Jpn J Clin Oncol 2009;39(9)588–594
doi:10.1093/jjco/hyp068

Clinical Impact of the Presence of the Worst Nucleolar Grade in Renal Cell Carcinoma Specimens

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Received March 12, 2009; accepted May 22, 2009; published online June 25, 2009

Objective: Renal cell carcinoma (RCC) with a high-nucleolar-grade component is considered to be an aggressive type of tumor. In the present study, we evaluated the impact of the presence of the worst-nucleolar-grade component and also tried to determine predictors for recurrence and prognosis in patients with the worst grade component.
Methods: We evaluated 314 patients with RCC. A three-graded system was used for nucleolar grading, the patients were classified into four groups according to the presence of the worst nucleolar grade (Grade 3) and the occupancy of each grade, and clinicopathological factors and clinical outcomes were compared. In patients of Grade 3 components (Groups 1 and 2), factors influencing on prognosis and recurrence were evaluated by multivariate analysis.
Results: There was no significant difference in clinicopathological factors between Group 1 (with Grade 3-dominant tumors) and Group 2 (with tumors in which Grade 1 or 2 was dominant and there were Grade 3 components). Neither did cause-specific survival or recurrence-free survival differ significantly between those two groups. In multivariate analysis, only distant metastasis was an independent predictor for prognosis in all patients with Grade 3 components. Moreover, an elevated C-reactive protein (CRP) level ($\geq$1 mg/dl) was the only independent predictor of recurrence in N0M0 patients.
Conclusions: Regardless of dominancy, the presence of the worst grade component has a significant clinical impact in RCC patients. N0M0 patients whose RCC has worst-grade components but whose CRP levels are $<$1 are expected to have longer recurrence-free intervals and to survive longer than those whose CRP levels are higher.

Key words: renal cell carcinoma – nucleolar grade – C-reactive protein – prognostic factor – recurrence

INTRODUCTION

Nucleolar grade generally reflects a biological activity of cancer cell and the aggressiveness of a tumor and is associated with the prognosis of patients with renal cell carcinoma (RCC) (1–3). A recent study evaluating 1801 RCC patients treated with radical nephrectomy found histological grade (Fuhrman nuclear grade), TNM stage, tumor size $\geq$5 cm and histological tumor necrosis to be independent predictors for cancer-specific survival (3). In that study, the patients with a worst-grade tumor (Grade 4) had a risk rate for cancer-specific death more than 10 times higher than that of the patients with a low-grade tumor (Grade 1 or 2). One interesting question is whether or not the clinical characteristics of RCC patients with a worst-nucleolar-grade-dominant tumor are similar to those of RCC patients whose tumor has only a minor component of the worst grade. It is unclear whether in RCC patients whose surgical specimen has the worst-grade component, the occupancy of the worst-grade component has an impact on the patient's prognosis and the likelihood of recurrence. Therefore, the present study was undertaken to evaluate the impact of the occupancy of the worst nucleolar grade on the clinical characteristics of and clinical outcome for patients with RCC. We also tried to identify predictive factors for the prognosis and the recurrence in patients with

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RCC containing the worst-grade component. Although nucleolar grade is reportedly an independent predictor for cause-specific survival (3) and patients with high-grade tumors have a higher risk for recurrence than do patients with low-grade tumors (4), a significant amount of patients with high-grade components in the primary tumor have an excellent prognosis. Therefore, it is clinically important to identify factors that have a significant impact on recurrence and prognosis in RCC patients with the worst-nucleolar-grade component.

