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Original contribution

High proliferation rate and TNM stage but not histomorphological subtype are independent prognostic markers for overall survival in papillary renal cell carcinoma☆,☆☆

Iris Polifka MD[a,*], Abbas Agaimy MD[a], Edwin Herrmann MD[b,1], Verena Spath MD[a,2], Lutz Trojan MD[c], Michael Stöckle MD[d], Frank Becker MD[d,3], Philipp Ströbel MD[e], Christian Wülfing MD[b,4], Andres J. Schrader MD[f,5], Peter Barth MD[f,6], Michael Staehler MD[g], Christian Stief MD[g], Markus Hohenfellner MD[h], Stephan Macher-Göppinger MD[i], Bernd Wullich MD[j], Joachim Noldus MD[k], Walburgis Brenner PhD, MD[l,7], Frederik C. Roos MD[m], Bernhard Walter MD[j,8], Wolfgang Otto MD[n], Maximilian Burger MD[n], Heinz Höfler MD[o,9], Axel Haferkamp MD[h,10], Carol I. Geppert MD[a], Christine Stöhr PhD[a,11], Arndt Hartmann MD[a,11], German Network Of Kidney Cancer

[a]*Institute of Pathology, University Hospital Erlangen, Friedrich Alexander University (FAU), 91054 Erlangen, Germany*
[b]*Department of Urology, University Hospital Muenster, 48149 Muenster, Germany*
[c]*Department of Urology, University Hospital Göttingen, 37075 Göttingen, Germany*
[d]*Department of Urology and Pediatric Urology, University of Saarland (UKS), 66421 Homburg, Germany*
[e]*Department of Pathology, University Hospital Göttingen, 37075 Göttingen, Germany*
[f]*Department of Urology, University of Marburg, 35037 Marburg, Germany*
[g]*Department of Urology, University Hospital Munich, 81337 Munich, Germany*
[h]*Department of Urology, University Hospital Heidelberg, 69120 Heidelberg, Germany*
[i]*Institute of Pathology, University Hospital Mainz, 55131 Mainz, Germany*

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* Corresponding author at: Institute of Pathology, University Hospital Erlangen, Krankenhausstraße 8-10, 91054 Erlangen, Germany.
E-mail address: iris.polifka@uk-erlangen.de (I. Polifka).
[1] Present address: Institute of Urology, Prosper-Hospital gGmbH, 45 659 Recklinghausen, Germany.
[2] Present address: Institute of Pathology, Caritas Hospital Bad Mergentheim, 97 980 Bad Mergentheim, Germany.
[3] Present address: Urological Group and Clinic Derouet/Pönicke/Becker, Boxberg Centre, 66 538 Neunkirchen, Germany.
[4] Present address: Department of Urology, Asklepios Clinics Altona, 22 763 Hamburg, Germany.
[5] Present address: Department of Urology, University Hospital Muenster, 48 149 Muenster, Germany.
[6] Present address: Institute of Pathology/Gerhard-Domagk Institute, University Hospital Muenster, 48 149 Muenster, Germany.
[7] Present address: Department of Gynecology, University of Mainz, 55 131 Mainz, Germany.
[8] Present address: Department of Urology, Kreiskliniken Altötting-Burghausen, 84 489 Burghausen, Germany.
[9] Emeritus.
[10] Present address: Department of Urology and Pediatric Urology, University Hospital Mainz, 55 131 Mainz, Germany.
[11] These authors contributed equally to this work.

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[j]Department of Urology and Pediatric Urology, University Hospital Erlangen, Friedrich Alexander University (FAU), 91058 Erlangen, Germany
[k]Department of Urology, Marien Hospital Herne, Ruhr University Bochum, 44625 Herne, Germany
[l]Department of Urology, University of Mainz, 55131 Mainz, Germany
[m]Department of Urology, University Hospital Frankfurt, 60590 Frankfurt/Main, Germany
[n]Department of Urology, University of Regensburg, 93053 Regensburg, Germany
[o]Institute of Pathology, Technical University of Munich (TUM), 81675 Munich

