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NEUTROPHIL TO LYMPHOCYTE RATIO AS A PROGNOSTIC MARKER FOR NON-METASTATIC RENAL CELL CARCINOMA — DOES IT ADD TO WHAT WE ALREADY KNOW?

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***Aim*: To study the correlation of pre-operative neutrophil to lymphocyte ratio (NLR) with pathological stage, Fuhrman grade, sarcomatoid differentiation, tumor necrosis and lymph node positivity and its prognostic role in non-metastatic renal cell carcinoma (non-mRCC). *Materials and Methods*: This retro-prospective, observational study was done at a tertiary care center in Mumbai, India. All patients with non-mRCC from July 2015 to April 2018 were included. Patients with co-existing systemic infection, prior immunotherapy, and long-term steroids were excluded. NLR closest to surgery, but within one month prior to surgery was used. Patients were stratified as NLR $\geqslant 3.0$ or < 3. NLR was correlated with known prognostic factors by Pearson's correlation. *Results*: 113 patients, aged 18–81 years (83 males and 30 females) were included. 75% had clear cell RCC. 62% had stage 1 disease. 58% patients had Fuhrman Grade 2. 10 patients had lymph node metastasis, 6 had sarcomatoid differentiation, 40 had tumor necrosis. The NLR was < 3 in 72 patients. Statistically significant correlation between NLR and tumor stage ($p = 0.0054$) as well as NLR and tumor necrosis ($p = 0.0128$) was shown. *Conclusions*: NLR correlates significantly with higher T stage and tumor necrosis. NLR may be integrated with well-established prognostic markers to improve the accuracy of prognostic scores.**

***Key Words*: neutrophil to lymphocyte ratio, prognostic factors, renal cell carcinoma.**

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Renal cell carcinoma (RCC) is the most common renal malignancy in adults and leads to a mortality of over 100,000 patients per year worldwide [1]. In the USA, RCC accounts for 2.3% of all cancer deaths [1]. The RCC incidence in Asian population is between 1.1 to 6.0/100,000 [2]. The worldwide incidence of RCC has slightly increased in the last three decades. However, because of the widespread use of radiological imaging techniques, a tendency towards diagnosing small and organ-confined tumors has been observed [3].

It is essential to optimize decision-making in the treatment of RCC to achieve a better prognosis. Until now, many tumor characteristics and a few patient characteristics have been suggested as possible prognostic factors. However, only a few, including pathological stage and Fuhrman grade, are undisputed prognostic factors for RCC, especially non-metastatic RCC (non-mRCC) [4]. The use of prognostic factors and models that can accurately predict clinical outcomes of RCC patients are of paramount interest, not only for patients' individualized risk assessment and optimized treatment but also for the comparison of the results from international clinical multicenter trials. Several prognostic models have been established to predict a patient's clinical outcome. The University of California Integrated Staging System incorporated performance status, pTNM stage and Fuhrman grade to predict overall survival in RCC patients [5]. Leibovich prognosis score integrates five clinicopathological features translated into a score and categorizes patients into eight-score categories, which are then assigned to one of three different risk groups [6].

The systemic inflammatory response, which is usually measured by surrogate blood-based parameters, such as erythrocyte sedimentation rate, C-reactive protein, neutrophil count, lymphocyte count and neutrophil to lymphocyte ratio (NLR) has been shown to independently predict the clinical outcome of various human cancer types. Of these inflammatory parameters, an increased NLR has been proposed as an easily accessible and reliable marker to predict cancer patients' survival [7].

Neutrophils represent the inflammatory response, whereas lymphocytes reflect cell-mediated immunity. It has been hypothesized that the synthesis of inflammatory cytokines triggered by the tumor microenvironment alters acute phase reactants and hematological components including serum neutrophil and leukocyte counts. Lymphocytosis has been observed to be predictive of oncologic outcomes in patients treated for advanced RCC [8].