PATIENTS AND METHODS

Patients and Design

We reviewed the records of patients with RCC treated by radical nephrectomy or partial nephrectomy between 1989 and 2006 at our institution. During this period, nephrectomies were performed in 343 patients. We reviewed the records of all the patients and in 314 patients all clinicopathological parameters including serum C-reactive protein (CRP) levels were available. The records of the 314 RCC patients, 88 female and 226 male, who were treated by radical nephrectomy ($n = 294$) or partial nephrectomy ($n = 20$) were reviewed. Their ages ranged from 26 to 86 years (mean = 60.3 $\pm$ 12.1 years). The tumor was on the right side in 152 patients and the left side in 162 patients. Local recurrence and metastasis were monitored by examining each patient post-operatively every 3–6 months for the first 5 years and every 6–12 months thereafter. Follow-up examinations included physical examination, chest radiography, abdominal and chest computed tomography, blood tests and, if indicated, radionuclide bone scanning. Follow-up intervals (from the date of nephrectomy to the last recorded follow-up) ranged from 1 to 245 months (mean = 46.4 months). Regional lymphadenectomy was performed when lymph node swelling was detected in pre-operative radiological examination or during the nephrectomy. Disease-free survival was evaluated using the date at which local recurrence or metastatic disease was identified, and cause-specific survival was evaluated using either the date of death due to disease progression or the date of the last follow-up examination. We extensively reviewed all pathological reports for the 314 patients. In our institute, surgical specimens were handled basically according to the Japanese guideline for RCC (5) with minor modification. Briefly, when pathologists examined specimens of radically nephrectomized kidneys, each tumor was incised at a portion where a maximal cross-section of tumor was obtained. Paraffin-embedded sections were made using all separated parts of the maximal cross-section which included surrounding perinephric fat and, if existed, grossly normal kidney. To check surgical margins, sections of renal artery and vein at renal hilus, and ureteral margin were made, respectively. If a tumor had a renal vein thrombus, sections of the thrombus were also made. Furthermore, sections were additionally made at the portion where extracapsular extension was macroscopically suspected. When pathologists examined specimens of partially nephrectomized kidneys, paraffin-embedded sections were made at a portion where maximal cross-section was obtained, similar to radically nephrectomized kidneys. An additional cross-section perpendicular to the maximal cross-section was also made and paraffin-embedded sections were made using this cross-section. All sections of each tumor were examined by two independent pathologists for the determination of pathological factors including nucleolar grade. The pathological tumor stage (pT stage) was determined according to the TNM classification system (6). Nucleolar grading in a three-grade system was determined according to the Japanese classification system for RCC (5). Although whether a three- or four-grade system should be used in RCC has been discussed (7) and this issue is still controversial, three-graded system was recently recommended to use for grading system in the UICC and AJCC (8). Standardized grading was performed according to following criteria. Briefly, Grade 1 tumors have tumor cell nuclei smaller than normal tubular cell nuclei, Grade 2 tumors have tumor cell nuclei similar in size to normal tubular cell nuclei and Grade 3 tumors have tumor cell nuclei larger than normal tubular cell nuclei. Pathologists evaluated all paraffin-embedded sections of each tumor, and a dominant grade, a secondary dominant grade and, if existed, a thirdly dominant grade were recorded.

The patients were classified into four groups according to the presence or absence of the Grade 3 component (the worst grade in three-graded system) and the occupancy of each grade (Table 1). Even if the Grade 3 component was in only a focal area in the tumor specimen, the patient with that was classified into Group 2. There were 31 patients in Group 1, 52 in Group 2, 141 in Group 3 and 90 in Group 4. The clinicopathological factors evaluated in this study are listed in Table 2. All factors were compared between the groups. The cause-specific survival for each of the four groups was calculated by the Kaplan–Meier method and disease-free survival was attributed to patients with neither lymph node metastasis (LNM) nor distant metastasis (DM) (N0M0 patients). Factors influencing the prognosis for the patients with the worst histological grade (Groups 1 and 2, $n = 83$) were evaluated, as were factors influencing the recurrence in N0M0 patients ($n = 41$).

Table 1. The classification of patients with RCC by using histological grade

Group 1: Grade 3 is dominant in RCC specimens ($n = 31$)
Group 2: Grade 1 or 2 is dominant but there is Grade 3 component in specimens ($n = 51$)
Group 3: Grade 2 is dominant and there is no Grade 3 component ($n = 141$)
Group 4: Grade 1 is dominant and there is no Grade 3 component ($n = 90$)

RCC, renal cell carcinoma.

Table 2. Comparison of clinicopathological factors in four groups

	Group 1 ($n = 31$)	Group 2 ($n = 52$)	P value (Group 1 vs. 2)	Group 3 ($n = 141$)	Group 4 ($n = 90$)
Age (years)	62.4 ± 11.1	60.4 ± 12.4	0.5563*	61.8 ± 10.5	57.2 ± 14.0
Gender (F/M)	13/18	14/38	0.1579#	36/105	25/65
Tumor side (R/L)	10/21	29/23	0.0379#	79/62	44/46
Tumor size (cm)	9.9 ± 4.4	8.5 ± 3.9	0.1875*	5.5 ± 2.9	4.2 ± 2.4
pT1 or 2/pT3 or 4	11/20	26/26	0.1981#	110/31	74/16
N−/+	23/8	44/8	0.2443#	136/5	90/0
M−/+	19/12	31/21	0.8801#	128/13	81/9
Stage I or II/III or IV	8/23	20/32	0.2382#	106/35	70/20
MVI−/+	5/26	8/44	0.9281#	90/51	71/19
CRP levels (mg/dl)	6.2 ± 6.2	3.8 ± 5.4	0.0561*	0.9 ± 1.7	0.5 ± 0.7

