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ANATOMICAL PATHOLOGY

Percentage grade 4 tumour predicts outcome for clear cell renal cell carcinoma

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Summary

Heterogeneity of tumour grading is common in clear cell renal cell carcinoma (ccRCC). WHO/ISUP grading specifies that RCC should be graded based on the highest grade present in at least one high power field. This does not take into account the proportion of high grade tumour present in a cancer, which may itself influence outcome. Cases of ccRCC accessioned by Aquesta Uropathology, Brisbane, Australia, between 2008 and 2015, were reviewed and grading assigned according to WHO/ISUP criteria. For tumours classified as grade 3 (G3) and 4 (G4), the percentage of tumour showing G3 and G4 morphology was assessed for each case. Survival analysis, with time to the development of metastases as the clinical outcome, was performed for six grading subclasses (G3 <10%, G3 10–50%, G3 >50%, G4 <10%, G4 10–50%, G4 >50%). Of the 681 cases of ccRCC in the series, there were 153 cases classified as G3 (91 cases) and G4 (62 cases) for which follow-up was available. During the follow-up period of <1–89 months, 19 (20.9%) patients with G3 and 30 (48.3%) patients with G4 cancers developed metastatic disease. The three subgroups of <10%, 10–50% and >50% G3 tumour were not significant in predicting outcome (p=0.47). Separating G3 into two groups of $\leq$50% vs >50% was also not significantly associated with outcome (p=0.22). For the three subgroups of G4 ccRCC (<10%, 10–50% and >50% G4) a higher percentage of G4 correlated with time to the development of metastases (p=0.01). Even though G4 tumours as a whole had a significantly worse outcome than G3 tumours (p=0.0004), the difference between G4 <10% and G3 tumours was not significant (p=0.27). On multivariate analysis, that included pT staging category and tumour size, there was a significant difference in survival between G4<10% and G4>50% tumours (p=0.018). The results of the study suggest that for ccRCC, WHO/ISUP G4 category should incorporate the percentage of G4 tumour present.

Key words: Renal cell carcinoma; grade; World Health Organization; International Society of Urological Pathology; prognosis.

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INTRODUCTION

The grading of renal cell carcinoma (RCC) has undergone considerable evolution since the first classification of grading of renal malignancies proposed by Hand and Broders in 1932.[1] More recent classifications have focused on nuclear features, with the recently adopted World Health Organization (WHO)/International Society of Urological Pathology (ISUP) classification being based upon nucleolar size for the first 3 grades, while grade 4 tumours require the identification of extreme nuclear pleomorphism, including atypical tumour giant cells, and/or sarcomatoid/rhabdoid differentiation.[2–4] Validation studies have shown this grading system to correlate with outcome for clear cell and papillary RCC.[5–8] Clear cell RCC usually does not show uniform grade throughout the whole tumour if sampled widely and currently WHO/ISUP grading is based upon the single high power field having the highest grade within the sampled component of the tumour. This implies that the tumour clone exhibiting the highest grade has the greatest influence on outcome. This recommendation is not unusual in pathology practice, although for some malignancies the volume of the specific components of a tumour are taken into account when assigning a final grade. The most obvious example of this is Gleason scoring of prostate adenocarcinoma. In the 2005 modification of Gleason score, as well as the recently developed ISUP grading system, grading of a needle biopsy is based upon both the highest-volume and highest-grade tumour pattern.[9]

The WHO/ISUP grading system for RCC does not take into account the extent of assigned grade and the assumption is that a small focus of high grade tumour has a similar outcome to a tumour that is predominantly high grade. In an earlier study the prognostic significance of percentage of high grade carcinoma in RCC was assessed,[10] with cases divided into 0% grade 3 + grade 4 (i.e., grade 1 and 2 tumours), 1–50% grade 3 + grade 4 and 51–100% grade 3 + grade 4 tumours. A significant difference in outcome, determined as time to metastases, time to cancer specific death or last follow-up, and overall survival of time to last follow-up, was demonstrated. The authors suggested that the incorporation of percentage of high grade tumour into the reporting of RCC may lead to the stratification of patients into prognostic

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groups and promote the development of individualised follow-up schedules. This study was based upon Fuhrman grading and consisted of a variety of RCC morphotypes. Further, this study failed to take into account the behaviour of tumours according to each of the constituent grades. This would mean that a tumour with 60% high grade cancer, consisting of 90% grade 3 and 10% grade 4 would be treated as being the same as a tumour with 60% high grade cancer consisting of 10% grade 3 and 90% grade 4. While both tumours would be classified as WHO/ISUP grade 4, it is uncertain if the higher proportion of grade 4 cancer in a tumour would influence the outcome.

