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Validation of the pre-treatment neutrophil–lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients

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Background: The neutrophil–lymphocyte ratio (NLR) has been proposed as an indicator of systemic inflammatory response. Several studies suggest a negative impact of increased NLR for patient's survival in different types of cancer. However, previous findings from small-scale studies revealed conflicting results about its prognostic significance with regard to different clinical end points in non-metastatic renal cell carcinoma (RCC) patients. Therefore, the aim of our study was the validation of the prognostic significance of NLR in a large cohort of RCC patients.

Methods: Data from 678 consecutive non-metastatic clear cell RCC patients, operated between 2000 and 2010 at a single centre, were evaluated retrospectively. Cancer-specific, metastasis-free, as well as overall survival (OS) were assessed using the Kaplan–Meier method. To evaluate the independent prognostic significance of NLR, multivariate Cox regression models were applied for all three different end points. Influence of the NLR on the predictive accuracy of the Leibovich prognosis score was determined by Harrell's concordance index.

Results: Multivariate analysis identified increased NLR as an independent prognostic factor for overall (hazard ratio (HR) = 1.59, 95% confidence interval (CI) = 1.10–2.31, $P=0.014$), but not for cancer-specific (HR = 1.59, 95% CI = 0.84–2.99, $P=0.148$), nor for metastasis-free survival (HR = 1.39, 95% CI = 0.85–2.28, $P=0.184$). The estimated concordance index was 0.79 using the Leibovich risk score and 0.81 when NLR was added.

Conclusion: Regarding patients' OS, an increased NLR represented an independent risk factor, which might reflect a higher risk for severe cardiovascular and other comorbidities. Adding the NLR to well-established prognostic models such as the Leibovich prognosis score might improve their predictive ability.

The worldwide incidence rates of renal cell carcinoma (RCC) have slightly increased within the last three decades (Siegel *et al*, 2012). However, because of the widespread use of radiological imaging techniques, a migration towards small and organ-confined

tumours has been observed (Pichler *et al*, 2012). The use of prognostic factors and models that can accurately predict clinical outcomes of RCC patients are of paramount interest, not only for patients' individualised risk assessment but also for the comparison

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of the results from international clinical multicentre trials (Meskawi *et al*, 2012). Several prognostic models have been established to predict patient's clinical outcome. Kattan *et al* (2001) integrated disease-related symptoms, histologic subtype, tumour size and pathologic tumour-node-metastasis (pTNM) stage into their model to predict 5-year treatment failure. The University of California Integrated Staging System (UISS) incorporated performance status, pTNM stage and Fuhrman grade to predict overall survival (OS) in RCC patients (Zisman *et al*, 2001). The Leibovich prognosis score integrates five clinicopathological features translated into a score and categorises patients into eight-score categories, which are then assigned to one of three different risk groups. According to this score, patients' assignment into the low-, intermediate- or high-risk group can accurately assess the risk for the occurrence of metastatic disease after radical nephrectomy (Leibovich *et al*, 2003). The accuracy of these models may be further improved by the incorporation of different prognostic biomarkers (Lam *et al*, 2008). In addition to individual's risk assessment, considering high costs and often toxic side-effects of novel drugs, an accurate identification and validation of prognostic factors will enable a better risk-stratified patient selection for adjuvant treatment modalities (Crispen *et al*, 2008). External validation of prognostic risk assessment tools using independent cohorts of patients is paramount before the general applicability of a prognostic marker or model (Altman and Royston, 2000; Bleeker *et al*, 2003).

