Histopathology

Histopathology 2019, **74**, 284–290. DOI: 10.1111/his.13737

Assessment of tumour-associated necrosis provides prognostic information additional to World Health Organization/International Society of Urological Pathology grading for clear cell renal cell carcinoma

Julien Dagher,[1,2,3] Brett Delahunt,[4] Nathalie Rioux-Leclercq,[2,3] Lars Egevad,[5] Geoff Coughlin,[6] Nigel Dunglison,[6] Troy Gianduzzo,[6] Boon Kua,[6] Greg Malone,[7] Ben Martin,[8] John Preston,[7] Morgan Pokorny,[6] Simon Wood[7] & Hemamali Samaratunga[1,9]

[1]*Aquesta Specialized Uropathology, Brisbane, Qld, Australia,* [2]*Rennes University Hospital, Rennes,* [3]*University of Rennes, Rennes, France,* [4]*Department of Pathology and Molecular Medicine, Wellington School of Medicine and Health Sciences, Wellington, New Zealand,* [5]*Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden,* [6]*Wesley Hospital, Brisbane,* [7]*Greenslopes Hospital, Brisbane,* [8]*Holy Spirit Northside Hospital, Brisbane, and* [9]*University of Queensland School of Medicine, Brisbane, Qld, Australia*

Date of submission 2 May 2018
Accepted for publication 18 August 2018
Published online *Article Accepted* 21 August 2018

Dagher J, Delahunt B, Rioux-Leclercq N, Egevad L, Coughlin G, Dunglison N, Gianduzzo T, Kua B, Malone G, Martin B, Preston J, Pokorny M, Wood S & Samaratunga H
(2019) *Histopathology* **74**, 284–290. https://doi.org/10.1111/his.13737

Assessment of tumour-associated necrosis provides prognostic information additional to World Health Organization/International Society of Urological Pathology grading for clear cell renal cell carcinoma

Aims: The aims of this study were to evaluate the impact of tumour-associated necrosis (TAN) on metastasis-free survival for clear cell renal cell carcinoma (RCC), and to determine whether TAN provides survival information additional to World Health Organization (WHO)/International Society of Urological Pathology (ISUP) grading.

Methods and results: The study consisted of 376 cases of clear cell RCC treated by nephrectomy, for which follow-up was available. WHO/ISUP grade was assigned, and sections were assessed for the presence of TAN. American Joint Committee on Cancer (AJCC) pT staging category and tumour size were also recorded. The development of metastatic disease was taken as the clinical endpoint, and survival analyses, utilising univariate and multivariate models, were performed. WHO/ISUP grades were: grade 1, 35 cases (9.3%); grade 2, 188 cases (50.0%); grade 3, 91 cases (24.2%); and grade 4, 62 cases (16.5%). Staging categories were pT1–pT2 [234 tumours (62.2%)] and pT3–pT4 [139 tumours (37.0%)]. TAN was seen in 128 cases (34.0%). Neither TAN nor metastases were seen in grade 1 tumours. Among grade 2–4 tumours, those with TAN had a significantly worse prognosis than those without TAN ($P = 0.017$, $P = 0.04$, and $P = 0.006$, respectively). Multivariate analysis (WHO/ISUP grade, pT staging category, and TAN) showed all three variables to be independently associated with outcome ($P = 0.009$, $P = 0.005$, and $P = 0.001$, respectively). For all tumour grades and pT staging categories, it was found that the presence of TAN was associated with a 2.91-fold greater risk of metastatic disease.

Conclusion: Tumour-associated necrosis is an important prognostic factor for clear cell RCC, independently of WHO/ISUP grade. This supports the suggestion that TAN could be incorporated into tumour grading criteria.

