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Markers of systemic inflammation predict survival in patients with advanced renal cell cancer

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Background: The host inflammatory response has a vital role in carcinogenesis and tumour progression. We examined the prognostic value of inflammatory markers (albumin, white-cell count and its components, and platelets) in pre-treated patients with advanced renal cell carcinoma (RCC).

Methods: Using data from a randomised trial, multivariable proportional hazards models were generated to examine the impact of inflammatory markers and established prognostic factors (performance status, calcium, and haemoglobin) on overall survival (OS). We evaluated a new prognostic classification incorporating additional information from inflammatory markers.

Results: Of the 416 patients, 362 were included in the analysis. Elevated neutrophil counts, elevated platelet counts, and a high neutrophil–lymphocyte ratio were significant independent predictors for shorter OS in a model with established prognostic factors. The addition of inflammatory markers improves the discriminatory value of the prognostic classification as compared with established factors alone (C-statistic 0.673 *vs* 0.654, $P=0.002$ for the difference), with 25.8% ($P=0.004$) of patients more appropriately classified using the new classification.

Conclusion: Markers of systemic inflammation contribute significantly to prognostic classification in addition to established factors for pre-treated patients with advanced RCC. Upon validation of these data in independent studies, stratification of patients using these markers in future clinical trials is recommended.

In renal cell cancer (RCC), inactivation of the von Hippel–Lindau tumour-suppressor gene results in abnormal accumulation of hypoxia-inducible factor, resulting in dysregulation of cellular growth and angiogenesis (Kaelin, 2004; Lynch *et al*, 2004). Although the genetic basis of this disease and many other cancers are well established, recent work across different cancer populations also identified that host inflammatory response has an important role in carcinogenesis and disease progression (Colotta *et al*, 2009; Hanahan and Weinberg, 2011). Initial *in-vitro* study findings have been supported by results from clinical studies that demonstrated a correlation between clinical outcomes and laboratory markers of systemic inflammatory response, including plasma C-reactive protein (CRP) concentration (Canna *et al*, 2004; Hilmy *et al*, 2005), hypoalbuminaemia (Forrest *et al*, 2003), and the Glasgow Prognostic Score (GPS, which combines CRP and albumin) (Murri *et al*, 2006; Ramsey *et al*, 2007; Crumley *et al*, 2008).

There is also a growing body of evidence demonstrating that haematological markers of systemic inflammatory response such as absolute white-cell count or its components (neutrophils, neutrophil–lymphocyte ratios (NLR) (Yamanaka *et al*, 2007; Halazun *et al*, 2008, 2009; Liu *et al*, 2010; Chua *et al*, 2011;

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Huang *et al*, 2011), platelets, and platelet–lymphocyte ratios (PLR) (Heng *et al*, 2009; Smith *et al*, 2009) are also prognostic indicators for cancer clinical outcomes. These markers are inexpensive to test and routinely measured in day-to-day oncological practice, and hence potentially provide readily available objective information to help oncologists to estimate patient prognosis.

The value of prognostic models to improve categorisation of patient risk by incorporating information from multiple pre-treatment factors is widely accepted in genitourinary oncology. In advanced RCC, one prognostic model in treatment-naive patients developed at the Memorial Sloan-Kettering Cancer Centre (MSKCC) (Motzer *et al*, 1999) has been widely adopted for enrichment and stratification of patients in clinical trials, and also used for patient counselling and risk-directed therapy. In patients who had prior cytokine therapy, a second prognostic model identified low Karnofsky Performance Status Scale (KPS), high corrected serum calcium, and low serum haemoglobin as predictors of shorter survival (Motzer *et al*, 2004b). In addition to the well-established MSKCC factors, some of the markers of systemic inflammatory response have also been identified as independent prognostic variables in treatment-naive patients (Heng *et al*, 2009; Huang *et al*, 2011). However, the value of these markers as independent prognostic factors and the extent these markers improve prognostic classification for patients with disease progression after front-line therapy remains unknown. In this study, we examined these questions in a population of patients treated with prior cytokine therapy; our hypothesis was that elevated inflammatory markers predicted for worse outcome.