pT stage, pathological tumor stage; MVI, microvascular invasion; CRP, C-reactive protein.
*Analyzed by Mann–Whitney U-test.
#Analyzed by χ^2 test.

Statistical Analysis

All statistical analyses were performed by using the StatView 5.0 software system for Windows (SAS Institute, Cary, NC, USA). Results are presented as mean ± standard deviation (SD), and the values of the variables of different groups were compared using the Mann–Whitney U-test. The independence of fit of categorical data was analyzed by using the χ^2 test. Survival curves were constructed by the Kaplan–Meier method, and the differences between them were assessed using the log-rank test. Cox's proportional hazard regression model was used for univariate and multivariate analyses. For all tests, a P value <0.05 was considered to indicate statistical significance.

RESULTS

Patient characteristics and pathological findings other than histological types are listed in Table 2. The predominant histological type was a conventional type in 294 patients, papillary in 6, chromophobe in 4, spindle cell in 8 and unclassified in 2. Twenty-one of the 314 patients (6.7%) had metastases in surgically resected lymph nodes, and 55 patients (17.5%) had DM that were confirmed by pathological diagnosis at operation or were obvious in pre-operative radiological examinations. The mean pre-operative level of CRP was 1.8 ± 3.6 mg/dl (median = 0.3 mg/dl), and 71 patients (22.6%) had pre-operative CRP levels ≥1 mg/dl.

None of the clinicopathological factors differed significantly between the patients in Group 1 (Grade 3 dominant) and the patients in Group 2 (Table 2). Although the CRP levels might seem to differ between those two groups, the difference is not statistically significant ($P = 0.0561$). The Kaplan–Meier method showed no significant difference in cause-specific survival between in Groups 1 and 2 (Fig. 1A, $P = 0.5564$), and for N0M0 patients, there was no significant difference in either disease-free or cause-specific survival between Groups 1 and 2 (Fig. 1B and C). These results suggest that Groups 1 and 2 were similar populations with regard to their clinicopathological characters and their recurrence and prognosis. Regardless of the occupancy of the worst-grade component, the presence of the worst-grade component appeared to have an impact on the clinical outcome in RCC patients.

We next tried to identify factors influencing prognosis and recurrence in patients with the worst nucleolar grade. Of the 83 patients with Grade 3 components, the Kaplan–Meier method revealed that the prognosis was poorer for those with a larger tumor, higher pT stage, LNM, DM, microvascular invasion or a CRP level ≥ 1 mg/dl (Fig. 2). Pre-operative CRP elevation was defined by a CRP level ≥1.0 mg/dl as reported previously (4). In multivariate analysis, only DM was an independent predictor for the prognosis ($P < 0.0001$, Table 3). In patients with both DM and Grade 3 components, the 2- and 3-year survival rates were 16.9% and 8.4%, respectively. Patients with both DM and Grade 3 components in the primary tumor thus had a quite poor prognosis. We further evaluated predictive factors for recurrence in N0M0 patients who had Grade 3 components in the primary tumor. The Kaplan–Meier method revealed that patients with pT stage ≥3, tumor size >10 cm and CRP ≥1 had recurrence rates higher than did those respective counterparts (Fig. 3). With regard to tumor size, there was a significant difference if 10 cm in size was used as cut-off (Fig. 3C). Multivariate Cox's proportional hazard model analysis evaluating these three factors showed only CRP elevation to be a significant ($P = 0.0112$, odds ratio: 6.098) predictor of disease-free survival (Table 4). A Grade 3-dominant tumor, in contrast, was not a predictive factor. For N0M0 patients with Grade 3 components in the primary tumor, the 5-year recurrence-free survival rate was 32.3% for those with a CRP ≥ 1 and was 80.8% for those with a CRP < 1. Also,