Address for correspondence: B Delahunt, Department of Pathology and Molecular Medicine, Wellington School of Medicine and Health Sciences, University of Otago, 23a Mein Street, Wellington, New Zealand. e-mail: brett.delahunt@otago.ac.nz

Keywords: grading, International Society of Urological Pathology, prognosis, renal neoplasia, tumour necrosis, World Health Organisation

Introduction

Clear cell renal cell carcinoma (RCC) is the most common form of renal malignancy in adults, causing significant morbidity and mortality.[1] To date, a wide variety of prognostic factors have been investigated for this tumour, but only tumour grade, the presence of sarcomatoid and/or rhabdoid differentiation and the presence of tumour necrosis are widely accepted, being recognised by both the World Health Organization (WHO) and the International Society of Urological Pathology (ISUP).[1,2]

It is clear from previous studies of RCC that these tumours may be associated with two separate types of necrosis. Tumours are reported as showing either diffuse coagulative necrosis or tumour-associated necrosis (TAN), which is also rather confusingly known as microscopic tumour coagulative necrosis, and it would appear that these two forms of necrosis are associated with different pathogenic mechanisms. Diffuse coagulative necrosis is usually macroscopic, being the result of thromboembolic tumour infarction. TAN is usually microscopic, and consists of well-demarcated foci of necrosis within tumour. The features are not typical of coagulative necrosis, as there is loss of architecture and an accumulation of granular nuclear and cytoplasmic debris. The pathogenesis of TAN is debated, and suggested mechanisms are an immune reaction, tumour outgrowth of blood supply, vascular immaturity, and hypoxia relating to tumour vascular remodelling.[2–5] The last of these mechanisms is supported by the observation that areas of TAN are associated with an increased density of small vessels.[6]

The existence of two apparently different mechanisms leading to tumour necrosis in clear cell RCC has led to confusion as to how necrosis should be assessed and recorded for prognostic purposes. The ISUP Vancouver Consensus Conference on Renal Cell Carcinoma recommended that the presence or absence of tumour necrosis should be routinely included in pathology reports.[2] It was also noted that, as our understanding of the prognostic significance of tumour necrosis is evolving, the presence of both macroscopic and microscopic necrosis should be recorded, and that the amount of necrosis present should be given as a percentage of the total tumour volume. It is unfortunate that this recommendation relating to necrosis type should focus on macroscopic and microscopic detection rather than the pathogenesis of the necrosis. Necrosis secondary to infarction is usually macroscopic, whereas TAN is usually microscopic but may be macroscopic. Although both the ISUP and the WHO recommend that the presence of necrosis should be reported for RCC, no methodology for interpreting the prognostic significance of this is presented by either of these two reference groups.[1,2]

The grading of clear cell RCC has evolved in recent years, with the current WHO/ISUP grading classification being based on nucleolar features for the first three grades of the four-tier grading system, and determination of grade 4 being dependent on the presence of marked nuclear pleomorphism and/or rhabdoid and/or sarcomatoid differentiation, which are features of extreme dedifferentiation.[2,7] Some studies have indicated that the presence of TAN has prognostic significance, and it has been recommended that this be incorporated into the grading of clear cell RCC,[2,3,5] whereas, in other studies, necrosis has been shown not to have prognostic predictive value.[8–14]

This study was undertaken to assess the prognostic importance of TAN in a series of clear cell RCCs that have been well sampled, handled and assessed according to contemporary guidelines,[1,2,15] and to determine whether any prognostic information relating to the presence of TAN is additional to grade, thus justifying the inclusion of TAN in a revised grading system for these tumours.

Materials and methods

In this study, we analysed 376 cases of clinically localised clear cell RCC accessioned by Aquesta Specialized Uropathology, Brisbane, Australia between the inclusive years 2008 and 2015 and for which follow-up was available. No patients had evidence of familial RCC, and all patients had been treated with curative intent by partial or radical nephrectomy. No patient had been treated by prior arterial embolisation of tumour or by neoadjuvant chemotherapy. In all cases, the submitted surgical specimens were handled according to the current guidelines of the Royal College of Pathologists of Australasia,[16] and complied, as a minimum, with the recommendations of the ISUP and the WHO for specimen handling, sampling, and reporting.[1,2,15] All tumours were liberally

sampled, with a minimum of 15 sections or complete tumour sampling (whichever was the greater) for small tumours, ranging up to 28 sections for larger tumours. In all cases, the samples were taken randomly to include areas of apparent tumour necrosis. Ethics approval was obtained from the Aquesta Pathology Institutional Ethics Committee (Ethics approval no. 2016-08).