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Summary Papillary renal cell carcinoma (PRCC) is currently divided in 2 subtypes. We reviewed a large cohort of PRCC and correlated subtype, morphological features and diagnostic marker expression with overall survival (OS) to uncover differences between the 2 subtypes. Three hundred seventy-six renal tumors initially diagnosed as PRCC with clinical and survival data were collected from the participating centers. Two hundred forty-six tumors were classified as PRCC1 (65.4%) and 130 as PRCC2 (34.6%) and graded according to the 2016 World Health Organization/International Society of Urological Pathology grading system. Morphological features (abundant cytoplasm, necrosis, fibrous stroma, foamy macrophages and psammoma bodies) were noted. Immunohistochemical stains (MIB1, p53, Racemase, EMA, CK7, CK20, E-Cadherin) were performed using tissue microarrays. χ^2-Tests, log-rank tests and uni- and multivariate Cox regression analysis were performed. Both subtypes displayed different morphological features and immunohistochemical profiles: abundant cytoplasm was more frequent in PRCC2, while foamy macrophages were more common in PRCC1. Abundant cytoplasm and presence of psammoma bodies were associated with poorer OS. PRCC1 showed more frequent CK7 expression, PRCC2 more frequent E-Cadherin, p53 and higher MIB1 expression (>15%). Expression of Racemase and CK7 was associated with better OS, while high MIB1 (>15%) was associated with poorer OS. In multivariate analysis, the only independent predictors of OS were proliferation (MIB1), tumor stage, metastasis and age at surgery. Subtype was not an independent prognostic factor. Therefore, PRCC subtype on its own is not suitable for estimating survival. More data focusing on PRCC tumor biology is needed to define prognostic subgroups, especially in PRCC2.

1. Introduction

Kidney cancer is the 9th most common cancer in men and the 14th most common in women worldwide (WHO Classification Tumors of the Urinary System and Male Organs 2016). Papillary renal cell carcinoma (PRCC), the second common renal cell carcinoma (RCC) after clear cell RCC, is derived from the renal tubular epithelium and represents a heterogeneous group [1]. According to the fourth edition of World Health Organization (WHO) Classification 2016 and The International Society of Urological Pathology (ISUP) Grading System for Renal Cell Carcinoma and Other Prognostic Parameters [2], PRCC is categorized into 2 subtypes. Papillary renal cell carcinoma type 1 (PRCC1) and type 2 (PRCC2). PRCC1 usually presents as a well circumscribed and encapsulated mass composed of papillae lined by a single layer of cuboidal neoplastic epithelial cells. The nuclei are arranged in a single line with little or no pleomorphism and often surrounded by pale scanty cytoplasm. Aggregates of foamy macrophages in the background stroma of the papillae are a common finding. Except for infarct-type necrosis, coagulative necrosis is rare in PRCC1. On the other hand, PRCC2 demonstrates a variety of aspects, with abundant eosinophilic cytoplasm, more pleomorphic nuclei and prominent nucleoli often accompanied by a diffuse infiltration of the peritumoral kidney tissue. Necrosis and pseudostratification are common in PRCC2. Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome-associated RCC is a hereditary form of aggressive RCC that was traditionally included in the spectrum of PRCC2. Because other renal cell neoplasms, eg, clear cell RCC, clear cell papillary RCC and MiT-family translocation-associated RCC can have variable papillary morphology, too [3], there is a need for immunoprofiling for correct differential diagnosis in difficult cases. PRCC often shows expression of EMA, racemase (AMACR), RCC, vimentin and CD10, which may help to distinguish them from other RCC subtypes [4]. Strong and homogeneous CK7 expression is more frequently detected in PRCC1 [5]. It has been suggested that PRCC2 is not a single entity but rather encompasses one or more subtypes of aggressive RCC with poorer outcome than PRCC1 [2,6,7]. Therefore, it is mandatory to exactly subclassify RCC in the morphological spectrum of PRCC2 in order to identify the tumor characteristics that are associated with poor prognosis. In this study we examined a large collection of RCC initially classified as PRCC by reviewing original histological slides and performing a wide range of immunohistochemical

stains. We describe morphologic features in order to assess trends in the morphological classification of PRCC in the light of recent developments, detect differences between the 2 PRCC subtypes and look for possible associations with clinical outcome.

