Effect of Systemic Inflammation on Survival in Patients With Metastatic Renal Cell Carcinoma Receiving Second-line Molecular-targeted Therapy

Effect of systemic inflammation on survival in patients with metastatic renal cell carcinoma receiving second-line molecular-targeted therapy

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Conflict of interest

Tsunenori Kondo has received honoraria from Pfizer, Bayer and Novartis. All other authors have no conflict of interest to declare.

Short title

Systematic inflammation on mRCC

Microabstract

We evaluated the relationship between systematic inflammatory markers (C-reactive protein, neutrophil/lymphocyte ratio, and platelet/lymphocyte ratio) and survival in a cohort of 63 patients with metastatic renal cell carcinoma receiving 2nd-lilne molecular-targeted therapy after 1st-line tyrosine kinase inhibitor failure. Each marker was associated with progression-free and overall survival. In particular, C-reactive protein was a strong independent predictive biomarker of prognosis.

Abstract

Background: The role of systemic inflammatory markers, including C-reactive protein (CRP), the neutrophil/lymphocyte ratio (NLR), and the platelet/lymphocyte ratio (PLR), in predicting survival for patients with metastatic renal cell carcinoma (mRCC) receiving 2nd-line molecular-targeted therapy (mTT) after 1st-line tyrosine kinase inhibitor failure remains unclear. Thus, we investigated the relationship between systemic inflammation and survival in such patients.

Patients and Methods: Sixty-three patients were evaluated. Progression-free survival (PFS) and overall survival (OS) after 2nd-line mTT initiation were evaluated based on inflammatory markers. In addition, the prognostic factors for survival were examined.

Results: The receiver operating characteristic curves for CRP, NLR, and PLR had areas under the curve of 0.779, 0.619, and 0.655, respectively; no significant differences were noted. The cutoff values were 0.48, 2.53, and 183, respectively. Patients with higher CRP (n = 40), NLR (n = 32), and PLR (n = 22) had significantly lower PFS and OS compared to those with lower CRP, NLR, and PLR. Multivariate analyses showed that CRP was the sole independent predictor for PFS and OS.

Conclusion: Systemic inflammation is associated with survival after 2nd-line mTT. In particular, CRP was a strong independent predictive biomarker of prognosis.

Keywords

C-reactive protein; neutrophil/lymphocyte ratio; platelet/lymphocyte ratio; biomarker; prediction

Introduction

The current treatment strategy for metastatic renal cell carcinoma (mRCC) consists of molecular-targeted therapy (mTT) to improve and prolong patient survival [1]. To further improve treatment strategy and, consequently, patient survival, identifying predictive factors or risk classifications are necessary.

The functional relationship between systemic inflammation and cancer is well recognized [2, 3]. The tumor microenvironment, which is orchestrated by inflammatory cells, is an indispensable factor in the neoplastic process, fostering cell proliferation, survival and migration [4]. In renal cell carcinoma (RCC), an association between systemic inflammation and prognosis has been demonstrated. C-reactive protein (CRP) has been identified as an independent predictive biomarker for survival in patients with RCC with or without metastasis [5-9]. Moreover, the neutrophil/lymphocyte ratio (NLR) has been identified as an effective predictor in patients with mRCC receiving mTT [10, 11]. These relationships have been attributed to the mechanism in which RCC cells produce inflammatory cytokines, such as interleukin-6, inducing CRP and neutrophils [12-15]. Furthermore, the platelet/lymphocyte ratio (PLR) has been identified as a predictive biomarker in patients with cancers, including RCC [16-18]. Several platelet-derived cytokines

related to tumor angiogenesis regulation, such as vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived growth factor, have been found to be elevated in patients with cancer [19, 20].

In a recent retrospective large-scale study, neutrophil and platelet levels were found to be independent predictive factors of prognosis in patients with mRCC who received 2nd-line mTT after progression from 1st-line therapy [21]. Therefore, we hypothesized that NLR and/or PLR would also be associated with patient survival after 2nd-line mTT. Indeed, to the best of our knowledge, the effect of NLR or PLR in predicting survival in a 2nd-line setting remains unclear, although this has been demonstrated in 1st-line therapy [10, 18]. Moreover, the association among systematic inflammatory markers, including CRP, NLR, and PLR, in a 2nd-line setting is unknown.

Thus, we investigated the correlation among systemic inflammatory markers, (CRP, NLR, and PLR), as well as influence of these biomarkers in the predicting of survival in patients with mRCC receiving 2nd-line mTT after 1st-line TKI failure.

Patients and Methods

Patient and study design

Of the 115 patients who received 2nd-line mTT at our department between January 2007 and August 2016, those who had received prior cytokine therapy (n = 23), received dialysis therapy (n = 6), shifted to 2nd-line mTT because of adverse events in 1st-line therapy (n = 11), received the mammalian target of rapamycin inhibitor (mTORi) as a 1st-line agent (n = 7), and had missing data (n = 22) were excluded. The remaining 63 patients were evaluated. Clinical and laboratory data were obtained from an electronic database and the patients' medical records.

The Internal Ethics Review Board of Tokyo Women's Medical University approved this retrospective study (ID: 4109), which was performed in accordance with the principles outlined in the Declaration of Helsinki.

Systemic inflammatory markers

We evaluated three systemic inflammatory markers; CRP, NLR, and PLR, which were examined within 1 month prior to the initiation of 2nd-line mTT. All markers were obtained from the same blood sample, and all laboratory tests were performed at the same facility (i.e., Tokyo Women's Medical University).