This present study was undertaken to determine if the quantitation of WHO/ISUP grade 3 and/or grade 4 is of prognostic significance, utilising an extensively sampled and well-characterised series of clear cell RCC.

MATERIALS AND METHODS

Cases of clear cell RCC accessioned by Aquesta Specialised Uropathology between the inclusive years 2008 and 2015 were retrieved from file. Tumours from those patients who had been treated surgically with curative intent, by partial or radical nephrectomy, were identified and sections from these cases were independently reviewed by two urological pathologists (HS and JD) in order to confirm the diagnosis and assure adequacy of tumour sampling. All tumours had been liberally sampled, with small tumours being sampled in entirety or a minimum of 15 sections taken, whatever was the greater. For larger tumours the number of sections of tumour taken ranged from 15 to 28 per case.

Specimen handling and reporting, as a minimum, satisfied the published guidelines of the Royal College of Pathologists of Australasia (RCPA),[11] the ISUP Vancouver Consensus Conference on Renal Neoplasia[12] and the International Collaboration on Cancer Reporting (ICCR),[13] with respect to sampling of the renal sinus, renal vasculature and perinephric fat. Tumours were graded according to the criteria of the WHO/ISUP grading system[4] and those tumours containing components of grade 3 or grade 4 carcinomas were selected for further study. The proportion of the highest grade component (grade 3 or 4) present was assessed subjectively, with cases divided into three groups representing the percentage of either grade 3 or grade 4 in all sections, i.e., <10%, 10–50% and >50% of total tumour. pT staging category was assigned according to the recommendations of the American Joint Committee on Cancer TNM Staging (8th edition).[14] Clinical findings and follow-up data were provided by the attending clinician, with follow-up ranging from <1 to 89 months (mean 34 months) and the development of metastatic disease being taken as the clinical endpoint.

Survival curves were estimated by the Kaplan–Meier product limit method and where appropriate, subgroup differences in survivor functions were assessed using the log rank test. Multivariate analyses were undertaken utilising multivariate Cox proportional hazards models.

Approval for this study was obtained from the Aquesta Pathology Ethics Committee (Ethics approval number 2016/06).

RESULTS

During the study period 681 cases of clear cell RCC were accessioned by Aquesta Specialised Uropathology. Of these, follow-up was available for 376 cases with 153 cancers containing foci of tumour that satisfied grade 3 or grade 4 criteria of the WHO/ISUP grading system for RCC. The patient population was predominantly male (73%) with a mean age at diagnosis of 63 years. The mean tumour diameter of all cases was 6.3 cm (range 1.2–16.0 cm). Tumours were localised to the kidney in 57 cases (pT1 56 cases, pT2 1 case), 92 cases showed regional spread (pT3) while four cases were pT4. On formal grading of tumours, 91 were WHO/ISUP grade 3 and 62 were WHO/ISUP grade 4. Follow-up data were available for all 153 patients and the clinical and pathological characteristics of the cases, divided according to the percentage of grade 3 or grade 4 tumour present, are shown in Table 1.

During the follow-up period 19 of 91 (20.9%) patients with grade 3 cancers developed metastatic disease, while 30 of 62 (48.3%) patients with grade 4 cancers had metastases at the time of diagnosis or at follow-up. For patients with grade 3 tumours, metastases were seen in two cases with <10%, seven with 10–50% and 10 with >50% grade 3 tumour. On univariate analysis the division of cases according to percentage of grade 3 tumour showed no significant association with outcome (p=0.47). Similarly, division of cases according to ≤50% and >50% grade 3 components showed no significant association with outcome (p=0.22).

For patients having a WHO/ISUP grade 4 component to their tumour, with division of cases according to percentage of grade 4, metastases were seen in eight cases with <10%, 11 cases with 10–50% and 11 cases with >50% grade 4 component. The time to the development of metastases differed significantly between these groups (p=0.01) (Fig. 1). Simple Cox regression of tumours with a grade 4 component showed pT staging category and tumour size to also be statistically significant predictors of outcome in this series (p=0.0001 and p=0.003, respectively).