2. Materials and methods

2.1. Tissue collection and pathological review

Four hundred eighty-six formalin-fixed, paraffin-embedded tissue samples from RCC cases initially diagnosed as PRCC and their corresponding clinical data (Table 1) including follow up were collected from the participating institutions (in alphabetical order: Erlangen, Heidelberg, Herne, Homburg, Mainz, Mannheim, Marburg, Muenster, Munich and Regensburg; all centers are in Germany). Patients' material was anonymized, part of the study (Erlangen cases) was approved by the Ethical Committee of the University Hospital Erlangen and the whole study was performed according to the standards established in the Declaration of Helsinki. Renal surgery was performed between 1993 and 2007. Median follow-up was 38 month (mean 52.4 months, minimum 0 and maximum 204). The collected papillary tumors were reviewed according to their morphology and with the help of immunohistochemistry (IHC) as

Table 1 Distribution of clinical data and morphological features of papillary renal cell carcinoma type 1 (PRCC1) and papillary renal cell carcinoma type 2 (PRCC2) in percent and number of cases/all evaluable cases

Variable	PRCC1	PRCC2	*P*
Tumor stage (T)			
T1	66.2% (135/204)	41.7% (40/96)	<.0001
T2	23.0% (47/204)	14.6% (15/96)	
T3	10.8% (22/204)	40.6% (39/96)	
T4	0% (0/204)	2.1% (2/96)	
Nodal status (N)			
N0	97.0% (194/200)	77.5% (69/89)	<.0001
N1	3.0% (6/200)	22.5% (20/89)	
Metastasis (M)			
M0	98.5% (197/200)	78.3% (72/92)	<.0001
M1	1.5% (3/200)	21.7% (20/92)	
2016 WHO/ISUP grade			
G1	28.4% (61/215)	0% (0/104)	<.0001
G2	58.6% (126/215)	17.3% (18/104)	
G3	11.6% (25/215)	73.1% (76/104)	
G4	1.4% (3/215)	9.6% (10/104)	
Necrosis			
No	45.8% (98/214)	35.9% (37/103)	.115
Yes	54.2% (116/214)	64.1% (66/103)	
Psammoma bodies	95.3% (204/214)	97.1% (100/103)	.558
No	95.3% (204/214)	97.1% (100/103)	.558
Yes	4.7% (10/214)	2.9% (3/103)	
Fibrous stroma			
Scanty	88.3% (188/213)	84.5% (87/103)	.374
Abundant	11.7% (25/213)	15.5% (16/103)	
Cytoplasm			
Small	85.0% (181/213)	24.3% (25/103)	<.0001
Abundant	15.0% (32/213)	75.7% (78/103)	
Foamy macrophages			
No	29.1% (62/213)	51.0% (52/102)	<.0001
Yes	70.9% (151/213)	49.0% (50/102)	
Sex			
Male	79.5% (151/190)	77.3% (75/97)	.761
Female	20.5% (39/190)	22.7% (22/97)	
Age at surgery (range 15–92 y), median 63 y	54.7% (110/201)	44.3% (43/97)	.108
<63 years	54.7% (110/201)	44.3% (43/97)	.108
≥63 years	45.3% (91/201)	55.7% (54/97)	
Surgical treatment	27.5% (56/204)	19.4% (19/98)	.155
Enucleation/ heminephrectomy	27.5% (56/204)	19.4% (19/98)	.155
Nephrectomy	72.5% (148/204)	80.6% (79/98)	

NOTE. $P < .05$ was considered as statistically significant.

necessary according to the 2016 WHO classification for tumors of the urinary system and male genital organs [4]. Original grading was done according to Fuhrman et al but all tumors were re-graded according to the 2016 WHO/ISUP grading system, which relates to the size of the nucleoli only [2]. We also examined different morphological features including quantity of cytoplasm, stratification of cells, presence or absence of necrosis, fibrous stroma, presence of foamy macrophages and psammoma bodies.