Endpoints

The endpoints included progression-free survival (PFS) and overall survival (OS) after 2nd-line mTT. PFS was defined as the time from 2nd-line mTT initiation to the date of progression or death from any cause, while OS was defined as the time from 2nd-line mTT initiation to death from any cause.

Statistical analysis

PFS and OS were calculated using the Kaplan-Meier method, and statistical significance was determined using the log-rank test. The ability of the three systemic inflammatory markers to predict PFS was evaluated using the are under curve (AUC) in a receiver operating characteristics (ROC) curve analysis. The cutoff values for the three markers were defined using the maximum Youden index [22]. Moreover, to examine the relationships between CRP and NLR, CRP and PLR, and NLR and PLR, the three markers were plotted against each other in scatter plots, and the Pearson's correlation coefficient was calculated. PFS and OS were compared based on cutoff values. Univariate and multivariate analyses for PFS and OS were performed using the Cox proportional hazards model. To manage larger statistical effects for the categorical classification based on

dichotomous values in CRP, NLR and PLR, we performed multivariate analyses modeling these markers both as a categorical classification (Model 1) and as a continuous variable (Model 2). Survival risk was expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). All statistical analyses were performed using JMP software (version 11; SAS Institute Inc., Cary, NC, USA), and a *P*-value <0.05 was considered statistically significant.

Results

Patient background

Table 1 shows the patients' characteristics. Thirty-six patients were ≥65 years old (57.1%), and 44 patients were male (69.8%). Clear-cell carcinoma (CCC) was observed in 48 patients (76.2%), and non-CCC, including papillary renal cell carcinoma, CCC with spindle, Bellini duct carcinoma, medullary carcinoma, mucinous tubular and spindle cell carcinoma, and unknown, were observed in 5 (7.94%), 4 (6.35%), 1 (1.59%), 1 (1.59%), 1 (1.59%), and 3 (4.76%) patients, respectively. Prior nephrectomy was performed in 58 patients (92.1%), including partial and radical nephrectomy in 1 (1.59%) and 57 (90.5%) patients, respectively. Metastasis was found in the lung, bone, liver, and lymph node in 52

(82.5%), 13 (20.6%), 11 (17.5%), and 19 (30.2%) patients, respectively; 42 patients (66.7%) had multiple metastasis. Sorafenib, sunitinib, and pazopanib were administered as 1st-line TKI in 22 (34.9%), 38 (60.3%), and 3 (4.76%) patients, respectively. TKI and mTORi were administered as a 2nd-line mTT agent in 52 (82.5%) and 11 (17.5%) patients, respectively. TKI included sorafenib (n = 2, 3.18%), sunitinib (n = 15, 23.8%), axitinib (n = 32, 50.8%), and pazopanib (n = 3, 4.76%); mTORi included temsirolimus (n = 3, 4.76%) and everolimus (n = 8, 12.7%). Based on the 1st-line Memorial Sloan Kettering Cancer Center (MSKCC) risk classification, 9 (14.3%), 47 (74.6%), and 7 (11.1%) patients were classified as favorable, intermediate, and poor, respectively. Thirty-six patients (57.1%) had ≥8 months of 1st-line PFS. Based on the 2nd-line MSKCC risk classification, identified according to Motzer's risk classification [23], 5 (7.94%), 42 (66.7%), and 16 (25.4%) patients were classified as favorable, intermediate, and poor, respectively. Means ± standard deviation (SD) for the absolute value of CRP, neutrophil count, lymphocyte count, platelet count, NLR, and PLR at the time of 2nd-line initiation were 2.16 ± 3.27 mg/dL, 3300 ± 1600/mm^3, 1300 ± 510/mm^3, 190,000 ± 84,000/mm^3, 3.09 ± 2.71, and 166.5 ± 101.7, respectively. According to the Common Terminology Criteria for Adverse Events ver. 4.0, due to

myelosuppression from 1st-line therapy evaluated at the time of 2nd-line initiation, grade 2 neutropenia, lymphocytopenia, and thrombocytopenia were observed in 8 (12.7%), 2 (3.18%), and 3 patients (4.76%), and grade 3 were observed in 1 (1.59%), 3 (4.76%), and 1 patient (1.59%), respectively. Mean ± SD at follow-up was 16.3 ± 12.6 months.

Association between systemic inflammatory markers and survival

Using the maximum Youden index, 0.48, 2.53, and 183 were selected as the optimal threshold value for CRP, NLR, and PLR, respectively. Based on these cutoffs, ROC analyses were conducted to evaluate the association between the three markers and PFS. The AUC for CRP, NLR, and PLR was 0.779 (95% CI 0.604-0.891), 0.619 (0.463-0.754), and 0.655 (0.513-0.775), respectively. No significant differences among the AUCs for the markers (*P* = 0.251) were observed (Figure 1). Patients were classified into high systemic inflammatory marker and low marker groups (i.e., high CRP, NLR, and PLR groups). Patients in the high CRP group (n = 40) had significantly lower PFS and OS compared to those in the low CRP group (median PFS: 4.74 vs. 19.9 months; OS: 9.6 vs. 38.7 months, *P's* < 0.0001; Figures 2A and 2B). Similarly, patients in the high NLR

group (n = 31) had significantly lower PFS and OS compared to those in the low NLR group (median PFS: 5.07 vs. 8.19 months, $P = 0.0059$; OS: 10.4 vs. 28.8 months, $P = 0.0006$; Figures 2C and 2D). In addition, patients in the high PLR group (n = 22) had significantly lower PFS and OS compared to those in the low PLR group (median PFS: 3.69 vs. 8.82 months, $P < 0.0001$; OS: 9.11 vs. 28.0 months, $P < 0.0001$; Figures 2E and 2F).