The systemic inflammatory response, which is usually measured by surrogate blood-based parameters, such as C-reactive protein, neutrophil or platelet count, has been shown to independently predict the clinical outcome of various human cancer types (Roxburgh and McMillan, 2010). Of these inflammatory parameters, an increased neutrophil–lymphocyte ratio (NLR) has been proposed as an easily accessible and reliable marker to predict cancer patients survival (Roxburgh and McMillan, 2010). Cumulating evidence in metastatic RCC suggests that a high NLR might represent an independent adverse prognostic factor in interferon-treated (Atzpodien *et al*, 2003), interleukin-2-treated (Donskov and von der Maase, 2006), as well as in sunitinib-treated (Keizman *et al*, 2012) patients. However, data regarding the prognostic significance of the NLR in non-metastatic RCC are sparse, and controversy still exists about how discriminating the NLR might potentially be as an independent risk factor in non-metastatic RCC. For instance, in a prospective cohort study including 83 localised RCCs, Ramsey *et al* (2008) found no statistically significant association of preoperative neutrophil count and patients' relapse-free or CSS. In addition, when evaluating 228 non-metastatic RCC patients, Jagdev *et al* (2010) also failed to demonstrate an increased NLR to be of independent prognostic value in their cohort study. In contrast, a study from Japan including 192 patients with non-metastatic RCC, identified a high preoperative NLR as an independent prognostic indicator of patients' relapse-free survival (Ohno *et al*, 2010). These previously published reports analysed relatively small numbers of patients, heterogeneously histologic subtypes and examined the NLR with regard to different clinical end points. Therefore, the aim of our study was to further clarify the prognostic significance of the preoperative NLR in non-metastatic clear cell RCC and to evaluate whether this parameter provides additional prognostic information to well-established clinicopathological parameters and to the Leibovich prognosis score (Leibovich *et al*, 2003).

PATIENTS AND METHODS

This retrospective analysis included data from 873 consecutive non-metastatic RCC patients who underwent a curative radical or

partial nephrectomy at the Department of Urology at the Medical University of Graz, between January 2000 and December 2010. Of this data set, we excluded 195 cases with non-clear cell histology and restricted our analyses to clear cell RCC only, as the evaluated prognostic model (i.e., Leibovich prognosis score) has been previously established on clear cell RCC (Leibovich *et al*, 2003). All of the clinicopathological data were retrieved from medical records from the Department of Urology, as well as from pathology reports from the Institute of Pathology at the same institution. As the TNM classification system for RCC changed during the observational period, pathologic T stages were uniformly adjusted according to the seventh edition of the TNM classification system (Novara *et al*, 2010). Other documented clinicopathological parameters included histological RCC subtype, tumour grade, presence or absence (not quantitatively assessed) of histologic coagulative tumour necrosis (TN), as well as patients' age and gender. The laboratory data, including neutrophil and lymphocyte counts, were obtained by preoperative exploration one day before surgical intervention. Patients' postoperative surveillance included routine clinical and laboratory examination; regarding imaging methods, X-rays of the chest and abdominal ultrasound were predominantly used, especially in patients with a low relapse risk (pT-1 G1–2), whereas computed tomography or magnetic resonance imaging was performed in all other patients as previously reported (Pichler *et al*, 2011). Follow-up evaluations were performed every 6 months for the first 5 years and annually thereafter for locally advanced tumours. In organ-confined cancers, imaging was performed twice in the first year after surgery and annually thereafter. No neoadjuvant or adjuvant treatment was administered. Dates of death were obtained from the central registry of the Austrian Bureau of Statistics. Cancer-specific survival was defined as the time (in months) from date of surgery to a cancer-related death. Metastasis-free survival (MFS) was defined as the time (in months) from date of surgery to the recurrence of radiologically or histologically confirmed distant metastases. Overall survival was defined as the time (in months) from date of surgery to individuals' death of any cause. The study was approved by the local ethical committee of the Medical University of Graz (no. 24-330 ex 11/12).

Statistical analyses. The primary study end point was CSS, which was calculated from the date of diagnosis to the date of patients' cancer-related death. Secondary end points included OS (the time between diagnosis and death of any cause) and MFS (the time between diagnosis and occurrence of distant metastases). The ideal cutoff value for the continuous NLR was calculated by testing all possible cutoffs that would discriminate between survival and cancer-related death by Cox proportional analyses. The ideal cutoff value was then rounded to clinically relevant (convenient) values as previously reported (Atzpodien *et al*, 2003).