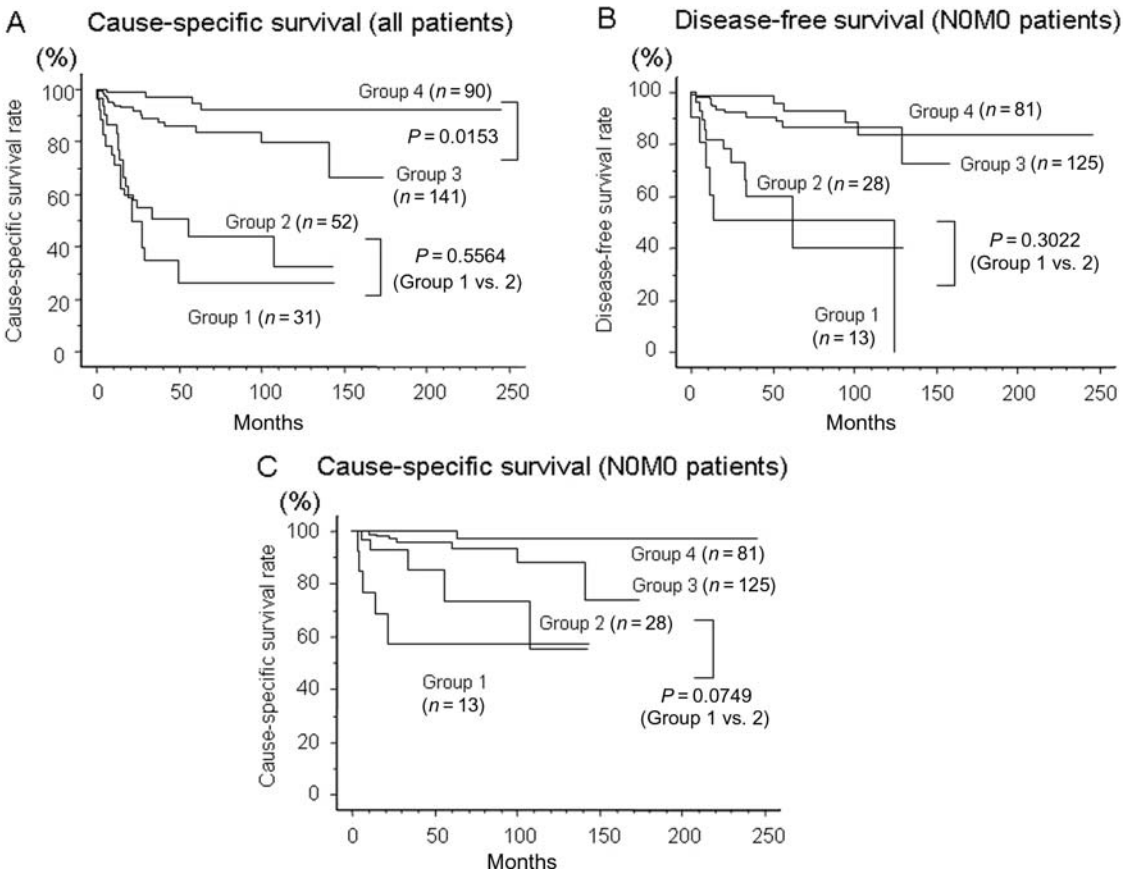

Figure 1. Disease-free survival and cause-specific survival in four groups. The Kaplan–Meier method showed no significant difference in cause-specific survival between Groups 1 and 2 (A), and for N0M0 patients, there was no significant difference in either disease-free (B) or cause-specific survival (C) between Groups 1 and 2.

the 5-year cause-specific survival rate was 53.4% for those with a CRP ≥ 1 and was 100% for those with a CRP < 1. Therefore, even RCC patients who have Grade 3 components in the primary tumor can be expected to have longer recurrence-free intervals and to survive longer if they have neither DM nor LNM and do not have elevated CRP levels.