The association between a host inflammatory response and cancer-specific outcomes remains complex. An elevated NLR reflects both a heightened neutrophil-dependent inflammatory reaction and a decreased lymphocyte-mediated antitumor immune response. Both of these factors may be related to aggressive tumor biology and may contribute to cancer progression, and poor prog-

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Abbreviations used: IL – interleukin; NLR – neutrophil to lymphocyte ratio; non-mRCC – non-metastatic renal cell carcinoma; RCC – renal cell carcinoma.

nosis. Circulating neutrophils have been shown to produce cytokines, such as tumor necrosis factor, interleukins (IL-1 and IL-6), and to secrete the proangiogenic factor vascular endothelial growth factor [9]. A relative lymphocytopenia may reflect a lower count of CD4 T-helper lymphocytes, resulting in a suboptimal lymphocyte-mediated immune response to malignancy. Thus, NLR may reflect the combined prognostic information of these two processes and be a stronger predictor of the outcome than either of them considered alone [8].

Clear cell RCC is currently one of the few malignancies in which immunotherapy has been employed (high-dose IL-2), in some cases, with remarkable responses. In fact, both lymphocytosis and C-reactive protein have been observed to be predictive of oncologic outcomes in patients treated for advanced RCC [8].

Cumulative evidence in metastatic RCC suggests that a high NLR might represent an independent adverse prognostic factor in interferon-treated, interleukin-2-treated, as well as in sunitinib-treated patients. However, data regarding the prognostic significance of the NLR in non-mRCC are sparse, and controversy still exists about how significant is NLR as an independent risk factor in non-mRCC.

We aimed to study the correlation of preoperative NLR with histopathological stage, Fuhrman nuclear grade, sarcomatoid differentiation, tumor necrosis and lymph node positivity and to evaluate whether this parameter provides additional prognostic information to already well-established clinicopathological parameters for non-mRCC.

MATERIALS AND METHODS

This study was done at a tertiary care center in Mumbai, India. The study was approved by the institutional review board. Written consent was obtained from all the participants.

This was a retro-prospective, observational study. All the patients with non-mRCC who were operated at our hospital from July 2015 to April 2018 were included in the study. Patients with coexisting systemic infection, who had received prior immunotherapy, and those on long-term steroids were excluded from the study.

The demographic details, NLR, type of surgery undertaken, the histopathology report and hospital stay data were collected from the medical records. The histopathology report included the type of tumor, Fuhrman nuclear grade, sarcomatoid differentiation, stage of tumor and lymph node status. Neutrophil and lymphocyte count closest to surgery, but within 1 month before the surgery were used for analysis. When multiple values existed for a patient, the value closest to the date of the surgery was utilized. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Patients were also stratified according to NLR $\geqslant 3.0$ or < 3.0 The ideal cut-off value for the continuous NLR was calculated by testing all possible cut-offs that would discriminate between survival and cancer related deaths by Cox proportional analyses. The ideal cut-off value was then rounded to clinically relevant value. Regression tree analysis for censored data was also used to find the best NLR cut-off value. The best cut-off value was found to be < 3 and $\geqslant 3$.

TNM 2009 staging system was used to stage the tumors. Fuhrman nuclear grading was used to grade the tumors.

Data recording was done using MS Excel. Descriptive statistics for quantitative variables (age) are represented as mean ± standard deviation. Qualitative variables (sex, presenting complaints — symptomatic/asymptomatic, operative procedure — radical nephrectomy/partial nephrectomy) are represented as frequency & percentages. The NLR is compared to T stage, Fuhrman nuclear grade, lymph node status, sarcomatoid differentiation and presence of necrosis by Pearson's correlation. *P*-value of < 0.05 was considered significant. Graphical representations are done wherever applicable. The Software used for analysis is MedCalc.

RESULTS AND DISCUSSION

A total of 116 patients were operated on during the study period. However, out of these, three were excluded as they were on long-term steroids. Thus, 113 patients were enrolled into analysis.

The age distribution is shown in Table 1.The youngest patient in our study was 18 years old and the oldest was 81 years old, the mean age (± standard deviation) was 55.12 ± 12.60 years.