The relationship between NLR and other clinicopathologic parameters was studied by non-parametric tests. Patients' clinical end points were calculated using the Kaplan–Meier method and compared by the log-rank test. Backward stepwise multivariate Cox proportion analysis was performed to determine the influence of pathologic T stage, grade, age, gender and histologic TN on CSS, MFS and OS. Hazard ratios (HRs) estimated from the Cox analysis were reported as relative risks with corresponding 95% confidence intervals (CIs). The patients were categorised according to the Leibovich prognosis risk groups developed for M0 clear cell RCC patients (Leibovich *et al*, 2003). Harrell's concordance index (c-index) was calculated using the individual Leibovich risk groups followed by the addition of the NLR (Harrell *et al*, 1982). All statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Of the 843 consecutive RCC patients, 678 (80.4%) had clear cell, 108 (12.8%) had papillary, 43 (5.1%) had chromophobe, 3 (0.4%) had collecting duct RCC and 11 (1.3%) were not otherwise specified. For all further analyses, we excluded non-clear cell histology cases resulting in 678 patients with clear cell RCC. Pathologic T stage was T1a in 334 (49.3%), T1b in 117 (17.3%), T2a in 32 (4.7%), T2b in 5 (0.7%), T3a in 170 (25.1%), T3b in 16 (2.4%), T3c in 2 (0.3%) and T4 in 2 (0.3%) patients. Tumour grading was G1 in 170 (25.1%), G2 in 411 (60.6%) G3 in 92 (13.6%) and G4 in 5 (0.7%) cases. Overall, the presence of histologic TN was noted in 165 (24.3%) patients with a mean age of 63.7 ± 11.9 years, a mean neutrophil count of 4828 ± 1974 , a mean lymphocyte count of 1580 ± 581 and a mean NLR of 3.51 ± 2.49 . Applying the criteria mentioned above, we determined a cutoff value of 3.3 for the NLR to be optimal to discriminate between patients' CSS that prompted us to select 3.3 as the optimal cutoff value for all subsequent analyses to differentiate between low (<3.3) and high (≥ 3.3) NLR. Overall, there were 398 (58.7%) patients with a low NLR and 280 (41.3%) patients with a high NLR. A high NLR was statistically significantly correlated with older age, high tumour grade, large tumour size, as well as with advanced tumour stage (all $P < 0.05$), but not with gender ($P = 0.552$), the presence of histologic TN ($P = 0.107$) or positive lymph node disease ($P = 0.136$).

To investigate whether the NLR is associated with the clinical outcome of RCC patients, univariable and multivariable analyses for all three end points were performed. Mean follow-up was 44 (range 0–130) months. Of the 678 clear cell RCC patients, 76 (11.2%) developed metastatic disease, of which 49 (7.2%) died because of their advanced disease state. Overall, 123 (18.1%) patients died by their most recent follow-up visit for any cause. Among the 678 RCC patients, metastatic disease was diagnosed in 29 out of 398 (7.3%) patients with a low NLR and in 47 out of 280 (16.8%) patients with a high NLR ($P < 0.001$). Regarding survival, cancer-related and overall deaths occurred in 26 (4.0%) and 52 (13.1%) patients with low NLR and in 33 (11.8%) and 71 (25.4%) patients with high NLR ($P < 0.001$). Figures 1, 2 and 3 show the Kaplan–Meier curves for CSS, MFS and OS and reveal that a high NLR is a consistent factor for poor prognosis in RCC patients ($P < 0.001$ for all three tested end points, log-rank test).

Univariate analysis identified age (≥ 65 vs < 65 years, $P = 0.044$), high tumour grade (G3 + G4 vs G1 + G2, $P < 0.001$), histologic TN (presence vs absence, $P < 0.001$), high pathologic T stage ($\geq pT3$ vs $< pT3$, $P < 0.001$) and a high NLR (≥ 3.2 vs < 3.2 , $P < 0.001$) as prognosticators of poor outcome for patients' CSS, whereas gender (male vs female, $P = 0.124$) was not statistically significantly associated with CSS (Table 1).