2.2. TMA construction and immunohistochemistry

From the identified PRCC (n = 376) a representative area per tumor (one tissue core, diameter 1.8 mm) was chosen and marked for construction of tissue microarrays (TMAs) [8]. Formalin-fixed, paraffin-embedded 3 μm sections of the TMA blocks were used for hematoxylin/eosin (H&E) staining and immunohistochemistry. The following antibodies and staining conditions were applied: Racemase (BioPrime Monoclonal AMACR (P504S) Rabbit clone 13H4, dilution 1:50), EMA (DAKO Monoclonal Mouse Anti-Human Epithelial Membrane Antigen, clone E29, dilution 1:20), CK7 (Bio-Genex Anti-Cytokeratin 7 [OV-TL12/30], species mouse, dilution 1:1000), CK20 (DAKO Monoclonal Mouse Anti-Human Cytokeratin 20, clone Ks 20.8, dilution 1:50), p53 (DAKO Monoclonal Mouse Anti-Human p53 Protein, clone DO-7, dilution 1:50), MIB1 (DAKO Monoclonal Mouse Anti-Human Ki-67 Antigen, clone MIB-1, dilution 1:100), E-Cadherin (BD Transduction Laboratories Purified Mouse Anti-E-Cadherin, 610 182, dilution 1:2000). Fumarate hydratase staining was performed on selected cases to display fumarate hydratase deficiency (Santa Cruz, clone J-13, dilution 1:50), [9].

Pictures were taken with Case Viewer program after scanning the slide with Panoramic P250, 3DHistech, Hungary.

2.3. Statistical analyses:

For statistical analyses Racemase, EMA, CK7, CK20 and E-Cadherin staining results were categorized by immunoreactivity scores (IRS) according to Remmele and Stegner [10]: Percentage of stained tumor cells was categorized and given points 0 to 4 as follows: 0%: 0, 1%-9%: 1, 10%-50%: 2, 51%-80%: 3, 81%-100%: 4. This score value was then multiplied by points given to staining intensity (0: no staining, 1: weak staining, 2: moderate staining, 3: strong staining). The resulting values (0–12) were categorized into negative (0–2) and positive [3-12], and used for statistical tests. For p53 IHC only intense positive nuclear staining (overexpression) was considered positive and divided in 2 categories (0–9% = 0/negative and 10–100% = 1/ positive). MIB1 was counted at tumor hotspots (positive nuclei/ 100 cells and documented in percent). As there is no standard threshold defined for MIB1 positivity in RCC, we tested several different values (10%, 15%, 20%, 25%) to define high and low proliferation indices.

We also categorized morphological features and patients' age at surgery for statistical analysis. Necrosis and presence of foamy macrophages were noted as present or absent. Median age at surgery (63 years) was used to compose groups of younger and older patients (<63 years versus ≥63 years).