MATERIALS AND METHODS

Patients. The patient population for this analysis comprised 416 patients with locally advanced or metastatic RCC who were treated with lapatinib or hormonal therapy after prior failure of immunotherapy in a randomised phase III trial (EGF20001) (Ravaud *et al*, 2008). The eligibility, methods, treatment plan, and outcome for this phase III trial have been previously published (Ravaud *et al*, 2008). Key eligibility criteria included a diagnosis of locally advanced or metastatic RCC not amenable to curative surgery or radiotherapy, measurable disease, progression after or intolerance to first-line cytokine-based therapy, adequate haematologic, renal, and hepatic function, KPS $\geqslant$ 70%, and life expectancy $\geqslant$ 12 weeks. Lapatinib was administered orally at 1250 mg daily; hormonal therapy was also administered daily, and consisted of megestrol acetate or tamoxifen as decided by the investigator. All patients provided written informed consent to participate in the randomised trial.

Baseline demographic, clinical, and laboratory data were collected prospectively on all patients as part of the clinical trial. Date of death or last follow-up was also recorded for all patients.

Statistical analysis. The end point of interest was overall survival (OS), defined as the time from randomisation to the date of death or date of last follow-up. We first evaluated the discriminative value of MSKCC-defined prognostic factors: Karnofsky Performance Status, haemoglobin level, and corrected serum calcium (Motzer *et al*, 2004b), in patients from the EGF20001 trial. These factors were examined individually in univariate analyses, and then in combination as multivariate models. Using the previously defined MSKCC classification (Motzer *et al*, 2004b), patients were grouped into good, intermediate, or poor risk groups based on the presence of none, one, or two to three baseline factors. Low Karnofsky Performance Status ($<$80%), low haemoglobin level ($<13\,g\,dl^{-1}$ for males and $<11.5\,g\,dl^{-1}$ for females), and high corrected serum calcium ($\geqslant 10\,mg\,dl^{-1}$) were considered to be risk factors for shorter OS.

We also individually examined the impact of each of the baseline markers of systemic inflammation (albumin, neutrophils, lymphocytes, platelets, NLR, and PLR) on OS. These markers were analysed as categorical variables. Dichotomisation of these variables was based on the upper (neutrophils and platelets) and the lower (albumin and lymphocytes) ranges of normal measurements for these markers.

For NLR and PLR, no widely accepted cutpoints have been adopted; therefore, we used the medians of distribution as cutpoints for dichotomisation. Previous studies examining NLR thresholds in advanced malignancy have used a cutpoint ranging from 2.5 to 5.0 (Yamanaka *et al*, 2007; Kao *et al*, 2010; Chua *et al*, 2011; Huang *et al*, 2011). A cutpoint ranging from 150 to 300 has been used for PLR (Aliustaoglu *et al*, 2010; Asher *et al*, 2011; He *et al*, 2013). We further examined the impact of other cutpoints as sensitivity analyses.

We then built a second multivariate model combining markers of systemic inflammation and MSKCC factors. A significant relationship between an inflammatory marker and OS (defined as $P<0.15$) in univariate analyses was used as the criterion for including that marker in the multivariate backward stepwise elimination procedure. The final multivariate model retained all MSKCC factors; only markers of systemic inflammation with $P<0.05$ were retained. Patients were then reclassified using the new classification based on the presence of baseline factors identified to be significant in the final model.

We compared the discriminatory value of the two prognostic classifications (MSKCC classification and new classification with MSKCC factors and markers of systemic inflammation). The Kaplan–Meier curves were used to illustrate the differences in survival distribution for the different prognostic groups. We also compared the concordance statistics (C-statistic) (Harrell *et al*, 1996) to evaluate the differences in discrimination for the two classifications. The C-statistic is equivalent to the area under the receiver operating characteristic curve for censored data, in which a value of 0.5 indicates no discrimination, and a value of 1 represents a perfect ability to correctly rank randomly selected pairs of patients according to their survival times.