To determine the independent prognostic significance of the NLR for CSS, a multivariate analysis using a Cox proportional hazard model was performed. In our multivariate analysis that included age, pathologic T stage, tumour grade, NLR and presence of histologic TN, we identified age, pathologic T stage, tumour grade, as well as histologic TN as independent prognostic factors for CSS and MFS, whereas the NLR was not statistically significantly associated with either CSS (HR = 1.59, 95% CI = 0.84–2.99, $P = 0.148$, Table 1) or MFS (HR = 1.39, 95% CI = 0.85–2.28, $P = 0.184$, Table 2). Regarding OS, a high NLR was identified as an independent prognostic factor for poor survival (HR = 1.59, 95% CI = 1.10–2.31, $P = 0.014$, Table 3). Before analysing the addition of the NLR to the Leibovich prognosis model, patients were categorised into risk groups (low, intermediate and high risk) according to Leibovich *et al* (2003). For MFS, the c-index was 0.79 when assessed with each individual Leibovich risk score and improved to 0.81 when the NLR was added. Regarding CSS, the c-index of the original Leibovich model

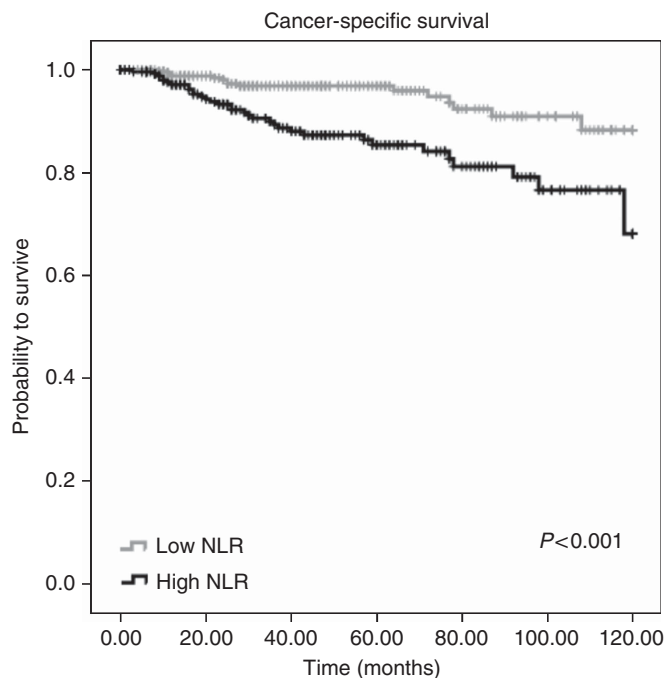


Figure 1. Kaplan–Meier curves for renal cell carcinoma patients cancer-specific survival groups categorised by the neutrophil-lymphocyte ratio (NLR).

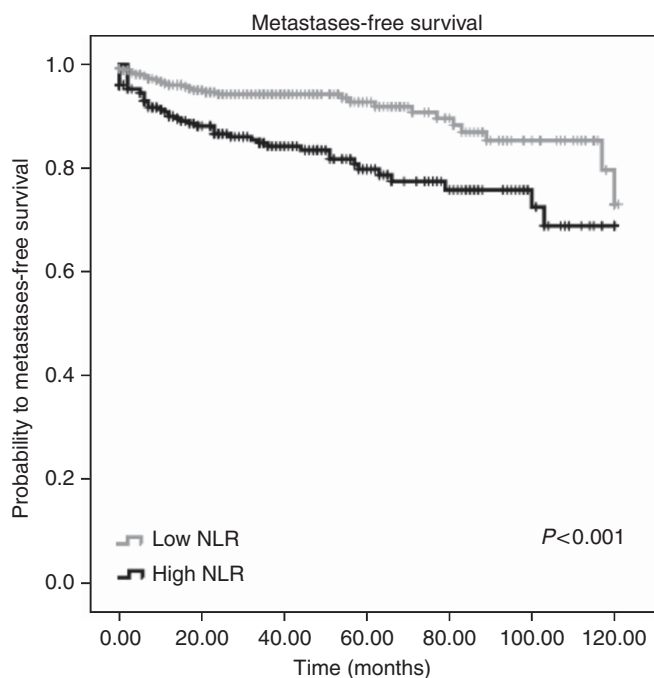


Figure 2. Kaplan–Meier curves for renal cell carcinoma patients metastasis-free survival groups categorised by the neutrophil-lymphocyte ratio (NLR).

was 0.83 compared with 0.86 when NLR was supplemented. Finally for OS, the c-index improved from 0.63 compared with 0.67 when the NLR was added to the Leibovich prognosis groups.