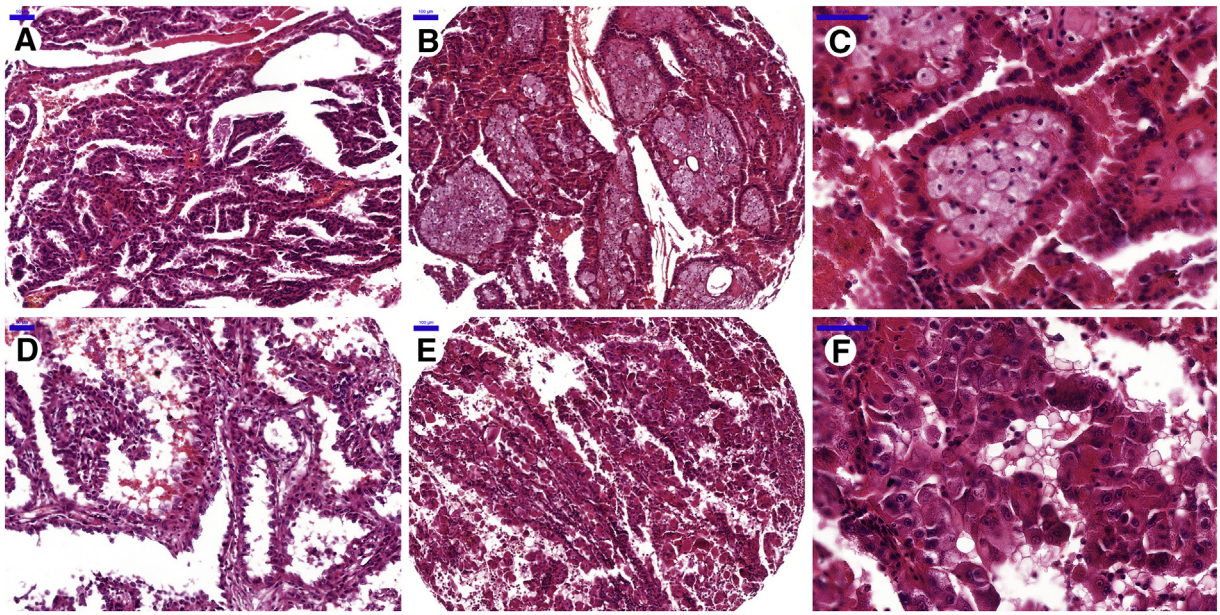

Fig. 1 Papillary renal cell carcinoma (PRCC) subtype 1 and 2 in different magnification. A, PRCC1 in H&E 20×, scale bar 50 μm. B, PRCC1 with foamy macrophages in H&E 10×, scale bar 100 μm. C, PRCC1 with foamy macrophages in H&E 40×, scale bar 50 μm. D, PRCC2 in H&E 20×, scale bar 50 μm. E, PRCC2 with cytological atypia in H&E 10×, scale bar 100 μm. F, PRCC2 with cytological atypia in H&E 40×, scale bar 50 μm.

For statistical analysis we used IBM SPSS for Windows, version 21. Differences were regarded significant at $P < .05$. χ^2-Test was used for distribution of features and staining among subtypes. Log-rank test, Kaplan–Meier plots and univariate Cox analyses were used to investigate associations of clinical data, tumor features and protein expression to patient overall survival (OS). Multivariate Cox regression analyses were performed to test for independency of prognostic factors. We tested online for the total number of events needed with the PRCC subtype distribution at hand by an algorithm called "Sample size – Survival analysis" which was provided by the UCSF (University of California San Francisco) Clinical & Translational Science Institute [11].

3. Results

3.1. Classification of the different PRCC

After histopathological review, 41 of the total cohort of 486 cases had insufficient tumor tissue on the submitted block and were thus excluded. 69 cases were re-classified as other specific

Table 2 Univariate Cox analysis of the significant parameters

Variable	Univariate Cox		
	Hazard ratio	95% CI	*P*
PRCC subtype			
PRCC1	1		
PRCC2	2.457	1.497–4.034	<.0001
Tumor stage (T)			
T1	1		
T2	2.092	1.042–4.202	.038
T3	6.796	3.725–12.397	<.0001
T4	24.902	3.222–192.437	.002
N	11.275	6.086–20.887	<.0001
M	15.936	8.781–28.923	<.0001
2016 WHO/ISUP 2016 grade			
G1	1		
G2	2.223	0.921–5.365	.076
G3	2.723	1.103–6.724	.030
G4	3.441	0.970–12.204	.056
2016 WHO/ISUP grade (2 groups)			
G1	1		
G2-G4	2.471	1.062–5.748	.036
Age at operation (median)			
<63 years	1		
≥63 years	2.499	1.480–4.218	.001
Surgical treatment			
Enucleation/heminephrectomy	1		
Nephrectomy	4.674	1.697–12.877	.003
Foamy macrophages			
Yes	1		
No	2.024	1.203–3.406	.008
Cytoplasm			
Small	1		
Abundant	1.846	1.103–3.092	.020
Psammoma bodies			
No	1		
Yes	2.421	1.036–5.658	.041
Racemase expression			
Yes	1		
No	2.504	1.287–4.870	.007
CK7 expression			
Yes	1		
No	2.543	1.482–4.364	.001
MIB1 expression (cutoff >15%)			
No	1		
Yes	4.280	2.005–9135	<.0001

NOTE. $P < .05$ was considered as statistically significant. CI indicates confidence interval.

tumor entities (Supplementary Table 1) and were excluded as well. The remaining 376 PRCC cases were further classified into PRCC1 (n = 246; 65.4%) and PRCC2 (n = 130; 34.6%), see Fig. 1. Within the category of PRCC1, nine tumors were identified as oncocytic PRCC with inverted nuclei [12,13] and 2 tumors as biphasic squamoid alveolar renal cell carcinoma (BSARCC) [14-16].