While tumours with a grade 4 component had a worse outcome than grade 3 tumours (p=0.0004) (Fig. 2), the gap between the Kaplan–Meier curves of patients with tumours having >50% grade 3 component and <10% grade 4 component was not significantly different (p=0.75). Although patients with <10% grade 4 component appeared to have a slightly worse outcome than those with grade 3 tumours as a whole, the difference did not reach statistical significance (p=0.27).

On multivariate analysis the outcome between <10% grade 4 tumours, when compared to tumours with 10–50% grade 4 was not significant (p=0.636), and tumours with 10–50% grade 4 just failed to achieve a significant difference in outcome when compared to tumours with a >50% grade 4 component (p=0.051). Conversely, the difference in outcome between tumours with <10% and >50% grade 4 component, along with tumour size, were significantly associated with time to the development of metastases (p=0.018 and p=0.006, respectively).

Table 1 Clinical and pathological parameters for clear cell renal cell carcinomas in the study

	WHO/ISUP Grade	
	Grade 3 (n=91)	Grade 4 (n=62)
Mean age, years	63.8	61.8
Tumour size, cm	5.5	7.6
Gender		
Male	68	43
Female	23	19
pT category		
pT1	42	14
pT2	1	0
pT3	48	44
pT4	0	4
% grade		
<10%	13	25
10–50%	42	22
>50%	36	15

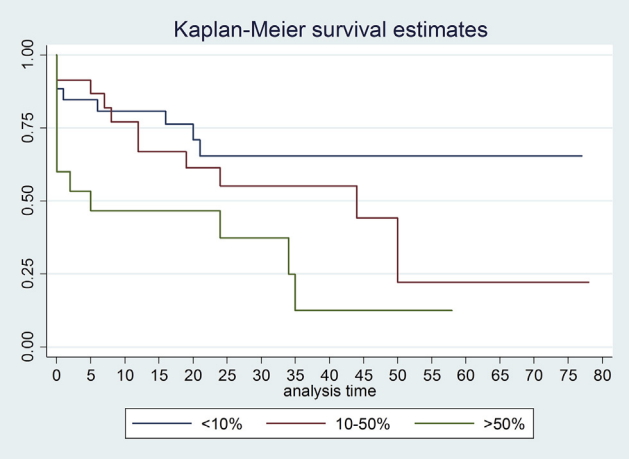

Fig. 1 Kaplan–Meier survival curve for WHO/ISUP grade 4 clear cell renal cell carcinoma with cases divided according to percentage of grade 4 component.

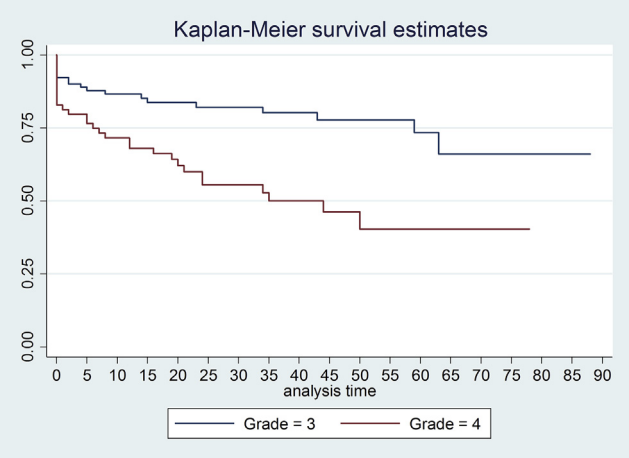

Fig. 2 Kaplan–Meier survival curve comparing outcome for WHO/ISUP grade 3 and 4 clear cell renal carcinoma.