Relationships among systemic inflammatory markers

A strong correlation was found between NLR and PLR ($R^2 = 0.675$, $P < 0.0001$). The relationship between CRP and NLR ($R^2 = 0.119$, $P = 0.0056$) and between CRP and PLR ($R^2 = 0.132$, $P = 0.0034$) were weak, although statistical significance was found (Figures 3A-C).

Independent predictors for survival

Univariate analyses for PFS and OS showed that pathology, 1st-line PFS, the 2nd-line MSKCC risk classification, CRP, NLR and PLR were significant predictors (all $P < 0.05$), whereas other factors, including age, sex, 2nd-line agent, or the 1st-line MSKCC risk classification were insignificant predictors (all $P > 0.05$).

Multivariate analysis for PFS showed that CRP was an independent predictor both as a categorical classification in Model 1 (HR 3.72, p = 0.0007) and as a continuous variable in Model 2 (HR 1.27, p < 0.0001), while PLR was an independent predictor only as a categorical classification in Model 1 (HR 2.81, p = 0.0233) (Table 2). Multivariate analysis for OS showed that CRP was an independent predictive biomarker both as a categorical classification (HR 2.67, p = 0.0268) and as a continuous variable in Models 1 and 2 (HR 1.31, p < 0.0001) (Table 3).

Discussion

A close relationship between systemic inflammation and prognosis in RCC has been recognized. Chronic systemic inflammation caused by CRP or neutrophils produced by cancer cells can affect the host's nutritional condition, resulting in a poor prognosis, by directly accelerating tumor growth/dissemination or impairing treatment tolerability [24, 25]. Moreover, lymphocytes play a role in the host's immunity against cancers and are thought to possess an antitumor effect by inducing cell apoptosis, suppressing tumor growth and migration, and mediating cytotoxicity [3]. Although CRP, NLR, and PLR have been identified as effective

biomarkers, the correlation among them in patients with mRCC remains unclear, and the role of these markers as predictors for survival with 2nd-line mTT is also controversial. To the best of our knowledge, this is the first study demonstrating that pre-treatment values of NLR, and PLR are closely related, and that patients with high CRP, NLR, and PLR have lower PFS and OS with 2nd-line mTT after 1st-line TKI failure in mRCC. Finally, CRP appears to be a strong, independent predictive biomarker for PFS and OS.

CRP has been identified as an independent biomarker for survival in patients with RCC with or without metastasis [5-9]. In mTT, the predictive value of CRP has been demonstrated [9, 26]. However, clinical information on CRP as a predictive biomarker in 2nd-line mTT after 1st-line TKI failure is limited [27]. Similarly, studies evaluating the prognostic value of NLR and PLR with 2nd-line mTT are few. Ko et al. previously indicated that neutrophilia and thrombocytosis were independent factors for OS following the initiation of 2nd-line therapy after progression from 1st-line therapy for mRCC [21]. However, in the present study, after adjustment for CRP, PLR's predictive value was only observed for PFS as a categorical classification, while NLR was not significantly associated with PFS or OS; these findings seemed to be partially inconsistent with the previous report by Ko et al. [21]. We

speculate that two potential reasons for this discrepancy exist. First, different models in the multivariate analyses were used [21] (i.e., a factor of 'CRP' was not incorporated in the previous study). Therefore, a different analysis model might have resulted in a different statistical finding. Second, a possible myelosuppression due to 1st-line therapy may have caused neutropenia, lymphocytopenia or platelet depletion. A previous study reported that 15-20% of patients experienced myelosuppression after TKI treatment including sunitinib [28]. Although sorafenib is considered to be safer than sunitinib, 10% of patients experience cytopenia with 1st-line sorafenib therapy in the Japanese population [29]. In the present cohort, sunitinib and sorafenib were used in the majority of patients as a 1st-line agent; therefore, the myelosuppressive effect possibly existed. Indeed, a few patients displayed myelosuppression at the time of 2nd-line initiation, as shown in Table 1.

This study has several limitations. First, a retrospective single-center design with a relatively small cohort was used, possibly resulting in bias. Second, the enrolled patients received different targeted agents. Although a univariate analysis showed no statistical difference in survival based on the type of agent, a possible bias may still exist. Third, the rate of adverse events and relative dose intensity

were not evaluated.

Conclusion

Systemic inflammatory markers (specifically, CRP, NLR, and PLR) have prognostic value for PFS and OS in patients with mRCC receiving 2nd-line mTT after 1st-line TKI failure. Furthermore, CRP was demonstrated to be a strong independent predictive biomarker. Patients with systemic inflammation have a poor prognosis despite 2nd-line therapy initiation. Therefore, careful follow-up must be performed for such high-risk patients. In addition, these markers may be used to determine the best use of 2nd-line agents.

Clinical practice points

- We demonstrated the impact of systematic inflammatory markers, including CRP, NLR, and PLR on predicting PFS and OS in a cohort of 63 patients with mRCC who received 2nd-line mTT after 1st-line TKI failure.
- Patients with higher CRP, NLR, and PLR had significantly lower PFS and OS after 2nd-line mTT, compared to those with lower CRP, NLR and PLR (p's < 0.05).

- Pre-treatment NLR and PLR were closely related (R^2 = 0.675), whereas CRP was not strongly associated with NLR (R^2 = 0.119) or PLR (R^2 = 0.132).
- Pre-treatment CRP was a strong independent predictive biomarker of PFS and OS after 2nd-line mTT for mRCC as a categorical classification (PFS: HR 3.72 p = 0.0007, OS: HR 2.67 p = 0.0288) and as a continuous variable (PFS: HR 1.27 p < 0.0001, OS: HR 1.31 p < 0.0001), after adjusting for other factors including pathology, 1st-line PFS, 2nd-line MSKCC, NLR, and PLR.