3.2. Morphological aspects and clinical data

PRCC1 and PRCC2 showed significant differences in TNM stage and grading (2016 WHO/ISUP grading system) with PRCC2 tumors displaying higher TNM stage and a higher grade than PRCC1 ($P < .0001$, respectively). Other morphological features showed also different distribution between the subtypes: PRCC2 tumors demonstrated significantly more abundant cytoplasm with 75.7% of PRCC2 and 15.0% of PRCC1 cases (n = 316, $P = <.0001$).

Presence of foamy macrophages was more frequent in PRCC1 (70.9% of cases) than PRCC2 (49.0%), (n = 315, $P < .0001$).

Necrosis, abundant fibrous stroma and presence of psammoma bodies displayed no significant difference in distribution between PRCC subtypes.

Sex, median age and surgical treatment had a random distribution between the subtypes.

All results are summarized in Table 1.

In log-rank test and univariate Cox analysis, patients had poorer OS when the tumors showed morphological features like abundant cytoplasm ($P = .018$ and $P = .02$, respectively) and presence of psammoma bodies ($P = .035$ and $P = .041$, respectively). We found better OS was linked to presence of foamy macrophages in log-rank test ($P = .007$) and univariate Cox analysis ($P = .008$).

Furthermore, as expected in log-rank test and univariate Cox analysis high tumor stage, positive nodal status and metastasis (WHO 2016) were associated to poor OS ($P < .0001$; $P < .0001$; $P = .001$, respectively). Grading according to 2016 WHO/ISUP grading system showed no significance in OS ($P = .119$), but after categorizing into 2 groups (G1 versus G2–4) tumors with higher grade were associated to poorer OS in log-rank test ($P = .030$) and univariate Cox analysis ($P =$

