)	< 0.001
Histologic Grade (G3&4 vs. G1&2)	3.354 (2.082 - 5.404)	< 0.001	1.941 (1.182 - 3.190)	0.009
AMC	7.901 (3.562 - 17.526)	< 0.001	1.806 (0.442 – 7.380)	0.411
ANC	1.250 (1.160 - 1.348)	< 0.001	1.141 (1.020 - 1.278)	0.021

G

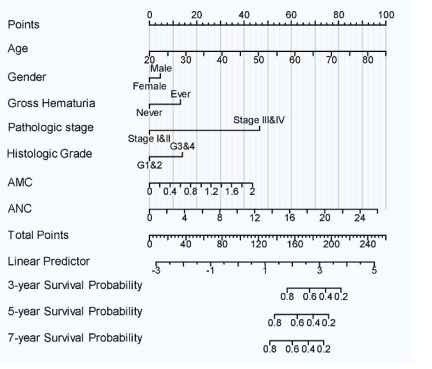


Fig. 5. Survival analysis of RCC patients with different AMC and ANC levels. Kaplan-Meier curve analysis based on AMC or ANC effect for overall survival (OS) (A, AMC; C, ANC) and progression-free survival (PFS) (B, AMC; D, ANC) of RCC, according to the 50th percentile of AMC or ANC level. Univariate and multivariate Cox regression analysis to investigate the independent prognostic value of AMC and ANC (E, OS; F, PFS). (G) Nomogram model for predicting survival rates of RCC patients at 3, 5, and 7 years. RCC, renal cell carcinoma; AMC, absolute monocyte count; ANC, absolute neutrophil count; OS, overall survival; PFS, progression free survival; HR, hazard ratio.

statistical significance (OS: HR = 2.168, P = 0.333; PFS: HR = 1.806, P = 0.411) (Fig. 5E-F). Additionally, we identified several significant prognostic factors through both univariate and multivariate analyses to develop a nomogram model. The corresponding scores for each variable were quantified by integrating the characteristics of each patient, and the 3-, 5-, and 7-year survival probabilities were estimated based on the total score obtained by summing up these variables. (Fig. 5G).

3.5. Expression of signature genes associated with TAN and TAM in ccRCC

Circulating monocytes and neutrophils are intricately associated with tumor-associated macrophages (TAM) and tumor-associated