DISCUSSION

Despite recent progress in the identification of genetic, epigenetic and common molecular alterations in RCC has been made

(Al-Ali *et al*, 2012; Gerlinger *et al*, 2012), the routine diagnostic and prognostic assessment of RCC currently relies on pathological tissue examination and traditional clinicopathological prognostic variables (Ficarra *et al*, 2010). The complexity of these molecular changes, as well as high costs of analyses, the time-consuming preparation required and the lack of evidence demonstrating how

these newly discovered molecular markers influence diagnostic or therapeutic decisions have rendered none of the markers available for routine testing. Regularly used blood-based parameters, such as the neutrophil or lymphocyte count and the resultant-derived NLR, are relatively easy to assess without additional laborious efforts, making them attractive parameters for patients' improved individualised risk assessment in RCC (Zahorec, 2001).

Heng *et al* (2009) detected 11.7% of patients with an increased blood neutrophil count (i.e., cutoff value defined as greater than the upper limit of normal range) and showed that the blood neutrophils only represent an independent prognostic factor in metastatic RCC patients. However, in our study we only could identify 15 (2.2%) patients with an increased blood neutrophil count only (i.e., greater than the upper limit of normal range), which represents a too low number of patients to perform a reliable multivariate prognostic analysis.

Beyond the neutrophil count only, an increased pre-treatment NLR has been previously demonstrated as a poor prognostic factor for different human cancer types, including gastrointestinal, soft tissue sarcoma, nasopharyngeal, as well as lung cancer (Roxburgh and McMillan, 2010). In non-metastatic RCC, only two studies have been published about the prognostic value of the pre-treatment NLR so far, and the reported findings are conflicting. In our validation study that included a large middle European cohort of 678 patients with non-metastatic clear cell RCC, we were able to demonstrate that an increased NLR was an independent negative predictor for patients' OS but not a predictor for direct cancer-related end points, such as CSS and MFS. To the best of our knowledge, our study represents one of the most comprehensive ones to date testing the independent prognostic significance of the NLR in clear cell RCC. Our findings are in agreement with the study of Jagdev *et al* (2010), who did not find an independent prognostic significance for the NLR in 228 non-metastatic RCC patients with regard to CSS and disease-free survival. In contrast to Jagdev *et al*'s and our negative findings, Ohno *et al* (2010) reported

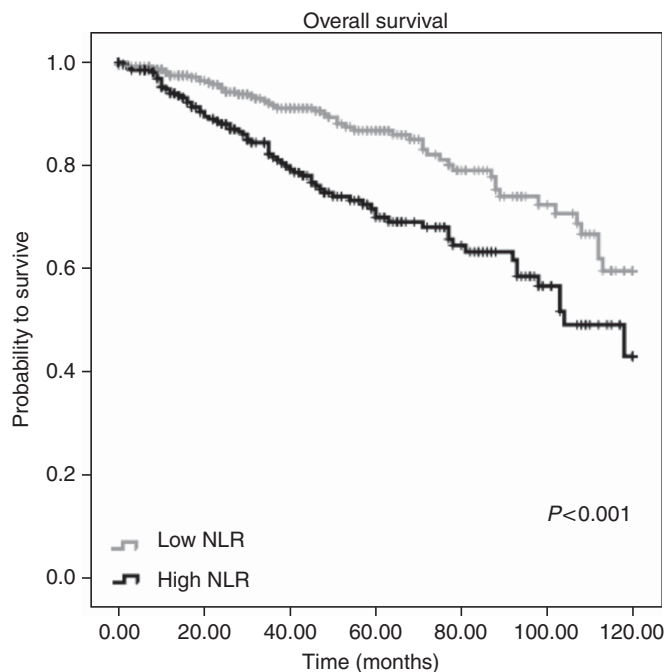


Figure 3. Kaplan-Meier curves for renal cell carcinoma patients overall survival categorised by the neutrophil-lymphocyte ratio (NLR).