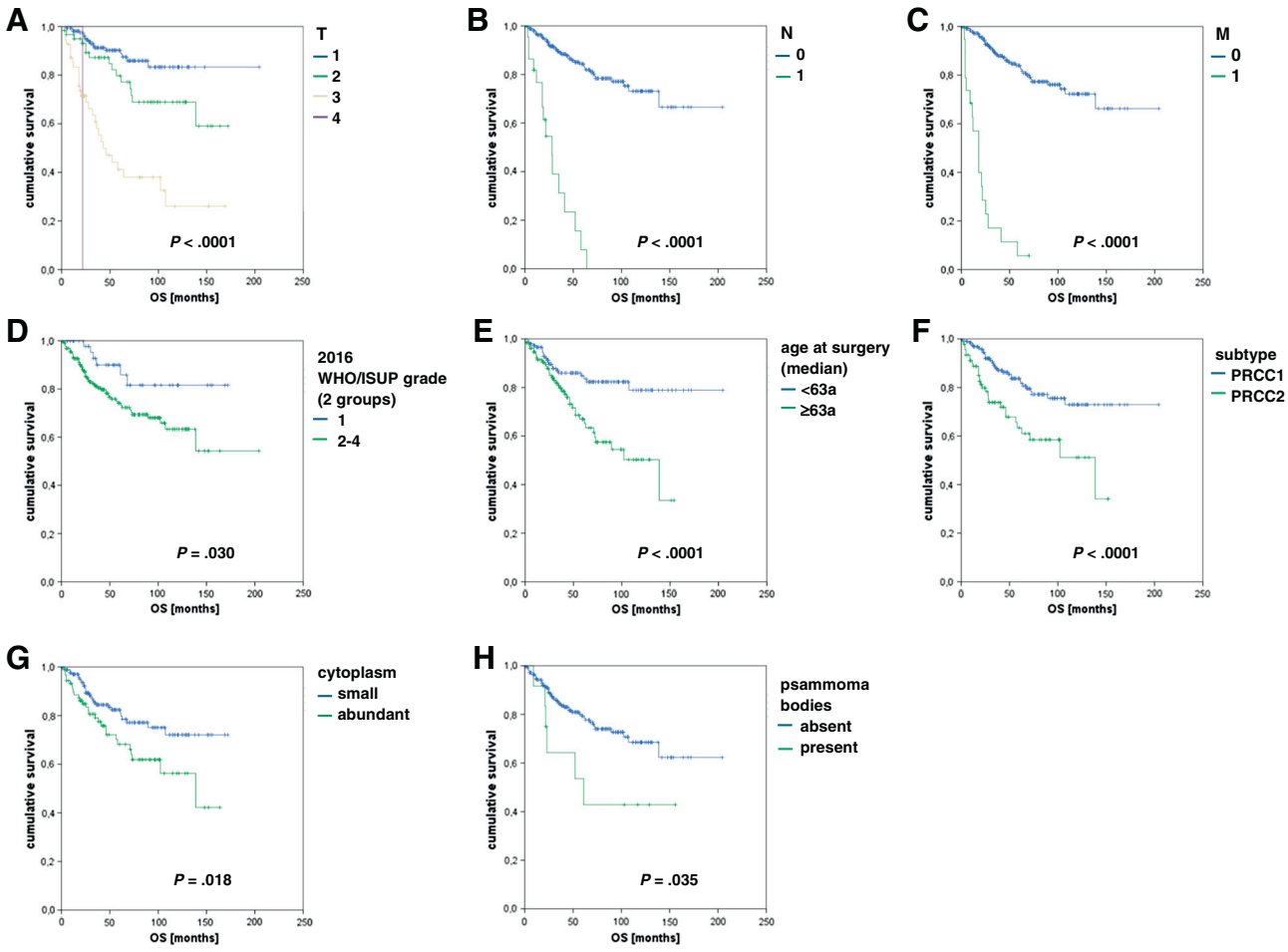

Fig. 2 Overall survival (OS) associated with PRCC features. A, Tumor-stage (T). B, Nodal-status (N). C, Metastasis (M). D, Grading (2016 WHO/ISUP grade). E, Age at surgery. F, Papillary renal cell carcinoma (PRCC) subtype. G, Aspect of cytoplasm. H, Presence of psammoma bodies.

Table 3 Staining profiles of papillary renal cell carcinoma subtype 1 (PRCC1) and papillary renal cell carcinoma subtype 2 (PRCC2).

Staining	PRCC1	PRCC2	*P*
E-Cadherin	40.2% (78/194)	52.8% (57/108)	.040
Racemase	91.9% (181/197)	85.2% (92/108)	.080
EMA	49.2% (96/195)	37.4% (40/107)	.053
CK7	79.6% (156/196)	52.8% (57/108)	<.0001
CK20	5.6% (11/197)	11.1% (12/108)	.111
p53	6.6% (13/196)	14.0% (15/107)	.039
MIB1 (cutoff >15%)	1.5% (3/196)	18.7% (20/107)	<.0001

NOTE. $P < .05$ was considered as statistically significant.
Marker expression in percent and number of positive cases/all evaluable cases

.036) than tumors graded as G1. Other categorizing showed no significance. Patients with ≥63a age at surgery showed significant poorer OS in log-rank test and univariate Cox analysis than patients with age below 63 years ($P < .0001/0.001$, respectively). Patients with nephrectomy as surgical treatment showed significant poorer OS in log-rank test and univariate Cox analysis than patients with enucleation/heminephrectomy ($P = .001/0.003$, respectively). Sex had no significant association to survival.

Patients with PRCC2 also showed poorer OS in log-rank ($P < .0001$) and univariate Cox analysis ($P < .0001$) than PRCC1. Significant results of univariate Cox analyses are summarized in Table 2. Kaplan Meier curves of significant parameters related to OS are shown in Fig. 2. According to our sample size calculation, our cohort had sufficient events (i.e, death by any cause) to detect the 2.457-fold HR of PRCC2 with a power of 90% and an error rate of 5% with the PRCC subtype distribution at hand.