The incremental benefit of adding markers of systemic inflammation to the MSKCC factors to improve prognostic classification was evaluated by calculating the net reclassification index (NRI) (Pencina *et al*, 2008). First, patients were stratified into one risk category using the MSKCC classification (Motzer *et al*, 2004b). Then, we used the new classification (MSKCC factors with markers of systemic inflammation) to determine the risk category, to ascertain whether there would be improvement in the NRI – that is, whether the proportion of patients who died would be assigned to a higher risk category and those who survived to a lower risk category.

RESULTS

Out of 416 patients enrolled in the EGF20001 randomised trial (Ravaud *et al*, 2008), a total of 362 patients (87%) with complete baseline information on the MSKCC factors (Motzer *et al*, 2004b) and markers of systemic inflammation were available for analysis (Supplementary Figure 1). Patients ($n=54$, 17%) excluded from this analysis had a higher incidence of bone metastasis at baseline than those included in the analysis. The other baseline characteristics were similar for patients included in this analysis, and for those excluded due to missing information (Table 1).

Impact of MSKCC factors on OS. In univariate analyses, low KPS, high corrected serum calcium, and low serum haemoglobin were predictors of shorter OS (Table 2). These three predictors

remained significant when they were examined together in a multivariate model.

The good-prognosis group (no risk factors) comprised 153 patients (42%) with a median OS of 18.5 months (95% CI 16.5–20.2). The intermediate-prognosis group (one risk factor) comprised 111 patients (31%) with a median OS of 10.6 months (95% CI 8.2–12.7). The poor-prognosis group (two or more risk factors) comprised 98 patients (27%) with a median OS of 5.8 months (95% CI 4.3–7.2) (Figure 1a).

Table 1. Baseline characteristics of patients included in and excluded from this study

	Patients included in this study ($n=362$)		Patients excluded from this study ($n=54$)		
Characteristics	***n***	**%**	***n***	**%**	***P*-value**
Age, years					
Median		62		60	0.11
Range		19–84		34–81	
Gender					0.39
Female	94	26	17	31	
Male	268	74	37	69	
KPS					0.95
70–80%	149	41	22	41	
90–100%	213	59	32	59	
Previous nephrectomy					0.20
Yes	339	94	48	89	
No	23	6	6	11	
Histology					0.84
Clear cell	315	88	47	87	
Non-clear cell	43	12	7	13	
No. of metastatic sites					0.64
⩽2	182	50	29	54	
>2	180	50	25	46	
Sites of metastasis					
Lung	295	81	41	76	0.33
Bone	104	29	24	44	0.02
Liver	81	22	15	28	0.38
Lymph nodes	174	48	25	46	0.79
Randomised treatment					0.80
Hormone therapy	181	50	26	48	
Lapatinib	181	50	28	52	

Abbreviation: KPS = Karnofsky Performance Status Scale.

Impact of markers of systemic inflammation on OS. In univariate analyses, low serum albumin, elevated neutrophil counts, elevated platelet counts, and low lymphocyte counts were predictors of shorter OS (Table 3). High NLR and PLR were also predictors of shorter survival.

In a multivariate model of markers of systemic inflammation combined with MSKCC factors, elevated neutrophil counts, elevated platelet counts, and a high NLR were significant independent predictors of shorter OS (Table 3). Low KPS and high corrected serum calcium remained significant factors but not low serum haemoglobin ($P=0.09$) in this model.

When other cutpoints for NLR and PRL were examined, similar outcomes were obtained (results not shown).