Table 1. Age distribution of patients

Age group (years)	Number of patients (%)
$\leqslant 20$	1 (0.88)
21–40	17 (15.04)
41–60	59 (52.21)
> 60	36 (31.86)

In our study, 83 of the 113 patients were males and 30 were females. The right kidney was affected in 58 patients and the left side in 55 patients. 78 of the 113 patients underwent radical nephrectomy and 35 patients underwent partial nephrectomy. 59 of 113 patients studied were asymptomatic, which means they were incidentally diagnosed to have a renal tumor. 54 patients were symptomatic, abdominal pain was the most common presenting complaint followed by hematuria. Other uncommon presentations were anorexia, loss of weight, pedal edema, abdominal lump, and weakness.

The absolute neutrophil count ranged between 2100–11 300 cells per mm^3, with a mean of 6037.79 ± 1544.67. The absolute lymphocyte count ranged between 600–4400 cells per mm^3, with a mean of 2403.84 ± 801.10. The NLR was < 3.0 in 72 patients and $\geqslant 3.0$ in 41 patients.

The histopathological type of tumors is shown in Table 2.

Table 2. Type of tumor

Type of tumor	Number of patients (%)
Clear cell RCC	84 (74.34)
Papillary RCC	16 (14.16)
Chromophobe RCC	7 (6.19)
Mixed types	3 (2.65)
Multilocular clear cell RCC	1 (0.88)
Carcinomas with Xp11 translocation	1 (0.88)
Collecting duct or Bellini's carcinoma	1 (0.88)

Three patients were found to have a mixed type of tumors, which showed features of two or more different types of carcinomas. Out of these three, one patient had features of carcinoma of collecting ducts and papillary, one patient had a hybrid tumor showing features of chromophobe RCC and oncocytoma and the third patient had mixed clear cell and papillary RCC features.

Stage distribution and Fuhrman grading of the tumors is shown in Table 3. The majority of the patients (62%) had an earlier stage at presentation. Only 10 patients of the 113 studied had lymph node metastasis on final histopathology. Sarcomatoid differentiation was found in 6 patients. Tumor necrosis was found in 40 patients, i.e. 35.4% of patients.

Table 3. Tumor stage and grade

Tumor stage	Number of patients (%)
1a	42 (37.17)
1b	28 (24.78)
2a	10 (8.85)
2b	5(4.42)
3a	17 (15.04)
3b	5 (4.42)
3c	2 (1.77)
4	4 (3.54)
Fuhrman grade	
1	13 (11.50)
2	66 (58.41)
3	29 (25.66)
4	2 (1.77)
NA	3 (2.65)

The NLR was correlated to various parameters using Pearson's correlation. NLR had a statistically significant correlation with tumor stage (T Stage) and tumor necrosis. NLR had a weakly positive correlation with Fuhrman grade, lymph node metastasis, and sarcomatoid differentiation, which did not reach statistical significance. The correlation of NLR with various parameters is shown in Table 4.

Table 4. Correlation of NLR with various parameters

Parameters	r	*p*-value
Tumor stage	0.2598	0.0054
Fuhrman grade	0.0181	0.8491
Lymph node	0.147	0.1202
Sarcomatoid differentiation	0.0527	0.5793
Tumor necrosis	0.2336	0.0128

Notes: calculated using Pearson's correlation; $p < 0.05$ considered significant; r – correlation coefficient wherein r = 1 means a perfect positive correlation and r = –1 means a perfect negative correlation.

Prognostic factors are important for any malignancy. They help in clinical decision-making and in pre-treatment counselling of the patients. Various decisions like whether to operate or not, whether to give neoadjuvant or adjuvant chemotherapy, whether to treat with curative or palliative intent depend upon the prognosis of the disease. There are already well-established clinical, pathological and molecular prognostic indicators in RCC. However, as science advances, new factors are being studied and added to the existing parameters. NLR is an easily measurable, simple to use, reliable and reproducible factor.

