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Lymphopenia is an Independent Predictor of Inferior Outcome in Clear Cell Renal Carcinoma

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Abstract

Purpose—Low absolute lymphocyte count (ALC), a likely index of poor systemic immunity, may be associated with aggressive features and inferior survival in clear cell renal cell carcinoma (CCRCC).

Materials and Methods—We retrospectively analyzed preoperative blood cell counts in 430 patients (mean age 60 years) undergoing primary surgical resection for CCRCC at Fox Chase Cancer Center. ALC values as a continuous variable and at a level below 1300/μl (our lowest reference value) were correlated with nuclear grade, pathologic stage (pT), and (TNM) stage. We used Kaplan-Meier method to estimate the overall survival (OS) stratified by ALC status.

Results—As a continuous variable, low ALC was associated with higher grade (p=0.009), higher pT stage (p =0.034), and TNM stage (p<0.0001). Lymphopenia below 1300/μl was associated with high grade (p=0.0043), pT stage (p =0.051) and TNM stage (p<0.0001). After a median follow-up of 33.5 months, lymphopenia was associated with inferior OS in univariate model (p<0.0001), and independent of pT, N, and M stages, age, grade, smoking history and comorbidities in multivariable analysis (p=0.0102). Lymphopenia was also associated with inferior OS in a subset of young patients (≤60) with no distant metastasis (p=0.014).

Conclusions—In 430 CCRC patients lymphopenia was associated with lower OS independent of pT and TNM stages, nuclear grade, age, tobacco smoking, and comorbidity index.

Keywords

Renal cell carcinoma; lymphopenia; immunity; outcome

Introduction

Cancers of the kidney and renal pelvis occur in 58,000 cases annually in the United States.[1] Renal cell carcinoma (RCC) comprises majority of these tumors and has been subdivided into five distinct subtypes.[2] Clear cell RCC (CCRCC) is the most common subtype accounting for approximately 85% of cases.[3]

Tumor, nodes, metastasis (TNM) staging system is one of the most important prognostic factors for RCC.[4, 5] Multiple prognostic models and nomograms in localized RCC have been proposed which focus primarily on TNM stage, nuclear grade [6, 7] and performance status. Others have incorporated pathological variates such as tumor necrosis and microvascular invasion.[8, 9] In advanced RCC, other clinical factors have been shown to be associated with poor prognosis including low hemoglobin, high serum calcium, elevated

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serum lactate dehydrogenase, thrombocytosis, elevated neutrophil count, hypoalbuminemia and high C-reactive protein.[10–18] Many of these factors highlight a pro-inflammatory, immune dysfunctional state. Suppression of the immune system is typically associated with suppressed and/or modulated lymphogenesis. Indeed, a high pretreatment neutrophil-to-lymphocyte ratio was recently found to be an independent predictor of recurrence in patients with nonmetastatic RCC[19]. Conversely, lymphocytosis, as a measure of better systemic immunity has been associated with a better response to IL-2 immunotherapy.[20, 21]

Altered immunity in RCC has long been recognized in its pathogenesis. Moreover treatment of renal cancers has focused on immune modulation. Based on these observations, we hypothesized that alterations in peripheral blood lymphocyte counts might be associated with prognostic pathological variables and outcome in CCRCC. Whereas this test is easily available preoperatively, absolute lymphocyte count (ALC) could help clinically stratify risk preoperatively.

Here we present the analysis of a large uniform single institution series of CCRCC patients examining the relationship between preoperative ALC and tumor grade, stage, and overall survival (OS).

Materials and methods

We identified patients undergoing evaluation for CCRCC from 1994–2008 using our IRB approved prospectively maintained kidney cancer database. We included patients in whom an ALC was available within 3 months prior to surgery. Most nephrectomy specimens were examined and graded by a single uro-oncologic pathologist (TAS). For RCC classification, hematoxylin eosin slides were classified using the WHO classification.[2] Immunohistochemical stains and cytogenetics were used as adjuncts as deemed necessary. Standard nuclear grading for clear cell carcinomas was basically according to the Fuhrman classification as adopted by the WHO[2]. Cases with any sarcomatoid component are considered grade IV. [2, 6, 7]

TNM staging is a collaborative of pathological and clinical findings from the patients' records, tumor registry and the kidney cancer database. Charlson comorbidity index (CCI) and self reported history of smoking were obtained from the kidney cancer database. Blood counts and follow-up were also obtained from the same sources. Collaborative (pathological plus clinical) staging was revised according to the 7th Edition of the AJCC cancer staging manual.[22] Pathological pT staging was available in all 430 patients. N staging is a mixture of pathological staging in patients with radical nephrectomy and lymph node dissection (about 36%) and clinical in those with nephrectomy but no node dissection (about 24%) or nephron sparing surgery (about 40%). M staging is mainly clinical based on imaging.