In this new prognostic model, the good-prognosis group (no risk factors) comprised 95 patients (26%) with a median OS of 18.8 months (95% CI 17.1–21.8). A new risk factor group, good-intermediate-prognosis group (one risk factor), comprised 85 patients (24%) with a median OS of 16.5 months (95% CI 12.3–19.3). The intermediate-prognosis group (two risk factors) comprised 74 patients (20%) with a median OS of 11.2 months (95% CI 7.8–13.2). The poor-prognosis group (three or more risk factor) comprised 108 patients (30%) with a median OS of 6.0 months (95% CI 4.3–7.2) (Figure 1b).

Performance of new prognostic classification. The C-statistic of the MSKCC classification was 0.654 (95% CI 0.623–0.685). With the new classification, the C-statistic improved to 0.673 (95% CI 0.643–0.703). The improvement of the C-statistic by 0.019 is statistically significant ($P=0.002$).

Net reclassification. Table 4 illustrates the reclassification of patients' prognostic category using the new classification. Among the patients who were alive at 12 months, 22% of patients were classified to a higher risk category and 29% of patients were classified to a lower risk category, with an NRI of 6.8% (11 of 161 patients). Among the patients who had died at 12 months, 16% of patients were classified to a higher risk category and 35% of patients were classified to a lower risk category, with an NRI of 19.0% (34 of 179 patients). The total overall net reclassification was 25.8% ($P=0.004$).

Table 2. Univariate and multivariate analyses of MSKCC factors[a] on overall survival

		Univariate analysis				Multivariate analysis			
Factors	***n***	**HR**	**95%**	**CI**	***P*-value**	**HR**	**95%**	**CI**	***P*-value**
KPS < 80	59	2.86	2.13	3.85	<.0001	2.33	1.71	3.18	<.0001
KPS ⩾ 80	303	1.00				1.00			
Low haemoglobin[b]	169	1.93	1.53	2.44	<.0001	1.41	1.09	1.83	0.009
Normal haemoglobin[b]	193	1.00				1.00			
Corrected calcium ⩾ 10 mg dl^{-1}	98	2.26	1.74	2.94	<.0001	1.89	1.44	2.49	<.0001
Corrected calcium < 10 mg dl^{-1}	264	1.00				1.00			

Abbreviations: CI = confidence interval; HR = hazard ratio; MSKCC = Memorial Sloan-Kettering Cancer Centre; KPS = Karnofsky Performance Status Scale.
[a]MSKCC factors are Karnofsky Performance Status Scale, haemoglobin, and corrected calcium.
[b]Haemoglobin normal > 13 g dl^{-1} (male); > 11.5 g dl^{-1} (female).