In our study, we have correlated the preoperative NLR with already established prognostic markers like T stage, Fuhrman nuclear grade, tumor necrosis, sarcomatoid differentiation, and lymph node metastasis. We briefly review our findings in comparison with other published series in the world.

The peak incidence of RCC is between 50 and 70 years of age [1]. However, diagnosis of renal cancer has increased more rapidly in those less than 40 years of age than any other age group. In accordance with these statistics, in our study too, the majority of the patients were between 40–60 years of age i.e. 52.21%, the next common age group was > 60 years accounting for 31.86% of the study population.

Siegel *et al.* [1] reported a male-to-female predominance of 3:2. In the 192 patients of non-mRCC studied by Ohno *et al.* [10], 149 were men and 43 were females. In our study, 83 of the 113 patients were males and 30 were females i.e. 73.45% were males.

In the study by Ohno *et al.* [10], 349 patients of non-mRCC were retrospectively reviewed, the majority of the patients were asymptomatic, only 16% of the patients had some symptoms. Viers *et al*. [11] studied 827 patients who underwent radical nephrectomy for RCC, out of these 518 i.e. 63% were symptomatic. In the study conducted by Otunctemur *et al*. [12] only 84 of 432 patients were symptomatic. These studies, however, did not mention the different presenting symptoms; all those patients that presented with abdominal pain, lump in abdomen, hematuria or fever were considered symptomatic. In our study, 59 (52.2%) of the 113 patients were asymptomatic. Abdominal pain was the most common presenting symptom, followed by hematuria. Other less common symptoms were anorexia, weight loss and a lump in the abdomen.

The median absolute neutrophil count was 5200 in the study [13]. The mean absolute neutrophil count was 4210 in [10], 4230 in [12], and in our study 6037. The median absolute lymphocyte count was 1700 in the study [13]. The mean absolute lymphocyte count was 1840 in [10], 1610 in [12] and in our study 2403. The NLR cut-off used for computing the relationship between high NLR and various parameters varied among different studies — 3.3 in [3], 2.7 in [10], 1.7 in [11, 14], while we used the NLR cut-off value of 3.0. The mean NLR was 2.44 in [10], 2.2 in [15], 3.5 in [3].

Vier *et al*. [11] retrospectively studied 827 patients of localized clear cell RCC using an NLR

cut-off value of ⩾ 4 or < 4. Pathologically, patients with an NLR ⩾ 4.0 had pathologically advanced (pT3/4) tumors, level I to IV tumor thrombosis, greater nuclear grade, and coagulative tumor necrosis or sarcomatoid differentiation. However, higher NLR was not associated with lymph node positivity. Pichler *et al.* [3] retrospectively studied 678 patients of non-metastatic clear cell RCC using an NLR cut-off value of 3.3. High NLR correlated significantly with higher tumor stage and higher Fuhrman nuclear grade, but NLR did not correlate significantly with tumor necrosis or lymph node positivity. Otunctemur *et al*. [12] retrospectively studied all subtypes of non-mRCC (432 patients) using NLR cut-off value of 3.01. They found a significant correlation of NLR with T stage and higher Fuhrman nuclear grade. De Martino *et al.* [16] prospectively studied 281 patients with non-clear cell RCC, mostly of papillary and chromophobe subtype, using NLR cut-off value of 3.6. They found a statistically significant correlation of NLR with positive lymph nodes but no correlation with T stage or Fuhrman nuclear grade. We studied 113 patients of all RCC subtypes using an NLR cut-off value of 3 to study the correlation of NLR with various parameters. 72 (63.72%) of the patients had NLR < 3 and 41 (36.28%) patients had NLR ⩾ 3. NLR correlated significantly with a higher T stage ($p = 0.054$) and presence of tumor necrosis ($p = 0.0128$), but showed no correlation with Fuhrman grade, lymph node positivity, and sarcomatoid differentiation.