Normal values for blood counts are established in our own laboratory from age-matched controls in our tertiary adult cancer center. Our normal ALC is 1300–4000/μl. Values below 1300/μl are considered as lymphopenia. Highest normal myeloid (non-lymphoid) cell count in our own lab, is 5800/μl.

We examined ALC as a continuous variable using one-way analysis of variance (ANOVA) to assess differences in mean ALC by grade and by TNM stage. We tested for linear trend using a regression model with the assumption of equal spacing between levels of grade. We also looked at ALC as a categorical variable, using ALC ≤1300 cells/μl to identify low ALC. Differences in stage and grade by low ALC were assessed using Fisher's exact test (FE) and trends were assessed using the Cochran-Armitage trend test.

We looked at OS and preoperative low ALC status (< 1300 cells /μl) using the Kaplan-Meier product-limit method to estimate the survival functions for overall survival by low ALC status. We censored patients who were alive at their last available follow-up date. Differences in the curves were assessed using the log rank test. We used Cox proportional hazards regression for inferences about the relationship of survival time with low ALC adjusting for age (≤60 yrs vs 60+ years at diagnosis), pathologic Tstage (pT_1p/T_2, pT3/pT4), Nstage (N0, N1, NX), Mstage (M0, M1), grade (I–II, III–IV), Charlson comorbidity index (0,1–2,3+) and smoking history as covariates. All analyses were conducted using SAS statistical software, and Kaplan Meier plots were generated using R, version 2.5.1.

Results

Patient characteristics

There were 430 patients who met inclusion criteria for this study. Twenty exclusions were due primarily to non-availability of ALC within 3 months of surgery. One patient with ALC above 5000/μl was excluded due to the possible diagnosis of chronic lymphocytic leukemia. Most patients had ALC within two weeks of surgery (mean 11 median 9 days). There were 296 males and 134 females. Median age was 61 years, mean 60.2 years (range 25–89). Follow-up was available for all 430 patients. Median follow up from surgery to death or last follow-up was 33.5 months (range 0.1–181 months). As of last follow-up, 100 patients had died.

Correlation of ALC and some of the most important known prognostic factors

ALC as a continuous variable revealed strong correlation with all pathologic prognostic factors analyzed. Low ALC was associated with higher grade (p= 0.009 ANOVA, 0.013 trend), higher T-stage (p=0.034 ANOVA, 0.0057 trend), presence of lymph node metastasis (p=0.032), presence of distant metastases (p <0.0001) and higher TNM stage (p <0.0001 ANOVA, <0.0001 trend). (Table 1)

ALC as a categorical variable also revealed equally strong correlations. Lower than normal ALC (<1300/μl) was associated with higher grade (p=0.0043 FE test. p=0.0029 trend), higher pT-stage (p=0.051 FE and 0.0099 trend), presence of distant metastasis (<0.0001) and higher TNM stage (p <0.0001, <0.0001 trend). Correlation with lymph-node metastases was not significant (p=0.18). Of the host-related factors, lymphopenia was also associated with age 60+ (p=0.009), high CCI (3–9), (p<0.0001) but not with smoking history (p=0.10) (Table 1)

ALC and overall survival

The median follow-up for overall survival of our cohort was 33.5 months. ALC distribution in our patients is demonstrated in fig.1a. Lymphopenia was significantly associated with worse survival (log rank p<0.0001). (Figure 1b) And by univariate analysis for Cox model with only lymphopenia vs no lymphopenia: (HR-2.27, 95% CI=1.52 to 3.38, p<0.0001). An estimate of OS at specific time points was also performed revealing worse survival for patients with low ALC at 3 years after diagnosis. 3 year estimate 68% vs 87%, (p=0.0002) (Table 2). Multivariable analysis of overall survival by ALC, pT, N and M stages, grade, age below or above 60 years and CCI revealed ALC to be an independent prognostic factor (p=0.0102). As a linear variable. ALC showed significant effect on OS (p=0.002), and 0.038 in the above multivariable model. (Table 3)