Sections from all cases were reviewed by two uropathologists (H.S. and J.D.), who assigned both a WHO/ISUP grade and an eighth edition of the American Joint Committee on Cancer (AJCC) TNM[17] pathological staging category to each case. Sections were then assessed for the presence of TAN, and the amount present was recorded as a percentage of the total area of the tumour present within the histological sections. Clinical follow-up data, consisting of the cancer-free survival interval for each patient, was obtained from attending clinicians. Outcome was determined with respect to the presence/absence of TAN and the percentage of tumour showing TAN, as well as WHO/ISUP grade, tumour size, and AJCC TNM pT category.

Statistical analysis was performed with univariate analysis on categorical variables by use of Kaplan–Meier techniques. For this part of the analysis, survival times were considered as observations on a continuous variable. Survival curves were estimated with the Kaplan–Meier product limit method, and, where relevant, subgroup differences in survivor functions were assessed with the log rank test. During this exploratory phase, a Cox proportional hazard model was used to examine continuous variables as single predictors. The significance of a single predictor was tested with a standard χ^2 test with one degree of freedom. Multivariate analyses were performed with multivariate Cox proportional hazard regression. The process was started by fitting a model with all relevant predictors; then, on the basis of their *z*-scores, the initial model was reduced. The final model was obtained with stepwise Cox regression procedures in STATA (Version 14). The assumption of proportionality was checked on the basis of the Schoenfeld residuals test.

Results

Adequate histological material and follow-up data were obtained for all 376 cases in the series. The clinical and pathological characteristics of the cases are shown in Table 1.

Tumour-associated necrosis (Figure 1) was identified in 34% of cases, whereas 66% of cases were necrosis-free. No grade 1 tumours showed TAN. When cases were divided according to grade, there was a significant association between an increasing percentage of TAN and tumour grade (Fisher's exact, $P < 0.0005$). The distribution of TAN by WHO/ISUP grade is shown in Table 2. Follow-up intervals ranged from <1 month to 89 months, with a mean of 35 months (standard deviation 22 months).

A detailed analysis of the comparison of Fuhrman grading with WHO/ISUP grade and the associations with clinical outcome has been previously reported for this series.[18] During the follow-up period, 56 patients developed recurrent and/or metastatic disease (Table 2). There were no events among patients with WHO/ISUP grade 1 tumours during the follow-up period. On both univariate analysis and multivariate analysis, which included AJCC pT staging category, there was a significant difference in cancer-free survival between grade 2 and grade 3 tumours, as well as between grade 3 and grade 4 tumours.[18]

Table 1. Clinical and histological features of cases studied

Gender	
Male	254
Female	122
Age (years)	Range 26–92 years Mean 61 years (SD = 11.0)
Tumour size (cm)	Range 0.5–14.5 cm Mean 4.9 (SD = 2.9)
pT staging category	
pT1a	158
pT1b	66
pT2	10
pT3	138
pT4	4
WHO/ISUP grade	
1	35 (9.3%)
2	188 (50.0%)
3	91 (24.4%)
4	62 (16.5%)
Tumour specific necrosis	
Present	128 (34%)
Absent	248 (66%)

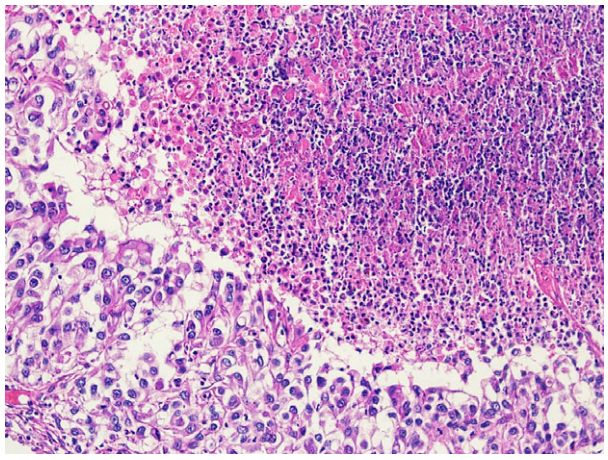

Figure 1. Tumour-associated necrosis within clear cell renal cell carcinoma. There is a sharp demarcation between apparently viable tissue and necrotic tissue. The underlying architecture of the necrotic tissue is lost, and widespread nuclear debris is present.