DISCUSSION

In the present study, there was no significant difference in clinicopathological background, recurrence or prognosis between Groups 1 and 2. These results suggest that in spite of the different occupancy of the worst-grade component in surgical specimens, the populations of Groups 1 and 2 are similar with regard to clinicopathological characteristic and clinical outcome. Therefore, even if a patient's surgical specimen contains a small component of Grade 3, clinicians should follow up the patient the same way they would do for a patient with a Grade 3 dominant tumor. Hand and Broders (9) used the dominant grade for the nucleolar grading of RCC, whereas the Fuhrman grading system used the worst nucleolar grade in the specimen even if the worst grade existed focally there (10). In addition, there has been a lack of consensus about the minimal size of the worst-grade component to be considered significant (7,11). From the results of the present study, it seems to be appropriate that the worst nucleolar grade is used for grading system of RCC because the presence of the worst grade influenced the patients' prognosis, regardless of its occupancy. Since $>50\%$ of RCCs are composed of cells with different nucleolar grades (12), it is important to determine which nucleolar grade in a heterogeneous specimen is the one we should focus on when we want to predict clinical outcome. From the results of the present study, even a small component of the worst grade is considered to be significant in the prediction of patient prognosis in RCC.

To identify factors having an impact on recurrence and prognosis in patients with the worst-grade tumors, we analyzed patients whose tumors had the worst-grade component and did multivariate analysis. In our 83 patients with Grade 3 components, the presence of DM was the only independent predictor for cancer-specific death. Patients who had both Grade 3 components in the primary lesion and DM had a quite poor prognosis. Their 1-, 3- and 5-year survival rates were 56.9%, 8.4% and 0%, respectively. In contrast, the 1-, 3- and 5-year survival rates for our patients with Grade 3 components and without DM were 87.7%, 68.6% and 61.0%, respectively. When we further evaluated our 41