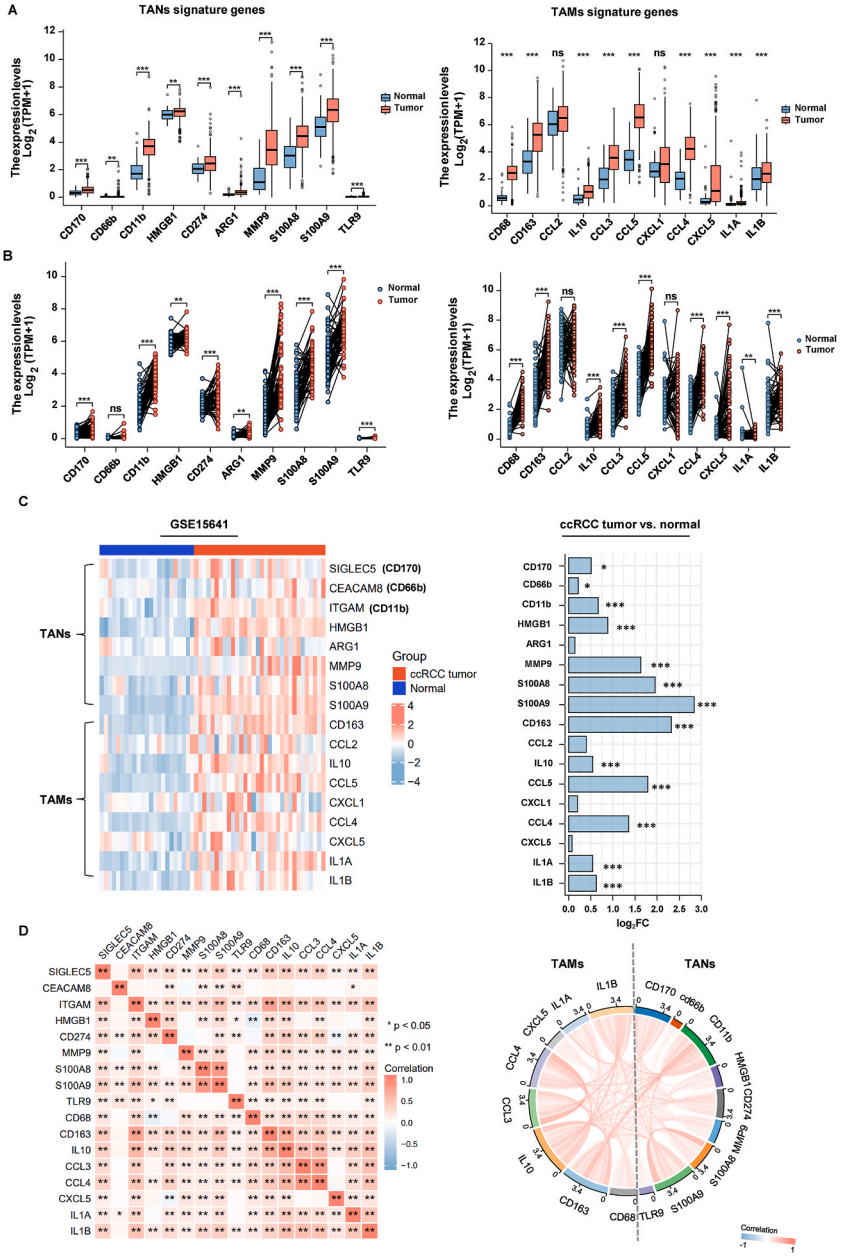


Fig. 6. TANs and TAMs signature gene expressions in ccRCC. (A) Expression of TANs (left panel) and TAMs (right panel) signature genes in normal and ccRCC tumor tissues based on TCGA database. (B) Expression of TANs (left panel) and TAMs (right panel) signature genes in tumor and adjacent normal tissue in ccRCC patients based on TCGA database. (C) Representative heatmap of TANs and TAMs signature genes using GSE15641 data (left panel). Bar graph showing the fold difference of all signature genes between ccRCC tumor and normal tissues (right panel). (D) Correlation analyses between the expression of TANs and TAMs signature gene expression in ccRCC based on TCGA database. TANs, tumor-associated neutrophils; TAMs, tumor-associated macrophages; ccRCC, clear cell renal cell carcinoma; *P < 0.05, **P < 0.01, ***P < 0.001.

neutrophils (TAN) within the tumor microenvironment; specifically, chemokines released by tumor foci serve to attract peripheral monocytes and neutrophils into TAN and TAM [22,23]. Hence, we endeavored to gain a deeper insight into the roles of AMC and ANC in ccRCC through the utilization of TAN and TAM. First, we conducted an analysis of the expression patterns of signature genes associated with TAN and TAM using data from the TCGA and GSE15641 databases. These signature genes include CD170, CD66b, CD11b, HMGB1, CD274, ARG1, MMP9, S100A8, S100A9, and TLR9 for TAN; and CD68, CD163, CCL2,IL10, CCL3, CCL5, CXCL1, CCL4, CXCL5, IL1-A and IL1-B for TAM [23,24]. Interestingly, the majority of these signature genes exhibited higher expression levels in tumor tissues compared to normal or adjacent tissues, as indicated by the expression profile data obtained from the TCGA database (Fig. 6A-B). Furthermore, in order to validate the differential expression of these genes between tumor and normal tissue, a heat map was generated to visually illustrate their expression differences across various tissues using the GES15641 dataset. It was observed that these signature genes were generally highly expressed in tumor tissues. The results of gene differential expression analysis were presented in a bar chart format based on the GSE15641 expression profile, from which we can see that the expression level of S100A9 had the highest fold change in ccRCC versus normal tissue (Fig. 6C). The study investigated the associations between S100A9 expression and other genes, encompassing both TAN and TAM signature genes. Our analysis unveiled a predominantly positive correlation with the majority of genes, except for TLR9. (Fig. 6D).

4. Discussion

In the current investigation, we have demonstrated that hematological parameters such as AMC, ANC, MLR, and NLR, particularly the former two, can serve as robust indicators for distinguishing RCC from RAML, a prevalent benign renal tumor. Furthermore, we have conducted an analysis of the prognostic role of AMC and ANC in predicting outcomes for RCC patients. The findings indicate that higher levels of AMC and ANC are associated with poorer prognosis in RCC patients.

Peripheral neutrophils and monocytes, commonly used parameters for patient evaluation, have been thoroughly investigated in hematologic malignancies and solid tumors with the exception of RCC [25]. Previous research has demonstrated a correlation between elevated peripheral monocyte and neutrophil counts and diminished overall survival in various malignancies, including gastric cancer, hepatocellular carcinoma, colorectal cancer, and prostate cancer [18,26–28]. As a result, the role of peripheral monocytes and neutrophils in predicting the prognosis of malignant events has been established. Consistent with previous studies, it was hypothesized that high levels of AMC and ANC are indicative of poor survival in RCC. However, this hypothesis was confirmed not by chance but through a retrospective cohort study with a large sample size. In terms of differentiating between RCC and RAML, Computed tomography(CT) is generally effective due to the presence of significant adipose tissue in most renal angiomyolipomas, resulting in low signal on CT images. Nevertheless, when distinguishing RCC from RAML without adipose tissue, there is a substantial decrease in imaging efficacy [29]. For example, in the majority of cases of epithelioid RAML, there is a paucity or absence of fat content, leading to potential confusion with classical renal cell carcinoma. Pathologically, classic renal cell carcinomas typically exhibit well-defined boundaries with fibrous pseudocapsules, whereas RAML lacks a capsule and often presents with indistinct boundaries, which can aid in its diagnosis [30]. Furthermore, several studies have suggested that the apparent diffusion coefficient (ADC) value in magnetic resonance imaging(MRI) demonstrates a strong capability to differentiate between benign and malignant renal lesions [31,32]. Nevertheless, to date, there have been almost no reports on the use of specific hematological indicators for distinguishing between the two diseases. Our study has provided the first confirmation of the distinct roles of monocyte and neutrophil counts in these two diseases.