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Reference

1. Figlin R, Sternberg C, Wood CG. Novel agents and approaches for advanced renal cell carcinoma. *The Journal of urology.* 2012;188:707-715.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet.* 2001;357:539-545.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008;454:436-444.

4. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420:860-867.

5. Steffens S, Kohler A, Rudolph R, et al. Validation of CRP as prognostic marker for renal cell carcinoma in a large series of patients. *BMC cancer.* 2012;12:399.

6. Saito K, Tatokoro M, Fujii Y, et al. Impact of C-reactive protein kinetics on survival of patients with metastatic renal cell carcinoma. *European urology.* 2009;55:1145-1153.

7. Lamb GW, McArdle PA, Ramsey S, et al. The relationship between the local and systemic inflammatory responses and survival in patients undergoing resection for localized renal cancer. *BJU Int.* 2008;102:756-761.

8. de Martino M, Klatte T, Seemann C, et al. Validation of serum C-reactive protein (CRP) as an independent prognostic factor for disease-free survival in patients with localised renal cell carcinoma (RCC). *BJU Int.* 2013;111:E348-353.

9. Beuselinck B, Vano YA, Oudard S, et al. Prognostic impact of baseline serum C-reactive protein in patients with metastatic renal cell carcinoma (RCC) treated with sunitinib. *BJU Int.* 2014;114:81-89.

10. Na N, Yao J, Cheng C, et al. Meta-analysis of the efficacy of the pretreatment neutrophil-to-lymphocyte ratio as a predictor of prognosis in renal carcinoma patients receiving tyrosine kinase inhibitors. *Oncotarget.* 2016.

11. Templeton AJ, Knox JJ, Lin X, et al. Change in Neutrophil-to-lymphocyte Ratio in Response to Targeted Therapy for Metastatic Renal Cell Carcinoma as a Prognosticator and Biomarker of Efficacy. *European urology.* 2016;70:358-364.

12. Jabs WJ, Busse M, Kruger S, Jocham D, Steinhoff J, Doehn C. Expression of C-reactive protein by renal cell carcinomas and unaffected surrounding renal tissue. *Kidney international.* 2005;68:2103-2110.

13. Negrier S, Perol D, Menetrier-Caux C, et al. Interleukin-6, interleukin-10, and vascular endothelial growth factor in metastatic renal cell carcinoma: prognostic value of interleukin-6--from the Groupe Francais d'Immunotherapie. *Journal of clinical oncology.* 2004;22:2371-2378.

14. Song W, Yeh CR, He D, et al. Infiltrating neutrophils promote renal cell carcinoma progression via VEGFa/HIF2alpha and estrogen receptor beta

signals. *Oncotarget.* 2015;6:19290-19304.

15. Cuadros T, Trilla E, Sarro E, et al. HAVCR/KIM-1 activates the IL-6/STAT-3 pathway in clear cell renal cell carcinoma and determines tumor progression and patient outcome. *Cancer research.* 2014;74:1416-1428.

16. Ding N, Pang Z, Shen H, Ni Y, Du J, Liu Q. The Prognostic Value of PLR in Lung Cancer, a Meta-analysis Based on Results from a Large Consecutive Cohort. *Scientific reports.* 2016;6:34823.

17. Hu H, Yao X, Xie X, et al. Prognostic value of preoperative NLR, dNLR, PLR and CRP in surgical renal cell carcinoma patients. *World journal of urology.* 2016.

18. Gunduz S, Mutlu H, Tural D, et al. Platelet to lymphocyte ratio as a new prognostic for patients with metastatic renal cell cancer. *Asia-Pacific journal of clinical oncology.* 2015;11:288-292.

19. Pinedo HM, Verheul HM, D'Amato RJ, Folkman J. Involvement of platelets in tumour angiogenesis? *Lancet.* 1998;352:1775-1777.

20. Peterson JE, Zurakowski D, Italiano JE, Jr., et al. VEGF, PF4 and PDGF are elevated in platelets of colorectal cancer patients. *Angiogenesis.* 2012;15:265-273.

21. Ko JJ, Xie W, Kroeger N, et al. The International Metastatic Renal Cell Carcinoma Database Consortium model as a prognostic tool in patients with metastatic renal cell carcinoma previously treated with first-line targeted therapy: a population-based study. *The Lancet. Oncology.* 2015;16:293-300.

22. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biometrical journal.* 2005;47:458-472.

23. Motzer RJ, Bacik J, Schwartz LH, et al. Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. *Journal of clinical oncology.* 2004;22:454-463.

24. Ishihara H, Kondo T, Omae K, et al. Sarcopenia and the Modified Glasgow Prognostic Score are Significant Predictors of Survival Among Patients with Metastatic Renal Cell Carcinoma Who are Receiving First-Line Sunitinib Treatment. *Targeted oncology.* 2016;11:605-617.

25. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med.* 2006;119:526.e529-517.

26. Teishima J, Kobatake K, Kitano H, et al. The impact of change in serum C-

reactive protein level on the prediction of effects of molecular targeted therapy in patients with metastatic renal cell carcinoma. *BJU Int.* 2016;117:E67-74.