We also tested the association of low ALC with OS in localized and locally advanced disease (no distant metastasis) at the time of surgery (stages I–III). There was a trend of inferior OS in the whole cohort approaching statistical significance (p=0.11), (fig 2a),

however in the patients 60 years or younger (our cohort's median age), there was significantly inferior survival with lymphopenia (p=0.014), figure 2b. Younger patients with localized/locally advanced disease and with lymphopenia had inferior estimated survival 36 and 48 months compared to those with normal ALC (92 versus 98 and 85 versus 95 months respectively).

Since patients undergoing cytoreductive nephrectomy are substantially distinct from those undergoing nephrectomy for curative intent, Stage IV only (73 patients) were analyzed separately in the multivariable model with ALC. In this group, lymphopenia was also associated with inferior OS (p=0.045), hazard ratio 1.85 (95%CI 1.01 – 3.36).

High versus normal myeloid cell count did not show a significant association with overall survival (p= 0.41), (fig 2c). Myeloid to ALC ratio (M/L) was significant when analyzed for splits at approximate quartiles (p= 0.0008) as well as for split at approximate median of M/L 3.38 or L/M ratio of 0.3, (p=0.0072) (Figure 2d).

Because many host-related factors, in addition to age, may influence ALC or OS, we examined our database for other possible important confounding factors, Smoking history was available in 377 patients, 38% are non-smokers and 62% either current or ex-smokers. Current and ex-smokers had significantly higher ALC than non-smokers regardless of stage (Table 1). However, smoking is not a significant predictor of OS in univariate model (p=0.10) or in the multivariable which includes smoking history (any), or never smoked (p=0.74). High CCI (3–9) correlated with inferior survival in the univariate model (p=0.02) but not in the multivariable analysis (p=0.33) (Table 3)

Co-morbidities that may cause lymphopenia or affect OS were also examined. Hypothyroidism (30) or chronic kidney disease (23 including 8 uremic) did not have a statistically significant lower ALC than the rest probably due to small numbers. There were only few patients with sarcoidosis (2), lupus (2), and rheumatoid arthritis (3). There were 82 diabetics (out of 430 patients with information), but had no effect of survival either (p=0.25).

We also looked for medicines that may affect ALC. Other than various non-steroidal anti-inflammatory drugs and steroids (12 patients), few patients only used, possible immune modulatory drugs including methotrexate (1 patient), Prograf (1), Sutent (1) and Sorafenib (1).

Discussion

The incidence of RCC is on the rise. There is an ever increasing detection of small renal masses in patients with competing comorbities resulting in a great need for preoperative prognostication. Additionally, with the advent of newer, more effective systemic agents, there is need to identify patients with early stage disease who are at risk for poor outcome and hence may benefit form additional therapy.

The anatomic extent of disease identified in the TNM staging system remains one of the most important prognostic variables for RCC patients.[4, 5] The prognostic value of the Fuhrman grading system for clear cell tumors has been shown in a number of studies.[23, 24] Younger patients are more likely to have lower stage and grade tumors and higher cancer-specific survival than older patients.[25, 26] Although, histological subtypes of RCC have often been studied together, with improved understanding of their molecular and genetic profiles and potentially different therapeutic targets, there is need to identify prognostic markers specific to individual subtypes. To our knowledge, there are no studies to identify risk

factors in RCC subtypes separately. In this study, we have demonstrated, again, the prognostic significance of grade, stage, and age in OS in CCRCC.

Several different hematological indices have shown prognostic significance in patients with RCC. Thrombocytosis, for example, has been shown to be an indicator of poor prognosis in both advanced as well as localized RCC.[27] C-reactive protein (CRP), an acute phase reactant, has been found to be prognostic in both early and advanced disease.[28, 29] These, as well as a number of other laboratory variables noted previously represent a pro-inflammatory and immune dysfunctional state. Immune dysfunction or suppression may be expected to result in low peripheral blood lymphocyte counts.