Table 1. Univariate and multivariate analysis of clinicopathological parameters for the prediction of cancer-specific survival in patients with clear cell renal cell carcinoma (n = 678)

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age at operation (years)				
< 65	1 (Referent)	0.044	1 (Referent)	0.127
≥ 65	1.83 (1.01-3.29)		1.59 (0.87-2.89)	
Gender				
Female	1 (Referent)	0.124		
Male	1.55 (0.88-2.72)			
T stage				
pT1-2	1 (Referent)	< 0.001	1 (Referent)	< 0.001
pT3-4	6.47 (3.48-12.04)		3.77 (1.93-7.36)	
Tumour grade				
G1 + G2	1 (Referent)	< 0.001	1 (Referent)	0.003
G3 + G4	6.91 (3.89-12.29)		2.66 (1.40-5.04)	
Presence of tumour necrosis				
No	1 (Referent)	< 0.001	1 (Referent)	0.002
Yes	4.25 (2.42-7.47)		2.55 (1.41-4.63)	
Neutrophil-lymphocyte ratio				
< 3.3	1 (Referent)	< 0.001	1 (Referent)	0.148
≥ 3.3	2.89 (1.59-5.22)		1.59 (0.84-2.99)	

Abbreviations: CI = confidence interval; HR = hazard ratio.

Table 2. Univariate and multivariate analysis of clinicopathological parameters for the prediction of metastasis-free survival in patients with clear cell renal cell carcinoma (n = 678)

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age at operation (years)				
<65	1 (Referent)	0.357	1 (Referent)	0.747
≥65	1.23 (0.78–1.94)		1.07 (0.68–1.71)	
Gender				
Female	1 (Referent)	0.072		
Male	1.51 (0.96–2.37)			
T stage				
pT1–2	1 (Referent)	<0.001	1 (Referent)	<0.001
pT3–4	5.69 (3.52–9.19)		3.47 (2.06–5.84)	
Tumour grade				
G1 + G2	1 (Referent)	<0.001	1 (Referent)	<0.001
G3 + G4	6.08 (3.83–9.64)		2.69 (1.61–4.49)	
Presence of tumour necrosis				
No	1 (Referent)	<0.001	1 (Referent)	0.001
Yes	3.80 (2.42–5.96)		2.22 (1.38–3.59)	
Neutrophil–lymphocyte ratio				
<3.3	1 (Referent)	<0.001	1 (Referent)	0.184
≥3.3	2.37 (1.49–3.76)		1.39 (0.85–2.28)	

Abbreviations: CI = confidence interval; HR = hazard ratio.

Table 3. Univariate and multivariate analysis of clinicopathological parameters for the prediction of overall survival in patients with clear cell renal cell carcinoma (n = 678)

Parameter	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age at operation (years)				
<65	1 (Referent)	<0.001	1 (Referent)	<0.001
≥65	2.14 (1.46–3.14)		2.03 (1.39–2.99)	
Gender				
Female	1 (Referent)	0.894		
Male	1.02 (0.71–1.47)			
T stage				
pT1–2	1 (Referent)	<0.001	1 (Referent)	0.048
pT3–4	1.92 (1.34–2.75)		1.47 (1.00–2.18)	
Tumour grade				
G1 + G2	1 (Referent)	<0.001	1 (Referent)	0.027
G3 + G4	2.50 (1.64–3.84)		1.72 (1.16–2.78)	
Presence of tumour necrosis				
No	1 (Referent)	0.068	1 (Referent)	0.493
Yes	1.43 (0.97–2.11)		1.15 (0.76–1.73)	
Neutrophil–lymphocyte ratio				
<3.3	1 (Referent)	<0.001	1 (Referent)	0.014
≥3.3	1.93 (1.35–2.77)		1.59 (1.10–2.31)	

Abbreviations: CI = confidence interval; HR = hazard ratio.

that an increased NLR was an independent predictor for relapse-free survival in a smaller cohort of 192 RCC patients from Japan. However, in addition to the significantly smaller sample size and a different ethnic background, Ohno *et al* did not include commonly

used prognostic factors in their multivariate model, such as tumour grade or histologic TN (Sun *et al*, 2011). Interestingly, we found that the NLR was an independent prognostic factor for OS, which might possibly reflect a higher overall mortality rate for patients