27. Miyake H, Harada K, Ozono S, Fujisawa M. Prognostic Significance of Early Tumor Shrinkage Under Second-Line Targeted Therapy for Metastatic Renal Cell Carcinoma: A Retrospective Multi-Institutional Study in Japan. *Molecular diagnosis & therapy.* 2016;20:385-392.

28. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *The New England journal of medicine.* 2007;356:115-124.

29. Akaza H, Oya M, Iijima M, et al. A large-scale prospective registration study of the safety and efficacy of sorafenib tosylate in unresectable or metastatic renal cell carcinoma in Japan: results of over 3200 consecutive cases in post-marketing all-patient surveillance. *Japanese journal of clinical oncology.* 2015;45:953-962.

Figure legends

Figure 1: Comparisons among systemic inflammatory markers in the area under the ROC curves for PFS.

No significant differences in AUCs among CRP, NLR, and PLR (P = 0.251) were found.

ROC, receiver operating characteristics; PFS, progression-free survival; AUC, area under the curve; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio

Figure 2: (A-B) The patients were classified into high (n = 40) and low (n = 23) CRP groups, according to the cutoff of 0.48. PFS and OS were significantly lower in patients with high CRP (both, $P < 0.0001$). (C-D) The patients were classified into high (n = 31) and low (n= 32) NLR groups, according to the cutoff of 1.15. PFS and OS were significantly lower in patients with high NLR (PFS, $P = 0.0059$; OS, $P = 0.0006$). (E-F) The patients were classified into high (n = 22) and low (n = 41) PLR groups, according to the cutoff of 183. PFS and OS were significantly lower in patients with high PLR (both $P < 0.0001$).

CRP, C-reactive protein; PFS, progression-free survival; OS, overall survival; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio

Figure 3: Correlations between systemic inflammatory markers.

(A) CRP and NLR. (B) CRP and PLR. (C) NLR and PLR. A strong correlation was observed between NLR and PLR ($R^2 = 0.675$, $P < 0.0001$).

Table 1: Patient characteristics

Variable	All (n = 63)
Age at the time of 2nd-line mTT initiation, year	
≥ 65 (ref. < 65)	36 (57.1%)
Sex	
Male (ref. female)	44 (69.8%)
Pathology	
CCC	48 (76.2%)
Non-CCC	15 (23.8%)
Papillary renal cell carcinoma type2	5 (7.94%)
CCC with spindle	4 (6.35%)
Bellini duct carcinoma	1 (1.59%)
Medullary carcinoma	1 (1.59%)
Mucinous tubular and spindle cell carcinoma	1 (1.59%)
Unknown	3 (4.76%)
Prior nephrectomy	
With (ref. without)	58 (92.1%)
Partial/radical	1 (1.59%)/57 (90.5%)
Metastatic sites at the time of 2nd-line mTT initiation	
Lung	52 (82.5%)
Bone	13 (20.6%)
Liver	11 (17.5%)
Lympho node	19 (30.2%)
Others	3 (4.76%)
Number of metastatic lesion	
Multiple (ref. solitary)	42 (66.7%)
1st-line TKI	
Sorafenib	22 (34.9%)
Sunitinib	38 (60.3%)
Pazopanib	3 (4.76%)
2nd-line mTT	
TKI	52 (82.5%)
Sorafenib	2 (3.18%)
Sunitinib	15 (23.8%)
Axitinib	32 (50.8%)
Pazopanib	3 (4.76%)

mTORi	11 (17.5%)
Temsirolimus	3 (4.76%)
Everolimus	8 (12.7%)
1st-line MSKCC risk classification	
Favorable/intermediate/poor	9 (14.3%)/47 (74.6%)/7 (11.1%)
1st-line PFS, months	
≥ 8 (ref. < 8)	36 (57.1%)
2nd-line MSKCC risk classification	
Favorable/intermediate/poor	5 (7.94%)/ 42 (66.7%)/ 16 (25.4%)
[a]CRP at the time of 2nd-line initiation, mg/dL	2.16 ± 3.27
[a] Neutrophil count, /mm^3	3300 ± 1600
[a] Lymphocyte count, /mm^3	1300 ± 510
[a] Platelet count, /mm^3	190,000 ± 84,000
[a]NLR	3.09 ± 2.71
[a]PLR	166.5 ± 101.7
Neutropenia at the time of 2nd-line initiation	
Grade 2	8 (12.7%)
Grade 3	1 (1.59%)
Lymphocytopenia at the time of 2nd-line initiation	
Grade 2	2 (3.18%)
Grade 3	3 (4.76%)
Thrombocytopenia at the time of 2nd-line initiation	
Grade 2	3 (4.76%)
Grade 3	1 (1.59%)
[a] Follow-up, month	16.3 ± 12.6

[a] Mean ± standard deviation (SD)

CCC, clear-cell carcinoma; mTT; molecular-targeted therapy; TKI, tyrosine kinase inhibitor; mTORi, mammalian target of rapamycin inhibitor; MSKCC, Memorial Sloan Kettering Cancer Center; PFS, progression-free survival; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; ref., reference