Here we demonstrate that preoperative ALC may be an important, previously unknown independent prognosticator of clinical outcomes including survival in patients with CCRCC. Lymphopenia (defined in our Institution as ALC <1300/μl) is associated with higher grade/stage tumors and inferior OS independent of the stage, grade, age, smoking and CCI. We limited our analysis to CCRCC because it represents a majority of kidney cancers and the immunological features of papillary and chromophobe RCC are less well known.

Despite mounting evidence for anti-tumor immunity to RCCs[30] and use of peripheral blood lymphocyte count as a marker of response to immunotherapy in advanced RCC, to our knowledge, preoperative ALC has not been studied as a prognostic marker for tumor behavior and OS. This test is readily available in the form of a complete blood count, routinely requested by most urologists. Other prognostic factors such as pathologic stage and histologic grade are not available until after the surgical procedure. Based on our results, preoperative ALC may be used to select patients with high risk disease and test intensifying therapy as well as developing novel agents to improve patient outcome. Obviously, this is most important in younger patients and those with non-metastatic disease at diagnosis, a subset who seem to have an inferior OS if they were lymphopenic preoperatively. Again, we highlight the role of anti-tumor immunity and need for developing therapies targeted at enhancing this protective mechanism.

Neutrophil to lymphocyte ratio has been tested as a prognostic factor in various tumor types including RCC.[19] A high ratio reflective of pro-inflammatory and/or immune suppressed state is generally associated with worse outcome. Since other myeloid cells such as monocytes and eosinophils are also part of the inflammatory response, we used a myeloid cell count comprising neutrophils, monocytes and eosinophils to more accurately identify the ratio of peripheral blood inflammatory and immune cells. As expected, a higher ratio was found to be associated with worse OS. The myeloid cell count by itself was, however, not significant. Interestingly in the study neutrophil count was not associated with increased recurrence in univariate analysis. On the other hand their best fit value of 1700 ALC/ μl was. We selected a more specific value of 1300/ μl for our analysis to test for lymphopenia as we define it in our institution. We also found that a myeloid/lymphoid (M/L) ratio of about 3.4 (our median) or L/M of 0.3 is also associated with a poor OS survival. These findings may reflect a stronger impact of lymphocyte count in the neutrophil to lymphocyte or myeloid to lymphocyte ratio. We feel strongly that specific values, rather than ratios of two can be better utilized in this clinical setting.

In conclusion, our study shows that low preoperative peripheral ALC is associated with higher pathologic grade, higher T-stage, higher TNM stage, presence of metastasis and inferior OS in patients with CC RCC. Association with OS is not confounded by comorbidities or by cigarette smoking. Additional studies are needed to corroborate this finding and test it in other histologic subtypes of RCC. If the results are confirmed, this easily available test may be incorporated in future prognostic models for RCC. We believe

that the quantification of specific immune cells in peripheral blood and their association with other prognostic factors in clear cell and other types of RCC is an important avenue for future investigation. Such knowledge may hopefully enhance the development of specific immune modulatory approaches to RCC management.

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Abbreviations

ALC	absolute lymphocyte count
CCRCC	clear cell renal cell carcinoma
RCC	renal cell carcinoma
TNM	tumor, nodes, metastasisk
OS	overall survival
CCI	Charlson comorbidity index
FE	Fisher's exact test

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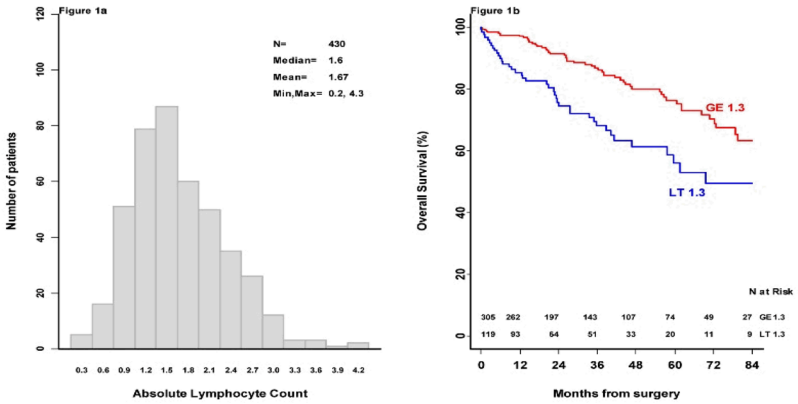

Figure 1.