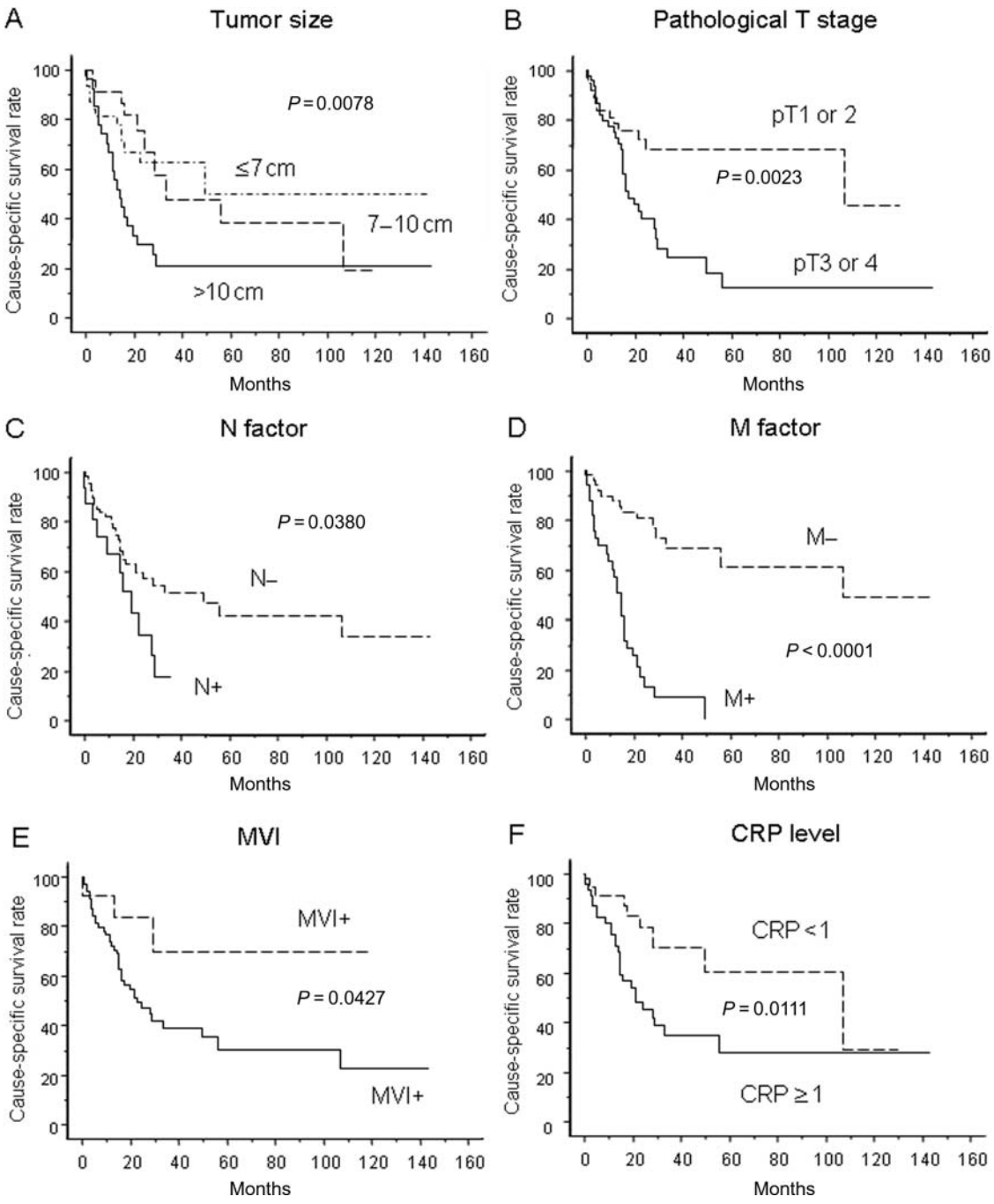

Figure 2. Cause-specific survival rates for 83 patients with Grade 3 components. The Kaplan–Meier method revealed that the prognosis was poorer for those with a larger tumor (A), higher pT stage (B), lymph node metastasis (C), distant metastasis (D), microvascular invasion (E) or a CRP level $\geq$1 mg/dl (F). LNM, lymph node metastasis; DM, distant metastasis; MVI, microvascular invasion; CRP, C-reactive protein.

N0M0 patients by the Kaplan–Meier method, CRP elevation (CRP $\geq$ 1 mg/dl) was an independent predictor for the recurrence but previously reported prognostic factors such as tumor size and pT stage were not. N0M0 patients with both Grade 3 components and pre-operative CRP $\geq$ 1 mg/dl had a high risk of recurrence. The 5-year recurrence-free survival rate for N0M0 patients with Grade 3 components and a CRP $\geq$ 1 mg/dl was 32.3%, whereas that for N0M0 patients with Grade 3 components and a CRP $<$ 1 was 80.8%. The 5-year cause-specific survival rate was also CRP-dependent: 53.4% in patients with CRP $\geq$ 1 mg/dl and 100% in patients with CRP $<$ 1. We previously reported that CRP level was also an independent predictor for recurrence in N0M0 RCC patients ($n = 142$) (4), and several authors have recently reported the significance of CRP elevation for RCC recurrence and patient prognosis (13,14). CRP was reportedly an informative predictor of cancer-specific mortality in a European study of 313 patients (14). In that report, the

Table 3. Cox's proportional hazard model for predicting prognosis of patients with the worst nucleolar grade ($n = 83$)

Variables	Univariate	Multivariate		
	P value	*P* value	Odds ratio	Relative risk ratio (95% CI)
Gender	0.7009			
Age	0.6285			
Side of tumor	0.9281			
Tumor size	0.0047	0.4093	1.035	0.954–1.122
G3 dominant	0.5576			
Pathological T3 or 4	0.0034	0.6829	1.202	0.345–2.008
N+	0.0426	0.1246	6.614	0.221–1.201
M+	<0.0001	<0.0001	9.804	0.043–0.246
MVI+	0.0552			
CRP ≥ 1	0.0111	0.1213	1.972	0.215–1.197

Table 4. Cox's proportional hazard model for predicting recurrence of N0M0 patients with the worst grade histology ($n = 41$)

Variables	Univariate	Multivariate		
	P value	*P* value	Odds ratio	Relative risk ratio (95% CI)
Gender	0.6107			
Age	0.8618			
Side of tumor	0.9680			
Tumor size >10	0.0254	0.5041	1.587	0.163–2.443
G3 dominant	0.1695			
Pathological T3 or 4	0.0886	0.6550	1.360	0.353–5.237
MVI+	0.2470			
CRP ≥ 1	0.0050	0.0112	6.098	1.508–25

model with CRP had better predictive accuracy for cancer-specific mortality at 2 and 5 years than the UCLA Integrated Staging System did. Saito et al. (15) reported an impact of CRP kinetics on the survival of patients with metastatic RCC. They divided RCC patients into three groups: patients whose pre-operative CRP levels were <0.5 mg/dl, patients whose pre-operative CRP levels were >0.5 mg/dl and normalized at least one time during treatment, and patients whose pre-operative CRP levels were >0.5 mg/dl and never normalized. CRP kinetics was an independent predictor for overall survival. Our results of multivariate analysis show that in RCC patients with Grade 3 components, CRP level