Table 2: Univariate and multivariate analyses for PFS

	Univariate HR (95% CI)	p	*Model 1 Multivariate HR (95% CI)	p	**Model 2 Multivariate HR (95% CI)	p
Age ≥ 65 (ref. < 65)	0.67 (0.38 – 1.18)	0.165				
Sex Male (ref. female)	0.75 (0.42 – 1.39)	0.349				
Pathology Non-CCC (ref. CCC)	2.82 (1.44 – 5.24)	0.0033	1.19 (0.52 – 2.60)	0.678	2.03 (0.93 – 4.20)	0.0725
1st-line agent Sorafenib (ref. sunitinib/pazopanib)	0.93 (0.49 – 1.68)	0.815				
2nd-line agent TKI (ref. mTORi)	0.67 (0.35 – 1.37)	0.256				
1st-line MSKCC Poor (ref. favorable/intermediate)	1.01 (0.35 – 2.32)	0.987				
1st-line PFS < 8 month (≥ 8 months)	2.76 (1.53 – 4.98)	0.0009	1.94 (0.99 – 3.76)	0.0541	1.95 (0.95 – 3.90)	0.0700
2nd-line MSKCC Poor (ref. favorable/intermediate)	2.82 (1.45 – 5.26)	0.0028	1.72 (0.77 – 3.66)	0.178	1.72 (0.77 – 3.59)	0.178
CRP ≥ 0.48 (ref. < 0.48)	5.42 (2.77 – 11.5)	<0.0001	3.72 (1.72 – 8.45)	0.0007	-	-
NLR ≥ 2.53 (ref. < 2.53)	2.20 (1.24 – 3.98)	0.0069	0.79 (0.34 – 1.77)	0.575	-	-
PLR ≥ 183 (ref. < 183)	3.75 (2.06 – 6.79)	<0.0001	2.81 (1.15 – 7.22)	0.0233	-	-
CRP (continuous variable)	1.32 (1.20 – 1.46)	<0.0001	-	-	1.27 (1.13 – 1.42)	<0.0001
NLR (continuous variable)	1.14 (1.03 – 1.23)	0.0154	-	-	1.10 (0.92 – 1.29)	0.264
PLR (continuous variable)	1.00 (1.00 – 1.01)	0.0057	-	-	1.00 (0.99 – 1.00)	0.929

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confident interval; CCC, clear-cell carcinoma; TKI, tyrosine kinase inhibitor; mTORi, mammalian target of rapamycin inhibitor; MSKCC, Memorial Sloan Kettering Cancer Center; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; ref., reference

*Model 1: Modeling with systematic inflammatory markers as a categorical classification

**Model 2: Modeling with systematic inflammatory markers as a continuous variable

Table 3: Univariate and multivariate analyses for OS

	Univariate HR (95% CI)	p	*Model 1 Multivariate HR (95% CI)	p	**Model 2 Multivariate HR (95% CI)	p
Age ≥ 65 (ref. < 65)	0.70 (0.38 – 1.31)	0.263				
Sex Male (ref. female)	0.78 (0.42 – 1.51)	0.451				
Pathology Non-CCC (ref. CCC)	2.45 (1.18 – 4.79)	0.0174	1.24 (0.56 – 2.67)	0.589	2.30 (1.07 – 4.67)	0.0330
1st-line agent Sorafenib (ref. sunitinib/pazopanib)	1.09 (0.56 – 2.04)	0.792				
2nd-line agent TKI (ref. mTORi)	1.26 (0.61 – 2.87)	0.548				
1st-line MSKCC Poor (ref. favorable/intermediate)	0.90 (0.22 – 2.54)	0.863				
1st-line PFS < 8 month (≥ 8 months)	2.41 (1.30 – 4.49)	0.0057	2.13 (1.11 – 4.10)	0.0239	1.68 (0.82 – 3.45)	0.157
2nd-line MSKCC Poor (ref. favorable/intermediate)	2.65 (1.31 – 5.10)	0.0076	1.20 (0.55 – 2.47)	0.639	2.46 (1.06 – 5.41)	0.0369
CRP ≥ 0.48 (< 0.48)	4.95 (2.37 – 11.6)	<0.0001	2.67 (1.12 – 6.91)	0.0268	-	-
NLR ≥ 2.53 (< 2.53)	3.10 (1.60 – 6.30)	0.0007	1.09 (0.39 – 2.86)	0.858	-	-
PLR ≥ 183 (< 183)	4.31 (2.25 – 8.39)	<0.0001	2.50 (0.91 – 7.58)	0.0750	-	-
CRP (continuous variable)	1.30 (1.18 – 1.43)	<0.0001	-	-	1.31 (1.16 – 1.49)	<0.0001
NLR (continuous variable)	1.12 (1.03 – 1.21)	0.0140	-	-	1.09 (0.91 – 1.30	0.343
PLR (continuous variable)	1.00 (1.00 – 1.01)	0.0081	-	-	1.00 (0.99 – 1.00)	0.629

*Model 1: Modeling with systematic inflammatory markers as a categorical classification