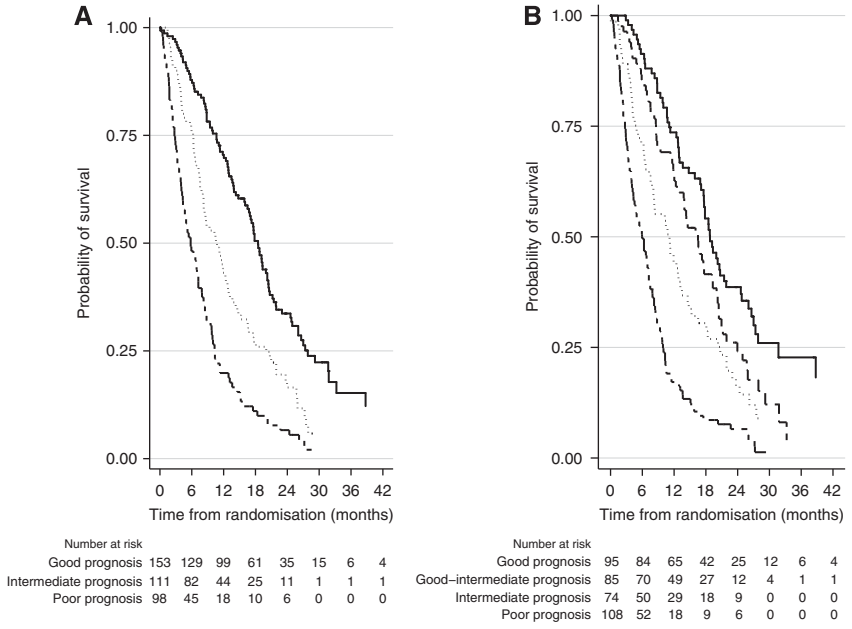

Figure 1. (**A**) Kaplan–Meier estimates of the probability of survival according to prognostic groups as defined by MSKCC* factors. (**B**) Kaplan–Meier estimates of the probability of survival according to prognostic groups as defined by markers of systemic inflammation[†] and MSKCC*. MSKCC = Memorial Sloan-Kettering Cancer Centre. *MSKCC factors are Karnofsky Performance Status Scale, haemoglobin, and corrected calcium. [†]Systemic inflammation markers are neutrophils, platelets, and neutrophils–lymphocytes ratio. (**A**) Good prognosis is represented by the solid line, intermediate prognosis by the dotted line, and poor prognosis by the solid-dashed line. (**B**) Good prognosis is represented by the solid line, good-intermediate prognosis by the dashed line, intermediate prognosis by the dotted line, and poor prognosis by the solid-dashed line.

DISCUSSION

In this study, we confirmed that the MSKCC-defined factors of low Karnofsky Performance Status, low haemoglobin level, and high corrected serum calcium are independent and significant predictors of shorter OS in patients with advanced RCC treated with prior cytokine therapy. We also found that markers of systemic inflammation (elevated neutrophil counts, elevated platelet counts, and a high NLR) significantly predict for shorter OS. We demonstrated that the addition of inflammatory markers improves the discriminatory performance of the prognostic classification based on MSKCC factors alone (C-statistics 0.673 *vs* 0.654, $P = 0.002$ for the difference), with 25.8% of patients more appropriately classified using the new classification (Table 4). This new prognostic classification also better discriminates the 'good' and 'intermediate' prognosis patients by extending the risk classification to include a new 'good-intermediate' risk group.

The development of prognostic models to allow more accurate classification of patient survival time has many important implications. In the treatment-naive setting, the first MSKCC model (Motzer *et al*, 1999) has already been widely used for enrichment of patients in clinical trials according to risk (Escudier *et al*, 2007; Hudes *et al*, 2007; Motzer *et al*, 2007). In clinical practice, risk-directed treatment strategies are widely employed in the management of patients with newly diagnosed advanced RCC (Motzer *et al*, 2004a). However, there remains no standard agent or combination therapies recognised as effective salvage therapy following failure of front-line therapy. With many of these patients who were initially treated with effective front-line therapies but developed disease progression subsequently, accurate prognostic models are now urgently needed to better stratify these patients as they are being enrolled into second-line clinical trials of novel therapy.

Albumin, neutrophils, platelets, and lymphocytes are among the most frequently requested clinical laboratory tests together with haemoglobin and calcium in the oncology outpatient setting. The modern day automated blood cell analyser is precise and accurate in quantification of haemoglobin, platelets, and various white blood cell populations present in peripheral venous blood (Buttarello and Plebani, 2008). Furthermore, there is a standardisation of laboratory measurements of albumin, with internationally agreed standards, on definition and application of a reference measurement system for calibration and validation of routine methods (Infusino *et al*, 2011). These widely available and inexpensive routinely performed tests, which are accurate and standardised in many settings, provide oncologists with convenient and objective information to estimate patient prognosis.

This study also provides insight into the role of the host inflammatory response in cancer progression. Our findings can be used to raise hypotheses about the complex interactions of host factors (poor performance status), tumour biology (low haemoglobin and high calcium), and systemic inflammation (elevated neutrophil count, elevated platelet count, and a high NLR) and their effects on poorer survival in patients with metastatic RCC. An inflammatory microenvironment has recently been described as one of the hallmarks of cancer (Hanahan and Weinberg, 2011). Almost 150 years since Virchow originally postulated the relationship between inflammation and carcinogenesis (Balkwill and Mantovani, 2001), contemporary studies have confirmed that mitogenesis originates in an inflammatory microenvironment, and chronic inflammation persists throughout the disease course (Lu *et al*, 2006). This inflammatory milieu allows tumour cells to evade host responses, contributing to angiogenesis, tumour growth, invasion, and metastasis.