Figure 1a) Distribution of ALC, n=430

Figure 1b) Overall survival by ALC, <1.3 vs ≥ 1.3, n=424, p<0.0001

LT: Less than

GE: greater/equal

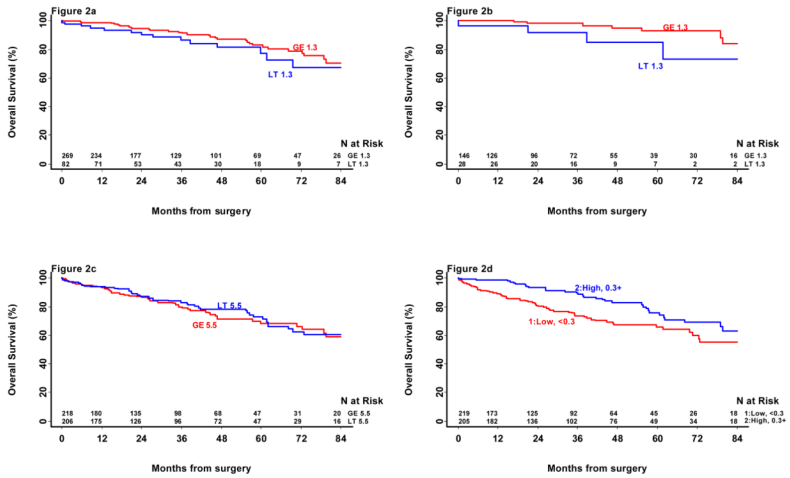

Figure 2.

Figure 2a) Overall survival by ALC, <1.3 vs ≥1.3, Stages 1–3, n=351, p=0.12

Figure 2b) Overall survival by ALC, <1.3 vs ≥1.3, Stages 1–3 and age ≤ 60,n=174, p = 0.014

Figure 2c) Overall survival by myeloid count, <5.8 vs > 5.8, n=424, p=0.41

Figure 2d) Overall survival by ratio of ALC to myeloid count, <0.3 vs ≥0.3, n=424, p = 0.003

Notes:

p-values are for log rank test

0.3 is median for all those with ALC and myeloid determined

Table 1

ALC and patient characteristics

	n (percent)	Absolute Lymphoctye Count (ALC) µl				ALC < 1.3 µl		
		Mean	Median	Range	p-value[1]	n	Percent	p-value[2]
All	430	1.67	1.6	0.2 – 4.3		119	27.7	
Grade	**(excludes n=6 ND)**				0.009			0.0043
I	51 (12.0)	1.66	1.6	0.3 – 2.9	*0.013*	13	25.5	*0.0029*
II	199 (46.9)	1.78	1.7	0.3 – 3.9		40	20.1	
III	131 (30.9)	1.62	1.5	0.2 – 4.3		45	34.4	
IV	43 (10.1)	1.44	1.3	0.6 – 3.2		18	41.9	
Pathological T stage					0.034			0.051
pT1	293 (68.1)	1.74	1.6	0.3 – 3.9	*0.0057*	70	23.9	*0.0099*
pT2	50 (11.6)	1.56	1.5	0.2 – 2.9		17	34.0	
pT3	83 (19.3)	1.55	1.5	0.2 – 4.3		30	36.1	
pT4	4 (0.9)	1.23	1.2	0.7 – 1.8		2	50.0	
N stage	**(excludes n=11 NX)**				0.032			0.18
N0	387 (92.4)	1.70	1.6	0.2 – 4.3		103	26.6	
N1	32 (7.6)	1.44	1.5	0.2 – 2.6		12	37.5	
M stage	**(excludes n=3 MX)**				<0.0001			<0.0001
M0	356 (83.4)	1.74	1.6	0.3 – 4.3		83	23.3	
M1	71 (16.6)	1.34	1.3	0.2 – 4.2		36	50.7	
TNM stage	**(excludes n=6 MX or NX/M0)**				<0.0001			<0.0001
I	277 (65.3)	1.76	1.7	0.3 – 3.9	*<0.0001*	62	22.4	*<0.0001*
II	36 (8.5)	1.63	1.5	0.9 – 2.9		11	30.6	
III	38 (8.9)	1.71	1.55	0.7 – 4.3		9	23.7	
IV	73 (17.2)	1.34	1.2	0.2 – 4.2		37	50.7	
Age at surgery								
<60 yr	200 (46.5)	1.74	1.7	0.3 – 3.9	0.0042	43	21.5	0.0094
60+ yrs	230 (53.5)	1.60	1.5	0.2 – 4.3		76	33.0	
CCI					<0.0001			<0.0001
0	180 (41.9)	1.77	1.7	0.6 – 3.9	*<0.0001*	34	18.9	*<0.0001*