**Model 2: Modeling with systematic inflammatory markers as a continuous variable

Figure 1

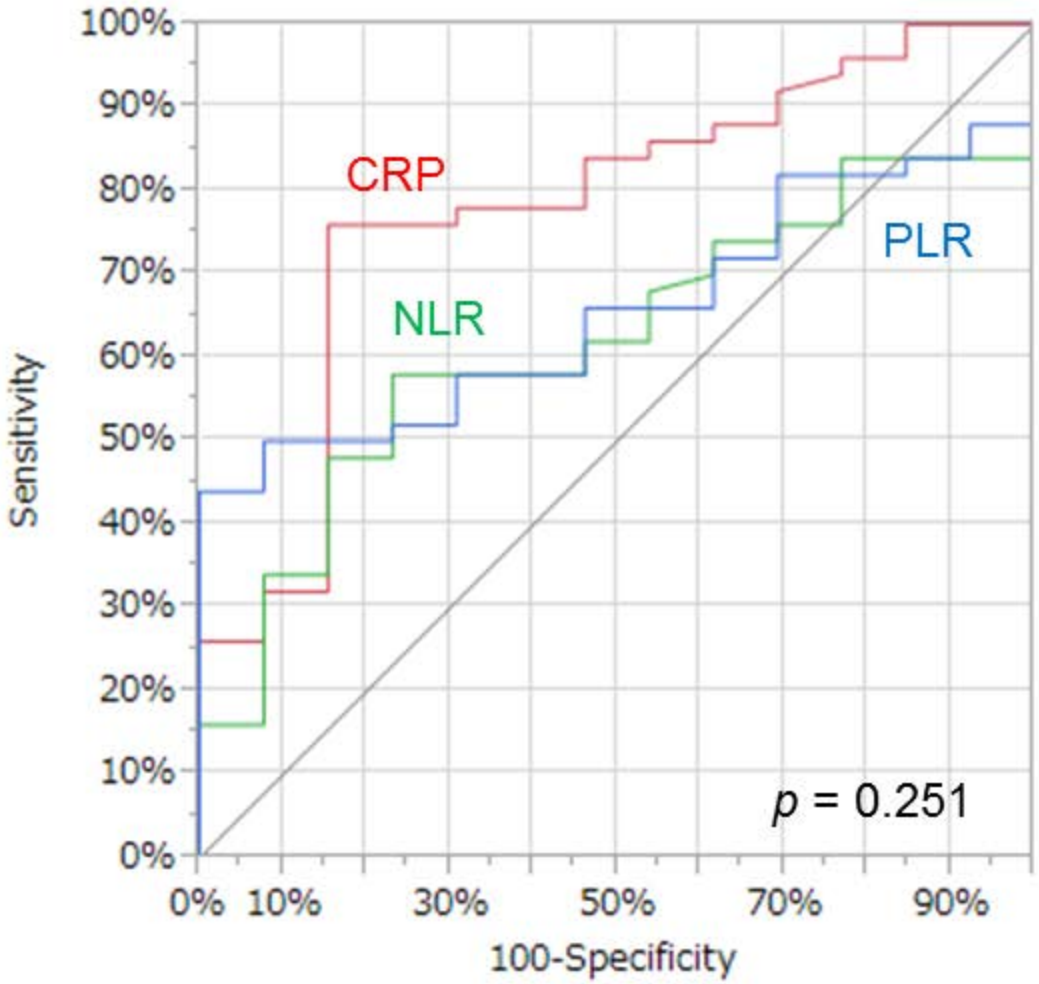

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sensitivity
CRP
PLR
NLR
p = 0.251
0% 10% 30% 50% 70% 90%
100-Specificity