Byun *et al.* [15] studied the prognostic role of NLR in the three major subtypes of RCC, i.e. clear cell, papillary and chromophobe RCC using NLR cut-off 3.7 High NLR correlated significantly with a higher T stage, presence of sarcomatoid differentiation and presence of tumor necrosis, but not with Fuhrman nuclear grade.

Our study did have some limitations. Being a retro-prospective non-randomized study, limitations are inherent to the study design. The study includes data from a single tertiary referral institute, therefore external validation is required. The sample size is relatively small and cannot be representative of the entire population. Different pathologists of the same institute evaluated the specimen, no central pathology review was used. Multivariate analysis was not done because of the small sample size.

Nevertheless, we managed to show that high NLR correlates significantly with higher T stage and tumor necrosis. NLR may help in pre-treatment patient risk stratification and patient counseling. NLR may be integrated into the already well-established prognostic markers and staging systems, improving their accuracy.

CONFLICT OF INTEREST

None of the contributing authors have any conflicts of interest, including specific financial interests, or relationship and affiliation relevant to the subject matter or materials discussed in the manuscript.

REFERENCES

1. **Siegel R, Naishadham D, Jemal A.** Cancer statistics, 2013. CA Cancer J Clin 2013; **63**: 11–30. doi: 10.3322/caac.21166.

2. Singapore Cancer Registry report no. 7 2017; https://www.nrdo.gov.sg/docs/librariesprovider3/ Publications-Cancer/inc_report_v745ce06a5c9d76bafab5aff000014cdee. pdf?sfvrsn=0.

3. **Pichler M, Hutterer GC, Stoeckigt C, *et al.*** Validation of the pre-treatment neutrophil–lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. Br J Cancer 2013; **108**: 901–7. doi: 10.1038/bjc.2013.28.

4. **Ljungberg B, Bensalah K, Canfield S, *et al.*** EAU Guidelines on Renal Cell Carcinoma: 2014 Update. Eur Urol 2015; **67**: 913–24. doi: 10.1016/j.eururo.2015.01.005.

5. **Meskawi M, Sun M, Trinh Q-D, *et al.*** A Review of integrated staging systems for renal cell carcinoma. Eur Urol 2012; **62**: 303–14. doi: 10.1016/j.eururo.2012.04.049.

6. **Leibovich BC, Blute ML, Cheville JC, *et al.*** Prediction of progression after radical nephrectomy for patients with clear cell renal cell carcinoma. Cancer 2003; **97**: 1663–71. doi: 10.1002/cncr.11234.

7. **Roxburgh CSD, McMillan DC.** Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. Futur Oncol 2010; **6**: 149–63. doi: 10.2217/fon.09.136.

8. **Hu K, Lou L, Ye J, Zhang S.** Prognostic role of the neutrophil-lymphocyte ratio in renal cell carcinoma: a meta-analysis. BMJ Open 2015; **5**: e006404. doi: 10.1136/bmjopen- 2014-006404.

9. **Cho H, Hur HW, Kim SW, *et al.*** Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. Cancer Immunol Immunother 2009; **58**: 15–23. doi: 10.1007/s00262-008-0516-3.

10. **Ohno Y, Nakashima J, Ohori M, *et al.*** Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of recurrence in patients with nonmetastatic renal cell carcinoma. J Urol 2010; **184**: 873–8. doi: 10.1016/j.juro.2010.05.028.

11. **Viers BR, Houston TR, Boorjian SA, *et al.*** Preoperative neutrophil-lymphocyte ratio predicts death among patients with localized clear cell renal carcinoma undergoing nephrectomy. Urol Oncol Semin Orig Investig 2014; **32**: 1277–84. doi: 10.1016/j.urolonc.2014.05.014.