	n (percent)	Absolute Lymphoctye Count (ALC) μl				ALC < 1.3 μl		
		Mean	Median	Range	p-value[1]	n	Percent	p-value[2]
1–2	149 (34.7)	1.73	1.6	0.2 – 4.3		37	24.8	
3–9	101 (23.5)	1.42	1.3	0.2 – 3.0		48	47.5	
Smoking Hx	(excludes n=53 missing smoking history)				0.0061			0.10
No	142 (37.7)	1.55	1.4	0.2–3.0		48	33.8	
Yes	235 (62.3)	1.74	1.6	0.3–4.3		60	25.5	

ND: Not done, NX: N stage unknown, MX:M stage unknown, CCI: Charlson Comorbidity Index

[1] p-value for 1-way ANOVA or t-test, additional p-values for linear trend are italicized

[2] p-value for Fisher's exact tests; additional p-values for Cochran-Armitage trend tests are italicized

Table 2

Overall survival estimates at 36 months post surgery by TNM stage

	ALC < 1.3/μI		ALC ≥ 1.3/μI	
	N	**36 mo survival (95% CI)**	**N**	**36 mo survival (95% CI)**
All	119	68.1 (57.6–76.4)	305	86.7 (81.5 –90.5)
Stage I–III	82	86.5 (75.3–92.8)	269	91.9 (87.0–95.0)
Stage IV	37	29.5 (14.5–46.2)	36	52.6 (33.7–68.5)
Stage I–III, age ≤ 60	28	92.0 (71.5–98.0)	146	98.0 (92.3–99.5)

Table 3

Results of Cox proportional hazards regression for time from surgery to all causes mortality, n=418 patients (6 without stage (i.e. MX or NX/M0) and 6 without grade (i.e. Grade=ND) excluded from analysis).

Parameter	Comparison	Univariate models			Multivariable model, ALC <1.3 vs >=1.3		
		Hazard Ratio (HR)	95% HR Confidence Limits	p-value	HR	95% HR Confidence Limits	p-value
ALC	**<1.3 vs >= 1.3**	**2.27**	**1.52–3.38**	**<0.0001**	**1.75**	**1.14–2.67**	**0.0102**
pTstage	T3/T4 vs T1/T2	6.53	4.39–9.72	<0.0001	2.96	1.75–5.00	<.0001
Nstage	N1 vs N0	7.20	4.43–11.71	<0.0001	1.62	0.88–3.01	0.12
	NX vs N0	9.16	3.92–21.4	<0.0001	4.06	1.58–10.43	0.0037
Mstage	M1 vs M0	7.57	5.02–11.41	<0.0001	2.60	1.51–4.50	0.0006
Grade	III–IV vs I–II	3.08	2.05–4.65	<0.0001	1.65	1.04–2.60	0.033
Age	60+ vs< 60 yr	1.90	1.26–2.88	0.0024	1.56	0.98–2.47	0.061
CCI	1–2 vs 0	1.39	0.87–2.23	0.17	1.02	0.61–1.70	0.95
	3–9 vs 0	1.79	1.09–2.95	0.022	1.31	0.76–2.24	0.33
Smk HX	Yes vs No	1.05	0.67–1.64	0.85	0.92	0.58–1.47	0.74
	Unk vs No	0.77	0.41–1.44	0.40	0.80	0.41–1.55	0.50