Promotion of the extrinsic pathway (pre-existing inflammation) or the intrinsic pathway (oncogene activation) results in mobilisation of transcription factors and inflammatory mediators, giving rise to recruitment of inflammatory cells including neutrophils, and megakaryocytes causing thrombocytosis (Mantovani *et al*, 2008). The resulting cascade of inflammatory mediators leads to

Table 3. Univariate and multivariate analyses of MSKCC[a] and systemic inflammation markers[b] on overall survival

	Univariate analysis					Multivariate model[c]			
Factors	***n***	**HR**	**95%**	**CI**	***P*-value**	**HR**	**95%**	**CI**	***P*-value**
Neutrophils $\geqslant 7.5 \times 10^9$/l	41	2.71	1.91	3.84	<.0001	1.66	1.12	2.45	0.01
Neutrophils $< 7.5 \times 10^9$/l	321	1.00				1.00			
Platelets $\geqslant 400 \times 10^9$/l	80	2.24	1.72	2.92	<.0001	1.48	1.09	2.00	0.01
Platelets $< 400 \times 10^9$/l	282	1.00				1.00			
Albumin $\leqslant 35$ mg dl^{-1}	69	2.48	1.87	3.30	<.0001				
Albumin > 35 mg dl^{-1}	293	1.00							
Lymphocytes $< 1.0 \times 10^9$/l	66	1.54	1.15	2.07	0.004				
Lymphocytes $\geqslant 1.0 \times 10^9$/l	296	1.00							
Neutrophils/lymphocytes ratio >3	188	1.87	1.48	2.37	<.0001	1.42	1.10	1.84	0.008
Neutrophils/lymphocytes ratio $\leqslant 3$	174	1.00				1.00			
Platelets/lymphocytes ratio >195	178	1.88	1.48	2.37	<.0001				
Platelets/lymphocytes ratio $\leqslant 195$	184	1.00							
KPS <80	59	2.86	2.13	3.85	<.0001	2.27	1.66	3.09	<.0001
KPS $\geqslant 80$	303	1.00				1.00			
Corrected calcium $\geqslant 10$ mg dl^{-1}	98	2.26	1.74	2.94	<.0001	1.53	1.15	2.04	0.003
Corrected calcium < 10 mg dl^{-1}	264	1.00				1.00			
Low haemoglobin[d]	169	1.93	1.53	2.44	<.0001	1.27	0.97	1.67	0.09
Normal haemoglobin[d]	193	1.00				1.00			

Abbreviations: CI = confidence interval; HR = hazard ratio; KPS = Karnofsky Performance Status Scale; MSKCC = Memorial Sloan-Kettering Cancer Centre.
[a]MSKCC factors are Karnofsky Performance Status Scale, haemoglobin, and corrected calcium.
[b]Systemic inflammation markers are neutrophils, platelets, and neutrophils–lymphocytes ratio.
[c]Multivariate model retained all MSKCC factors, and only systemic inflammation factors with $P<0.05$.
[d]Haemoglobin normal >13 g dl^{-1} (male); >11.5 g dl^{-1} (female).

Table 4. Reclassification of patients' prognostic classification after addition of markers of systemic inflammation[a] to MSKCC[b] factors[c]

	Reclassification			
	Good	**Good-intermediate**	**Intermediate**	**Poor**
Original prognostic classification	**Number of patients**			
Patients who died at 12 months				
Good	24	16	2	1
Intermediate	9	21	21	10
Poor	0	5	28	42
Patients alive at 12 months				
Good	65	30	3	1
Intermediate	16	17	9	2
Poor	0	6	8	4

Abbreviation: MSKCC = Memorial Sloan-Kettering Cancer Centre.
[a]Systemic inflammation markers are neutrophils, platelets, and neutrophils–lymphocytes ratio.
[b]MSKCC factors only are Karnofsky Performance Status Scale, haemoglobin, and corrected calcium.
[c]Twenty-two patients excluded because the follow-up time is <12 months and the survival status censored.

tumour promotion, invasion, and metastasis. The complex array of leukocytes and inflammatory mediators in the tumour microenvironment may be reflected in the peripheral circulation.