Figure 2

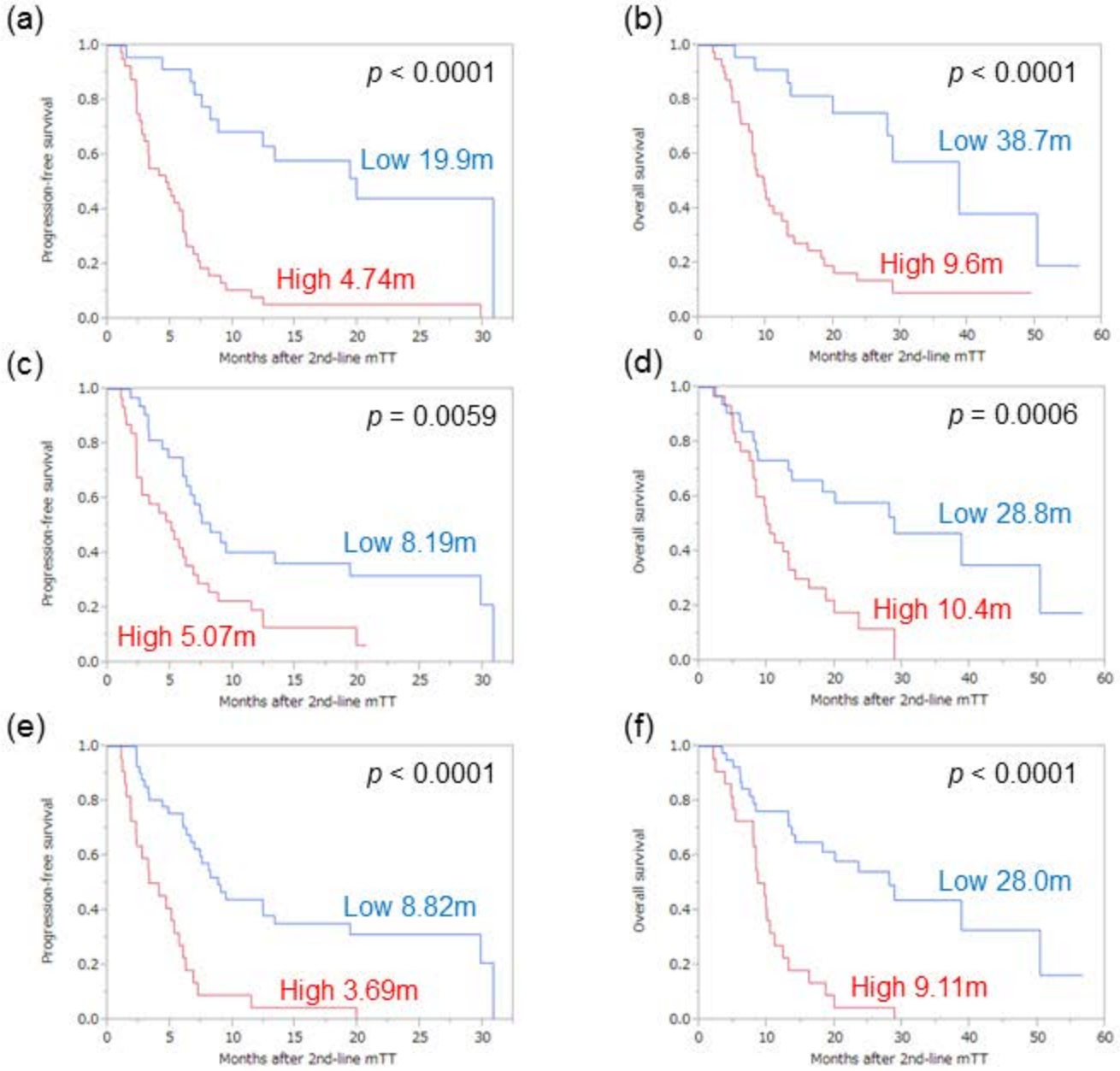
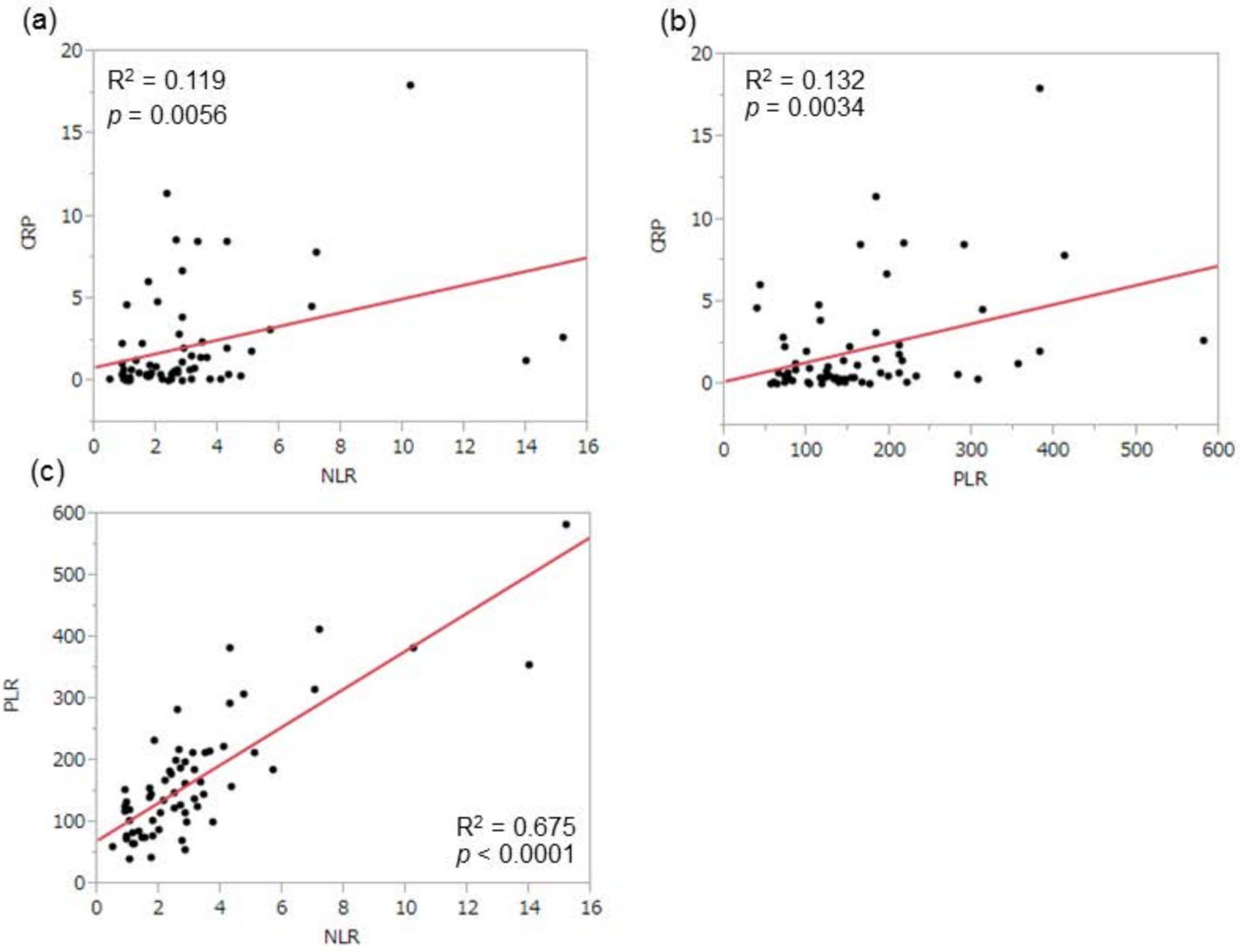

(a)
p < 0.0001
Low 19.9m
High 4.74m
Progression-free survival
Months after 2nd-line mTT
(b)
p < 0.0001
Low 38.7m
High 9.6m
Overall survival
Months after 2nd-line mTT
(c)
p = 0.0059
Low 8.19m
High 5.07m
Progression-free survival
Months after 2nd-line mTT
(d)
p = 0.0006
Low 28.8m
High 10.4m
Overall survival
Months after 2nd-line mTT
(e)
p < 0.0001
Low 8.82m
High 3.69m
Progression-free survival
Months after 2nd-line mTT
(f)
p < 0.0001
Low 28.0m
High 9.11m
Overall survival
Months after 2nd-line mTT

Figure 3


(a)
R² = 0.119
p = 0.0056
CRP
NLR
(b)
R² = 0.132
p = 0.0034
CRP
PLR
(c)
R² = 0.675
p < 0.0001
PLR
NLR