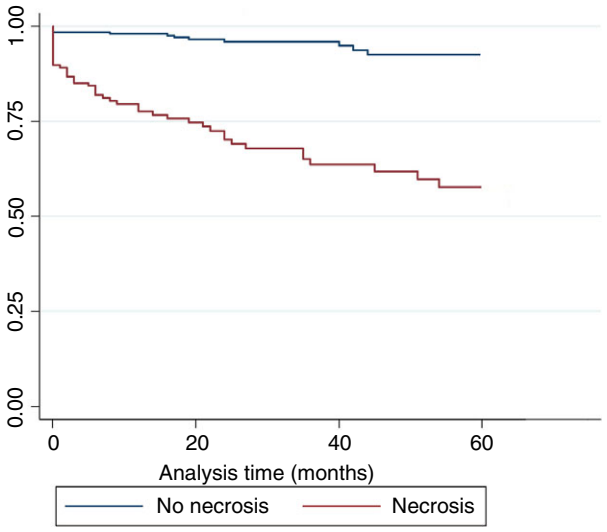

Figure 2. Kaplan–Meier survival curve comparing tumours with and without tumour-associated necrosis ($P = 0.0001$).

Table 2. Distribution of TAN and development of metastases by WHO/ISUP grade

WHO/ISUP grade	No TAN	TAN	Metastases
Grade 1	35	0 (0%)	0 (0%)
Grade 2	188	37 (19.6%)	7 (3.7%)
Grade 3	91	38 (41.7%)	19 (20.8%)
Grade 4	62	53 (85.4%)	30 (48.4%)

TAN, Tumour associated necrosis; WHO, World Health Organization; ISUP, International Society of Urological Pathology.

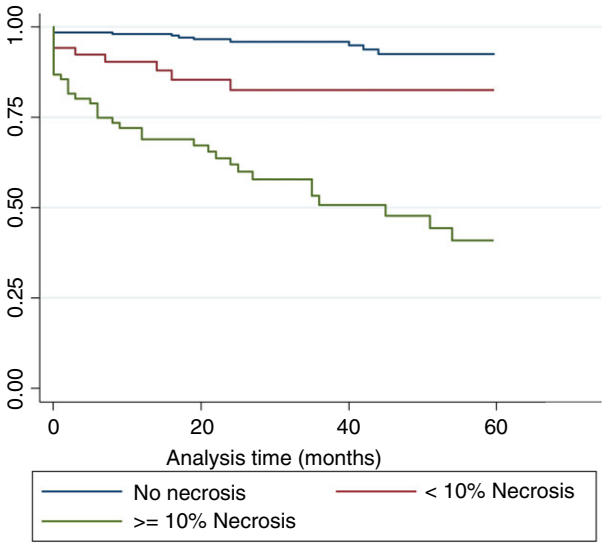

Figure 3. Kaplan–Meier survival curve comparing tumours stratified according to no tumour-associated necrosis and <10% and ≥10% tumour-associated necrosis ($P = 0.0001$).

For all cases, the presence of TAN was associated with outcome ($P < 0.0001$; Figure 2). The proportion of TAN within tumours ranged from <5% to 80%. Division of cases according to the percentage of TAN present showed 52 cases to have <10% TAN, 30 cases to have 10% to <20% TAN, 33 cases to have 20% to ≤50% TAN, and 13 cases to have >50% TAN. There were significant differences in outcome between tumours with no TAN and those with <10% TAN ($z = 2.44$, $P = 0.015$), and between tumours with <10% TAN and those with ≥10% TAN ($z = 3.41$, $P = 0.001$) (Figure 3).

A multivariate analysis model, incorporating WHO/ISUP grade, pT staging category, and TAN, showed that all variables were significantly associated with outcome ($P = 0.009$, $P = 0.005$, and $P = 0.001$, respectively). If TAN was present, and all other variables were held constant, the risk of metastasis was 2.91 times higher than for tumours without necrosis. On univariate analysis, grades 2, 3 and 4 with TAN showed a significantly worse association with outcome than tumours of similar grade without TAN ($P = 0.009$, $P = 0.046$, and $P = 0.006$, respectively). There were no significant differences in outcome between WHO/ISUP grade 2 tumours with TAN and WHO/ISUP grade 3 tumours without TAN, and between WHO/ISUP grade 3 tumours with TAN and WHO/ISUP grade 4 tumours without TAN.