Tumor-associated macrophages and neutrophils, integral components of the tumor microenvironment (TME), exhibit close correlation with circulating monocytes and neutrophils, which serve as the primary sources of TAM and TAN. The interaction between chemokine ligands expressed by tumor cells and chemokine receptors expressed by peripheral leukocytes facilitates the recruitment of monocytes and neutrophils to the tumor tissue. It has been documented that CCL2 binding to CCR2 in monocytes represents a pivotal mechanism for inducing monocyte trafficking, with inhibition of this interaction resulting in reduced enrichment [22,33]. After monocytes migrate to the tumor microenvironment and differentiate into tumor-associated macrophages, they can undergo polarization into two distinct phenotypes known as M1 and M2 macrophages. The M1 subtype is characterized by its ability to phagocytose and eliminate target cells, thereby exerting anti-tumor effects through the expression of nitric oxide synthase (iNOS), reactive oxygen species (ROS), and interleukin-12 (IL-12). In contrast, the M2 subtype is closely associated with heightened expression of interleukin-10 (IL-10), interleukin-1β (IL-1β), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs), thus promoting angiogenesis and tissue remodeling, ultimately contributing to tumorigenesis and disease progression [34]. However, despite TAM's dual role in tumors, extensive research has established a strong correlation between high TAM infiltration and an unfavorable prognosis [35–37].

Furthermore, the adverse effects of high TAM infiltration may be linked to the maintenance and self-renewal of tumor stem cells (TSC). Specifically, TAM release growth factors to support the survival and proliferation of TSCs, which in turn provide crucial signals for activating TAM [38]. The generation of TAN is analogous to that of TAM, both being influenced by the interaction between chemokine ligands and their respective receptors. Neutrophils exhibit elevated levels of the chemokine receptors CXCR1 (CXC motif chemokine receptor 1)and CXCR2, which interact with ligands expressed by tumor cells, tumor-infiltrating leukocytes, endothelial cells, and fibroblasts, including CXCL1 (CXC motif chemokine ligand 1), CXCL2, CXCL5, CXCL6, and CXCL8 [23]. With the exception of the CXC chemokine ligand family, CC chemokine ligands are known to play a crucial role in solid tumors. It has been reported that TANs expressing CCL2+ (C–C motif ligand 2)and CCL17+ positively correlate with tumor length, microvascular invasion, as well as the stratification of tumor differentiation and staging [39]. Interestingly, TAN can be further categorized into two distinct types: a pro-tumor state driven by transforming growth factor-β (TGFβ), and an antitumor state driven by interferon-β (IFNβ). It is noteworthy

that tumor-associated neutrophils not only play a role in the initiation and metastasis of primary tumors, but also release neutrophil extracellular traps (NETs) in distant tissues, which capture circulating cancer cells and facilitate their adhesion to endothelial cells, invasion, and proliferation at secondary sites [40].

Our current study collectively concludes that AMC and ANC can serve as reliable indicators for distinguishing RCC from RAML and predicting the prognosis of RCC based on a large dataset of patient data, with some insight into the underlying mechanisms. However, our study is subject to certain limitations. Firstly, as crucial inflammatory cells, the stability of AMC and ANC can be easily influenced by internal and external factors, thereby reducing their diagnostic utility. Secondly, despite our substantial sample size, there were still some patients lost to follow-up interviews. Additionally, we will investigate the potential therapeutic targets and pathways associated with these marker genes in clear cell renal carcinoma. This will entail conducting comprehensive studies on the molecular mechanisms underlying TAM and TAN, focusing on their interactions within the tumor microenvironment. Moreover, our objective is to examine the impact of these marker genes on disease progression, metastasis, and response to treatment in renal malignancies. Our ultimate aim is to contribute to the advancement of personalized medicine approaches for patients with renal clear cell carcinoma by identifying novel biomarkers and therapeutic strategies based on our research findings.

5. Conclusion

Elevated preoperative ANC and AMC levels are capable of distinguishing renal cell carcinoma from renal angiomyolipoma, and predicting a poor prognosis in patients with renal cell carcinoma.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

This study was reviewed and approved by the Medical Ethics Committee of Qilu Hospital of Shandong University [Approval number: KYLL-2021(KS)-1000]. All participants/patients provided informed consent to participate in the study.

CRediT authorship contribution statement

Jiajia Sun: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Qinzheng Chang:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Xiaoli He:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Shuo Zhao:** Writing – original draft, Methodology. **Nianzhao Zhang:** Writing – original draft, Data curation. **Yidong Fan:** Writing – review & editing, Writing – original draft. **Jikai Liu:** Writing – review & editing, Writing – original draft, Funding acquisition.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32360.

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