12. **Otunctemur A, Dursun M, Besiroglu H, *et al.*** Clinical significance of preoperative neutrophil — to — lymphocyte ratio in renal cell carcinoma. Int Braz J Urol 2016; **42**: 678–84. doi: 10.1590/S1677-5538.IBJU.2015.0397

13. **Jagdev SPK, Gregory W, Vasudev NS, *et al.*** Improving the accuracy of pre-operative survival prediction in renal cell carcinoma with C-reactive protein. Br J Cancer 2010; **103**: 1649–56. doi: 10.1038/sj.bjc.6605973

14. **Wen RM, Zhang YJ, Ma S, *et al.*** Preoperative neutrophil to lymphocyte ratio as a prognostic factor in patients with non-metastatic renal cell carcinoma. As Pac J Cancer Prev 2015; **16**: 3703–8. doi: 10.7314/apjcp.2015.16.9.3703.

15. **Byun SS, Hwang EC, Kang SH, *et al.*** Prognostic significance of preoperative neutrophil-to-lymphocyte ratio in nonmetastatic renal cell carcinoma: A large, multicenter

cohort analysis. Biomed Res Int 2016; **2016:** 5634148. doi: 10.1155/2016/5634148. doi: 10.1155/2016/5634148.

16. de **Martino M**, **Pantuck AJ, Hofbauer S**, ***et al***. Prognostic impact of preoperative neutrophil-to-lymphocyte ratio in localized nonclear cell renal cell carcinoma. J Urol 2013; **190**: 1999–2004. doi: 10.1016/j.juro.2013.06.082.

ІНДЕКС СПІВВІДНОШЕННЯ НЕЙТРОФІЛІВ ДО ЛІМФОЦИТІВ ЯК ПРОГНОСТИЧНИЙ МАРКЕР НЕМЕТАСТАТИЧНОГО НИРКОВО-КЛІТИННОГО РАКУ — ЧИ ЙОГО ВИЗНАЧЕННЯ МІСТИТЬ ДОДАТКОВУ ПРОГНОСТИЧНУ ІНФОРМАЦІЮ?

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Мета*:** Визначити кореляцію між доопераційним значенням індексу співвідношення нейтрофілів до лімфоцитів (ІСНЛ) та стадією, ступенем диференціювання за Фурманом, саркоматоїдним диференціюванням, некрозом пухлини та залученням до процесу лімфовузлів, а також прогностичним значенням у хворих на неметастатичний нирково-клітинний рак (НМНКР). ***Матеріали та методи: Ретроспективно-проспективне обсерваційне дослідження було виконано в спеціалізованому медичному центрі м. Мумбаї, Індія. У дослідження були включені хворі на НМНКР, які лікувалися в період з липня 2015 р. до квітня 2018 р. Пацієнтів із супутніми системними інфекціями, хворих, яким попередньо проводили імунотерапію або які одержували тривалий час кортикостероїди, у дослідження не включали. ІСНЛ визначали не пізніше, ніж за 1 міс до операції. Хворих розділяли залежно від критичного значення ІСНЛ, яке встановлювали на рівні 3. Визначали кореляцію за Пірсоном між ІСНЛ та відомими прогностичними факторами. ***Результати***: У дослідження було включено 113 хворих віком 18–21 рік (83 чоловіків та 30 жінок). У 75% пацієнтів було діагностовано світлоклітинний нирково-клітинний рак, у 62% хворих було визначено 1-шу стадію захворювання, у 58% хворих було визначено ступінь 2 диференціювання за Фурманом, у 10 хворих виявили метастазування в лімфовузли, у 6 — саркоматоїдне диференціювання, у 40 — некроз пухлини. У 72 хворих ІСНЛ був менше 3. Було виявлено вірогідну кореляцію між ІСНЛ та стадією пухлинного процесу ($p = 0.0054$), а також некрозом пухлини ($p = 0.0128$). ***Висновки***: Доопераційне значення ІСНЛ корелює зі стадією пухлинного процесу та наявністю некрозу пухлини. Виходячи з цього, визначення ІСНЛ може доповнити низку визнаних прогностичних маркерів, що може підвищити точність прогнозування.

Ключові слова: співвідношення нейтрофілів до лімфоцитів, прогностичні фактори, нирково-клітинний рак.