Discussion

The presence of tumour necrosis as a prognostic indicator for clear cell RCC has been debated since it was first investigated in 1974.[19] In this initial study, it was concluded that the presence of tumour necrosis was associated with a less favourable prognosis; however, the series consisted of mixed RCC morphotypes, and, moreover, the nature of the necrosis was not defined. Since the publication of this study, there have been several investigations that have corroborated these findings,[5,6,20–27] whereas others have suggested that the presence of necrosis has no prognostic significance or is associated with a more favourable outcome.[8–14,28] These contradictory results are unfortunate, and appear, in the main, to have resulted from methodological problems. One of the most significant issues regarding the prognostic significance of tumour necrosis relates to the nature of the necrosis itself. In some studies, TAN is investigated in isolation, whereas in others the nature of the necrosis is not fully defined, both forms of necrosis are combined, or infarctive coagulative necrosis is investigated in isolation.[10,11,13,14,20,23] This is of importance, as it has been suggested that massive coagulative necrosis, probably resulting from infarction, is associated with a more favourable outcome.[28] This would not appear to be the case for TAN. Interestingly, in some studies in which there appears to be confusion regarding the type of necrosis being assessed, the presence of necrosis still emerges as having prognostic significance. This is perhaps not surprising, as TAN is more commonly encountered than massive necrosis resulting from infarction, especially in high-grade tumours, and is therefore more likely to influence outcome studies.

Other methodological problems relate to the separation of RCCs according to morphotype for analytical purposes. In some studies, all morphotypes are grouped together.[8,11,14,22,28] This is clearly inappropriate, as each morphotype has its own genetic, morphological and clinical features, including outcome. In addition to this, some types, such as papillary RCC, are more prone to undergo infarction and/or develop TAN. In many of the previously published studies, the case series were retrospective, with many pre-dating the development of the current classification of RCC morphotypes.[6,8–12,14,20,22,25–27] This means that, rather than investigation of a single tumour type, several—as yet uncharacterised—RCC morphotypes are contained together within the series. This issue is also problematic in more contemporary studies involving retrospective series, when data are collected from pathology reports without formal case review. Other problems encountered in previous studies relate to our evolving understanding of the significance of other prognostic markers for RCC. Recent advances in our appreciation of the prognostic significance of renal sinus invasion by tumour, as well as the adoption of a novel grading system for both clear cell RCC and papillary RCC, have facilitated improved prognostic assessment for these tumours. A failure to incorporate these important prognostic factors into any multivariate analysis introduces uncontrolled variables. Problems also arise in relation to tumour sampling. This applies especially to retrospective series, in which there can be no assurance that foci of necrosis were sampled adequately. Here, areas of necrosis would probably have been avoided by pathologists taking blocks for diagnostic purposes alone. Furthermore, the collection of insufficient samples may also lead to undergrading and understaging of tumours. Finally, in some studies there are issues relating to sample size, as some series would appear to be underpowered because of insufficient case numbers.

In this study, which is based upon a contemporarily categorised series of tumours that have been graded and staged according to current criteria, we showed TAN to occur more frequently in higher-grade clear cell RCC. We showed that no necrosis or metastatic events occurred in grade 1 tumours, and that TAN was significantly associated with a worse outcome for grade 2–4 carcinomas. On multivariate analysis, this significance was retained in a model that included WHO/ISUP grade and AJCC pT staging category. As part of the study, tumours were sampled extensively in accordance with the protocols of Aquesta Uropathology. It may be argued that, if sampling is less rigorous, then foci of TAN may be overlooked, or the extent of TAN misclassified. Although we would recommend that tumours be well sampled, we also note that, as demonstrated in this study, the presence of even small amounts of TAN is of prognostic significance.

The quantification of necrosis in clear cell RCC has been previously investigated, with conflicting results. Surprisingly, a 20% cutpoint for extent of tumour necrosis was found to have prognostic significance, whereas the presence/absence of necrosis failed to achieve significance as a prognostic marker on multivariate analysis.[13] In this study, tumours were sampled widely; however, any form of necrosis was included in the analysis of the results. A larger retrospective series showed a similar result when the presence/absence of necrosis was considered as a

prognostic parameter, whereas a cutpoint of 50% tumour volume correlated with disease-specific and overall survival, but not with metastasis-free survival.[14] Unfortunately, this study consisted of a mixed series of RCC morphotypes, including clear cell, papillary, chromophobe and unclassified RCC, and, again, this study did not discriminate as to the nature of the necrosis. A more recent study by Renshaw and Cheville showed the presence of TAN, as well as a cutpoint of 10% TAN, to have prognostic significance, whereas tumours with <10% TAN had an outcome similar to those with no TAN.[29] A major issue with this study related to tumour sampling, with the area of necrosis being determined from a single section of tissue. Our results indicate that even <10% TAN within a tumour is associated with a worse prognosis that that of tumours with no necrosis, and that tumours with >10% TAN have a significantly worse prognosis than tumours with less TAN.