DISCUSSION

In this study we have shown the proportion of the grade 4 component of clear cell RCC to be significantly associated with time to the development of metastatic disease. Conversely, the proportion of grade 3 was not significantly associated with outcome. The only previous study to investigate the prognostic significance of the proportion of high grade tumour in RCC showed the sum of the percentage of grade 3 + grade 4 tumour in combination to correlate with the time to metastases, time to cancer-specific death and overall survival.[10] In that study, which was based upon a mixed series of RCC morphotypes and the now obsolescent Fuhrman grading system, the prognostic significance of grade 3 and grade 4 components were not tested separately against survival. In addition, the study included a category of cases showing 0% grade 3 + grade 4. Therefore, these were grade 1 and grade 2 tumours, which means that the analysis effectively compared tumours with low versus high Fuhrman grades, which would likely have influenced significance of the outcome analysis. From our results it is apparent that the observed prognostic significance of percentage higher-grade tumour in that study may also have been controlled by the percentage volume of the grade 4 component of the tumour.

In the ISUP/WHO grading system for RCC, the criteria for grade 4 tumour is the presence of any of the following features: high grade nuclear pleomorphism, atypical tumour giant cells and sarcomatoid/rhabdoid differentiation.[2–4] Of these components, the prognostic significance of the percentage of the sarcomatoid component in an RCC has been examined in several studies, with the percentage of sarcomatoid tumour being tested as a prognostic parameter at a variety of cut-points.[15–22] In all but one of these studies the percentage of the sarcomatoid component was not associated with outcome on either univariate or, if positive, on multivariate analysis. In the only study to demonstrate a significant relationship between percentage of sarcomatoid component of tumour and outcome, the proportion of sarcomatoid tumour was investigated at a cut-point of 30% and also when the percentage of sarcomatoid tumour was treated as a continuous variable.[22] This was a series of tumours that were examined retrospectively, being based on examination of 204 cases identified from a registry of 7687 cases with 25,008 histological sections available for review. This means that the registry held an average of 3.25 sections per case, which presumably included sections of non-neoplastic renal tissues sampled as part of the diagnostic process. This limited number of sections per case raises questions over adequacy of sampling for quantitative studies and sampling bias. Additionally, in this series an undisclosed number of patients received adjuvant therapy. This would appear to have introduced a further uncontrolled variable into the outcome studies as it has been shown tumours with low volume sarcomatoid components respond favourably to vascular endothelial growth factor receptor/tyrosine kinase inhibition.[23,24]

It was also suggested in this previous study that sarcomatoid RCC has a more aggressive clinical course than the other features that are utilised to characterise grade 4 tumours.[22] In addition to the sampling issues noted above, this study was based upon a mixed series of RCC rather than consisting of clear cell RCC only. The outcome of the cases with sarcomatoid tumour was tested against a control group of tumours of which 10% of the cases were papillary and chromophobe RCC, both of which are recognised as having a more favourable prognosis than clear cell RCC.[2]

Our rationale for treating all criteria for WHO/ISUP grade 4 tumours in unison for analytical purposes is based on the assumption that this is how grading is applied in clinical practice. Further, it is apparent that in high grade clear cell RCC, areas of pleomorphic epithelial tumour, sarcomatoid tumour and rhabdoid tumour often co-exist and that in tumours with sarcomatoid and rhabdoid components, the two high grade morphologies are often contiguous.[16] A further difficulty in separating the various grade 4 morphologies for analytical purposes is the lack of a universal definition for sarcomatoid tumours. At the ISUP Vancouver Consensus Conference there was no consensus as to the definition of sarcomatoid morphology with accepted definitions including: (1) consists of atypical cells and resembles any form of sarcoma; (2) spindle cell morphology need not be present, but the tumour is very atypical and resembles any form of sarcoma; and (3) spindle cell pattern.[2] Clearly these definitions would embrace tumours showing extreme atypia with giant

cells, tumours with a rhabdoid component, as well as tumours consisting of malignant cells resembling fibrosarcoma.

It is apparent from our study that the extent of WHO/ISUP grade 4 component in a tumour influences outcome for clear cell RCC. The comparison in outcome between tumours with a low percentage versus tumours with a high percentage of grade 4 component retained prognostic significance on multivariate analysis, while the outcome for clear cell RCC with a low percentage of grade 4 did not differ significantly from that of grade 3 tumours. These results suggest that the WHO/ISUP grading classification could be modified to incorporate the amount of grade 4 tumour present. This could be expressed as the percentage of grade 4 within a cancer. Further the WHO/ISUP grade 4 category could be re-defined, requiring the presence of grade 4 in >10% tumour, rather than a single high power field.

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