with increased systemic inflammatory responses. However, a definitive explanation for this observation remains speculative. Nevertheless, several studies evaluating the NLR as an adverse factor in cardiovascular diseases included several thousands of patients and have clearly established an increased NLR as a negative prognostic predictor for cardiovascular events. For instance, Tsai *et al* (2007) demonstrated in 1872 subjects that the NLR, in addition to metabolic syndrome, is strongly associated with the risk of ischaemic cardiovascular diseases. Other studies have indicated an increased NLR to be associated with poor survival after coronary artery bypass grafting (Gibson *et al*, 2007), to be an independent predictor of in-hospital and 6-month mortality in patients with acute coronary syndrome (Tamhane *et al*, 2008) and to be an independent predictor of in-hospital major adverse cardiac events in patients with ST-segment elevation myocardial infarction (Akpek *et al*, 2012). Imtiaz *et al* (2012) described an increased NLR in patients with hypertension and diabetes, and Terradas *et al* (2012) found that an increased NLR was an independent marker of mortality in patients with bacteraemia. Furthermore, one recently published report demonstrated that stage 4 chronic kidney disease patients with a high NLR had a worse prognosis and a significantly faster progression to dialysis compared with those with a low NLR (Kocyigit *et al*, 2012). In this context, Sun *et al* (2012) demonstrated that the status of chronic kidney disease is getting worse after nephrectomy, which might contribute to a higher overall mortality in patients with an increased systemic inflammatory response found in our study. Evidence also exists that an increased NLR might represent a marker for more severe gastrointestinal conditions, such as a higher risk for non-alcoholic steatohepatitis and fibrosis of the liver (Alkhoury *et al*, 2012), as well as adverse outcomes because of acute pancreatitis (Azab *et al*, 2011).

Taken together, these data support the hypothesis that an increased NLR seems to be associated with patients' increased risk of cardiovascular, infectious and gastrointestinal morbidity and mortality, and may underlie the increased overall mortality we observed in our cohort of RCC patients with an increased NLR. One caveat to consider is that although the NLR is easy to measure, the specificity of this parameter is influenced by several other conditions such as active infection, inflammatory diseases, smoking behaviour or stress at the time of blood collection (Pedersen *et al*, 1999; Alkhoury *et al*, 2012). In our opinion, these factors impair the use and generalisability of this marker as a specific predictor of survival in patient cohorts.

Several studies indicated that tumour infiltrating neutrophils or lymphocyte subtypes might have a meaningful role for the biological behaviour of tumour cells in RCC. For instance, Jensen *et al* (2009) demonstrated that a high number of intratumoural neutrophils is a strong and independent poor prognostic factor for the clinical outcome in localised clear cell RCC. In a study by Kondo *et al* (2004) the authors demonstrated a favourable outcome for RCC's where high levels of lymphocytic attractant chemokines are expressed. Hotta *et al* (2011) postulated that the intratumoural CD45RO+ memory T-cell status has a significant independent prognostic value, indicating that the adaptive immune response is functionally critical in human RCC. Cumulating data also indicated that T regulatory cells, which are detectable by different markers such as CD4CD25 positivity and intracytoplasmic-Foxp3 expression, are involved in RCC progression and RCC prognosis (Siddiqui *et al*, 2007; Jeron *et al*, 2009; Liotta *et al*, 2011).

As with all retrospective studies, limitations of our study are inherent to its design including the retrospective data collection. No data were available about the cause of death for calculation of patients' OS; hence, the reasons for an elevated death rate in the group of patients with an increased preoperative NLR remain elusive. In an attempt to control for the homogeneity of the study

population, we excluded patients with non-clear cell histology, hereditary RCC, patients with metachronous secondary RCC and those with competitive invasive cancers originating from other sites if metastatic spread was not assessed through histologic examination. In addition to the evaluation of the integration of the NLR to the Leibovich prognosis score, other prognostic models such as the Kattan nomogram (Kattan *et al*, 2001) or the UISS model (Zisman *et al*, 2001) should be evaluated.

Nonetheless, even considering these limitations, our data clearly indicate that an increased pre-treatment NLR might represent an independent prognostic factor for OS in non-metastatic clear cell RCC patients.

In conclusion, an increased NLR seems to represent an independent predictor with respect to patients' OS in non-metastatic RCC. As the NLR improves the prognostic accuracy of the Leibovich prognosis score, this parameter warrants further validation as a selection criterion for risk factor-stratified patient management in non-metastatic RCC.

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