Neutrophilia and elevated NLR convey a poor prognosis in a variety of clinical settings including critical illness, coronary interventions, and advanced malignancies (Zahorec, 2001; Poludasu *et al*, 2009; Proctor *et al*, 2012). Furthermore, these markers of inflammation are associated with increased risk of recurrence following surgical resection in localised cancers including RCC (Ohno *et al*, 2010). In colorectal cancer, normalisation of elevated NLR after one cycle of chemotherapy is associated with improved outcomes (Chua *et al*, 2011). These findings suggest that a systemic inflammatory state may be established long before metastases become clinically evident, and abrogation of systemic inflammation may occur in response to effective therapies. The NLR is a composite of both neutrophilia and lymphopenia, which together reflect the systemic inflammatory response in these white-cell lineages in malignancy (Leitch *et al*, 2007).

We demonstrated that an elevated platelet count is an independent predictor of poor prognosis in the second-line setting. A previous study also established thrombocytosis as an independent adverse prognostic factor in patients with RCC treated with VEGF targeted therapy (Heng *et al*, 2009). The activation and aggregation of platelets occurs in response to inflammatory cytokines and ADP released from tumour cells (Alexandrakis *et al*, 2003; Suzuki *et al*, 2004). The interaction between platelets and tumour cells facilitates invasion and metastasis (Suzuki *et al*, 2004). The association between tumour-related thrombocytosis and elevated inflammatory markers (IL-1, IL-6, TNFα, CRP, and ferritin) suggests that platelet activation may reflect a systemic inflammatory state (Alexandrakis *et al*, 2003).

This study has a number of strengths. We were able to validate the prognostic values of MSKCC factors and evaluate the role of new markers of systemic inflammation using high quality

randomised trial data. As the C-statistic is often criticised for its limited clinical interpretability (Vickers, 2011), we have further provided oncologists with the practical approach of using NRI to gauge the extent to which the new prognostic classification correctly reclassifies patients' levels of risk. As there was no significant difference in the treatment effect of the randomised agents in the EGF20001 trial, the baseline prognosis of patients would not have been modified by the treatment assignment.

This study has several limitations. The models were developed in patients previously treated with immunotherapy. Patients with metastatic RCC today have access to a larger number of effective therapies, such as sunitinib, sorafenib, and bevacizumab; these agents have revolutionised the treatment of metastatic RCC and have largely replaced immunotherapy as the first-line standard of care in routine clinical practice. For simplicity, we have assumed all factors identified in the multivariate models to have equal importance. This provides a straightforward extension of the widely accepted MSKCC approach. However, equal weighting of the importance of each factor could reduce precision and potentially misclassify some patients' prognosis. As the primary objective of the EGF20001 trial was not to investigate the impact of markers of systemic inflammation on OS, the trial protocol did not specify quantitative methods and reproducibility in the measurement of these markers, and this information was not collected to allow a complete assessment and reporting of assay methods to address the REMARK criteria (McShane *et al*, 2005). We have also not validated these markers as new prognostic factors in an independent cohort of similar patients. As patients in this data set were selected for the EGF20001 trial, the applicability of this new prognostic model in the wider non-trial population remains unknown.

In conclusion, an elevated neutrophil count, an elevated platelet count, and a high NLR contribute significantly to prognostic classification in addition to MSKCC factors for previously treated patients with advanced RCC. These markers reflect the importance of systemic inflammation in determining survival for these patients. Upon validation of these results in independent studies, stratification of patients using these markers in future clinical trials can be recommended.

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