It has been suggested that the presence of TAN should be incorporated into the grading criteria of clear cell RCC, as it does appear to provide prognostic information additional to grading alone and, in this study, a novel grading system combining WHO/ISUP grade and TAN was proposed and validated.[5] A recent study using WHO/ISUP grading found no significant difference in outcome for grades 1–3,[30] which is contrary to previous findings of significant differences in outcome between each grade.[2,18] They did, however, show a significant difference in survival between modified grade 1 and grade 3 tumours, and between grade 1 and grade 4 tumours when TAN was incorporated into the grading classification. It should be noted that, in this study, there were surprisingly few cases of WHO/ISUP grade 1 tumours, and it is uncertain whether grading conformed to strict WHO/ISUP grading guidelines.[18]

Our study has shown that the presence of TAN in clear cell RCC provides prognostic information that is independent of WHO/ISUP grading.

Acknowledgements

The University Hospital of Rennes, the University of Rennes and the University of Queensland are thanked for facilitating J. Dagher's tenure as a Post-Graduate Fellow at Aquesta Uropathology, Brisbane.

Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

J. Dagher, B. Delahunt, and H. Samaratunga: study design, data analysis, and writing of the manuscript. G. Coughlin, N. Dunglison, T. Gianduzzo, B. Kua, G. Malone, B. Martin, J. Preston, M. Pokorny, and S. Wood: data collection and manuscript revision. N. Rioux-Leclercq and L. Egevad: conceptual advice and manuscript revision.

References

1. Moch H, Amin MB, Argani P *et al.* Tumours of the kidney. In Moch H, Humphrey PA, Ulbright T, Reuter VE eds. *World Health Organization classification of tumors. Pathology and genetics: tumors of the urinary system and male genital organs*. 4th ed. Zurich: IARC Press, 2016; 14–21.
2. Delahunt B, Cheville JC, Martignoni G *et al.* The International Society of Urological Pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. *Am. J. Surg. Pathol.* 2013; **37**; 1490–1504.
3. Lohse CM, Gupta S, Cheville JC. Outcome prediction for patients with renal cell carcinoma. *Semin. Diagn. Pathol.* 2015; **32**; 172–183.
4. Cheville JC, Lohse CM, Zincke H, Weaver AL, Blute ML. Comparisons of outcome and prognostic features among histologic subtypes of renal cell carcinoma. *Am. J. Surg. Pathol.* 2003; **27**; 612–624.
5. Delahunt B, McKenney J, Lohse CM *et al.* A novel grading system for clear cell renal cell carcinoma incorporating tumor necrosis. *Am. J. Surg. Pathol.* 2013; **37**; 311–322.
6. Sengupta S, Lohse CM, Leibovich BC *et al.* Histologic coagulative tumor necrosis as a prognostic indicator of renal cell carcinoma aggressiveness. *Cancer* 2005; **104**; 511–520.
7. Delahunt B, Sika-Paotonu D, Bethwaite PB *et al.* Grading of clear cell renal cell carcinoma should be based on nucleolar prominence. *Am. J. Surg. Pathol.* 2011; **35**; 1134–1139.
8. Isbarn H, Patard J-J, Lughezzani G *et al.* Limited prognostic value of tumor necrosis in patients with renal cell carcinoma. *Urology* 2010; **75**; 1378–1384.
9. Delahunt B, Nacey JN. Renal cell carcinoma: histological indicators of prognosis. *Pathology* 1987; **19**; 258–263.
10. Sorbellini M, Kattan MW, Snyder ME *et al.* A post operative prognostic nomogram predicting recurrence for patients with conventional clear cell renal cell carcinoma. *J. Urol.* 2005; **173**; 48–51.
11. Foria V, Surdendra T, Poller DN. Prognostic significance of extensive necrosis in renal cell carcinoma. *J. Clin. Pathol.* 2005; **58**; 39–43.
12. Minervini A1, Di Cristofano C, Gacci M *et al.* Prognostic role of histological necrosis for nonmetastatic clear cell renal cell carcinoma: correlation with pathological features and molecular markers. *J. Urol.* 2008; **180**; 1284–1289.
13. Klatte T, Said JW, de Martino M *et al.* Presence of tumor necrosis is not a significant predictor of survival in clear cell renal cell carcinoma: higher prognostic accuracy of extent based rather than presence/absence classification. *J. Urol.* 2009; **181**; 1558–1564.
14. Katz MD, Serrano MF, Grubb RL III *et al.* Percent microscopic necrosis and survival after curative surgery for renal cell carcinoma. *J. Urol.* 2010; **183**; 909–914.