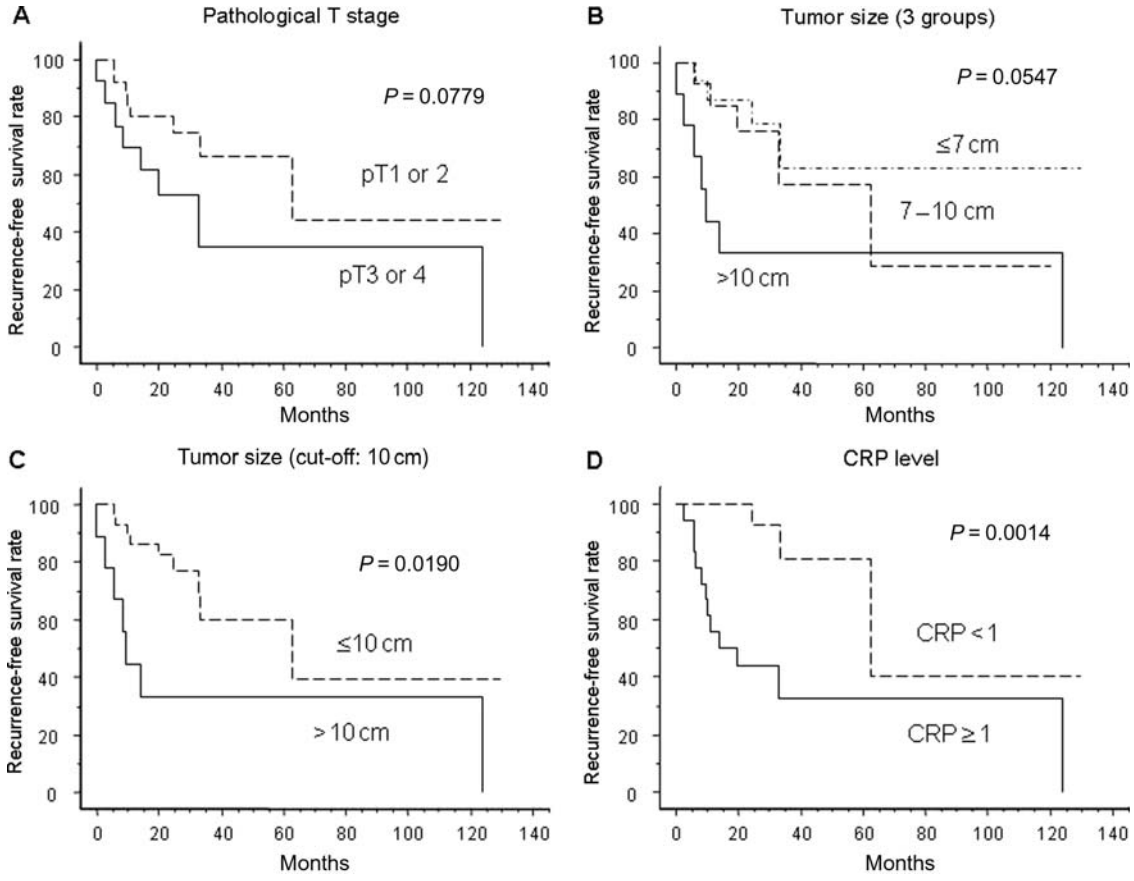

Figure 3. Recurrence-specific survival rates for N0M0 patients with Grade 3 components. The Kaplan–Meier method revealed that patients with tumor size >10 cm (B and C) and CRP ≥ 1 (D) had recurrence rates significantly higher than did those respective counterparts.

predicts recurrence better than tumor size and pT stage do. Pre-operative CRP level might reflect a biological potential of RCC and predict the presence of micrometastasis in N0M0 patients.

CONCLUSIONS

Patients with Grade 3-dominant RCC and those with RCC, in which Grade 1 or 2 is dominant and there are Grade 3 components, have similar clinicopathological characteristic and clinical outcomes. Even RCC patients who have Grade 3 components in the primary tumor have an excellent prognosis if they have neither DM nor LNM and do not have elevated CRP levels ($\geq$1 mg/dl). These results may provide important information for counseling RCC patients with the worst-grade component regarding their prognosis and risk of recurrence.

Conflict of interest statement

None declared.

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