15. Trpkov K, Grignon DJ, Bonsib SM *et al.* Handling and staging of renal cell carcinoma: the International Society of Urological Pathology Consensus (ISUP) conference recommendations. *Am. J. Surg. Pathol.* 2013; **37**; 1505–1517.
16. Delahunt B, Charles A, Clouston D *et al. Renal parenchymal malignancy (renal cell carcinoma) structured reporting protocol*. Sydney: Royal College of Pathologists of Australasia, 2011.
17. Amin MB, Edge S, Greene F *et al.* eds. *AJCC cancer staging manual*. 8th ed. New York: Springer Verlag, 2016.
18. Dagher J, Delahunt B, Rioux-Leclercq N *et al.* Clear cell renal cell carcinoma: validation of World Health Organisation/International Society of Urological Pathology grading. *Histopathology* 2017; **71**; 918–925.
19. Amtrup F, Hansen JB, Thybo E. Prognosis in renal carcinoma evaluated from histological criteria. *Scand. J. Urol. Nephrol.* 1974; **8**; 198–202.
20. Moch H, Gasser T, Amin MB *et al.* Prognostic utility of the recently recommended histologic classification and revised TNM staging system of renal cell carcinoma: a Swiss experience with 588 tumors. *Cancer* 2000; **89**; 604–614.
21. Frank I, Blute ML, Cheville JC *et al.* An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. *J. Urol.* 2002; **168**; 2395–2400.
22. Lam JS, Shvats O, Said JW *et al.* Clinicopathologic and molecular correlations of necrosis in the primary tumor of patients with renal cell carcinoma. *Cancer* 2006; **103**; 2517–2525.
23. Lee SE, Byun S-S, Oh JK *et al.* Significance of macroscopic tumor necrosis as a prognostic indicator for renal cell carcinoma. *J. Urol.* 2006; **176**; 1332–1338.
24. Ficarra V, Martignoni G, Lohse C *et al.* External validation of Mayo Clinic stage, size, grade and necrosis (SSIGN) score to predict cancer specific survival using a European series of conventional renal cell carcinoma. *J. Urol.* 2006; **175**; 1235–1239.
25. Tollenfon MK, Thompson JH, Sheinin Y *et al.* Ki-67 and coagulative tumor necrosis are independent predictors of poor outcome for patients with clear cell renal cell carcinoma and not surrogates for each other. *Cancer* 2007; **110**; 783–790.
26. Zigeuner R, Hutterer G, Chromecki T *et al.* External validation of the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score for clear-cell renal cell carcinoma in a single European centre applying routine pathology. *Eur. Urol.* 2010; **57**; 102–111.
27. Pichler M, Hutterer GC, Chromecki TF *et al.* Histologic tumor necrosis is an independent prognostic indicator for clear cell and papillary renal cell carcinoma. *Am. J. Clin. Pathol.* 2012; **137**; 283–289.
28. Collins J, Epstein JN. Prognostic significance of extensive necrosis in renal cell carcinoma. *Hum. Pathol.* 2017; **66**; 108–111.
29. Renshaw AA, Cheville JC. Quantitative tumour necrosis is an independent predictor of overall survival in clear cell renal cell carcinoma. *Pathology* 2015; **47**; 34–37.
30. Khor LY, Dhakal HP, Jia X *et al.* Tumor necrosis adds prognostically significant information to grade in clear cell renal cell carcinoma: a study of 842 consecutive cases from a single institution. *Am. J. Surg. Pathol.* 2016; **40**; 1224–1231.