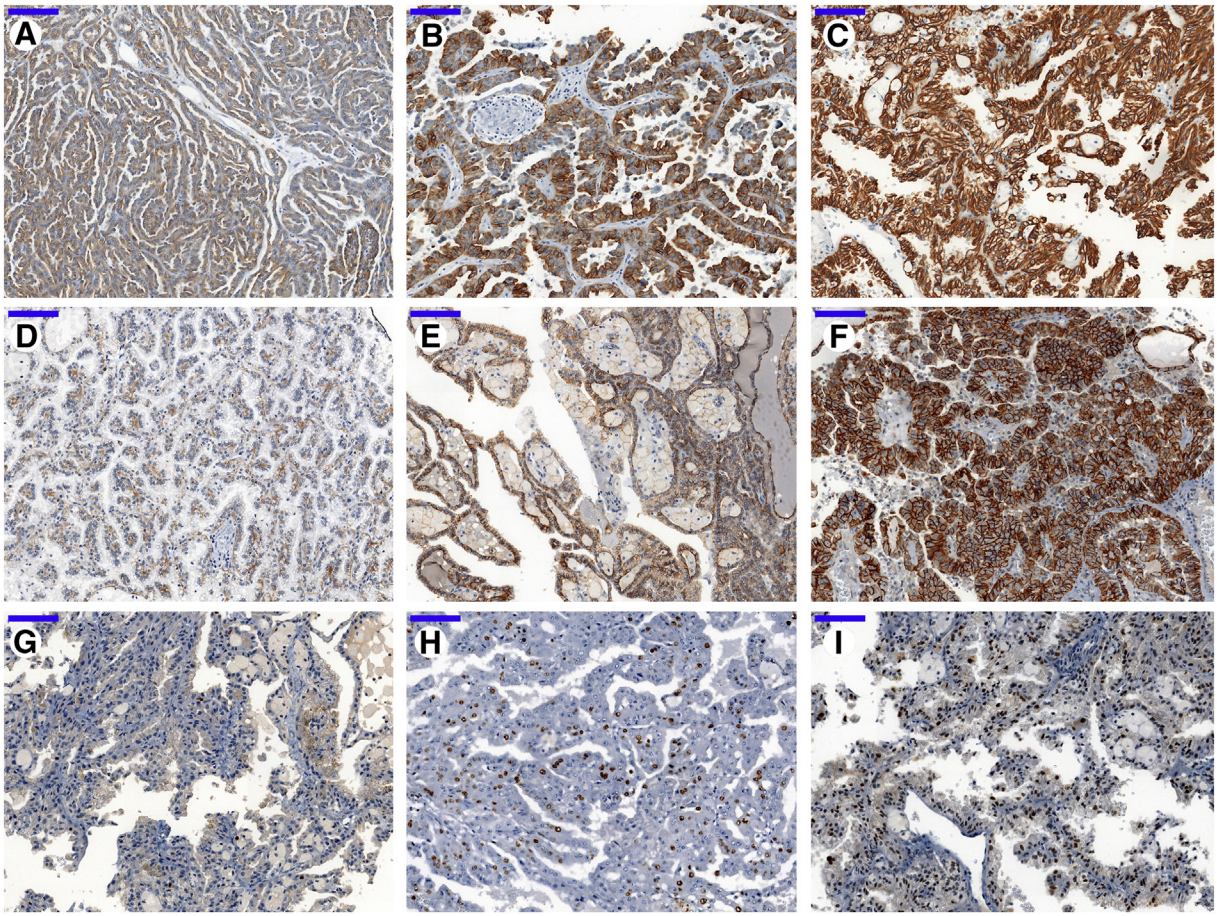

Fig. 3 CK7 expression low-medium-high. D-F, E-Cadherin expression low-medium-high. G and H, MIB-1 expression low-high. I, p53 positive expression. Scale bar in all panels = 100 μm.

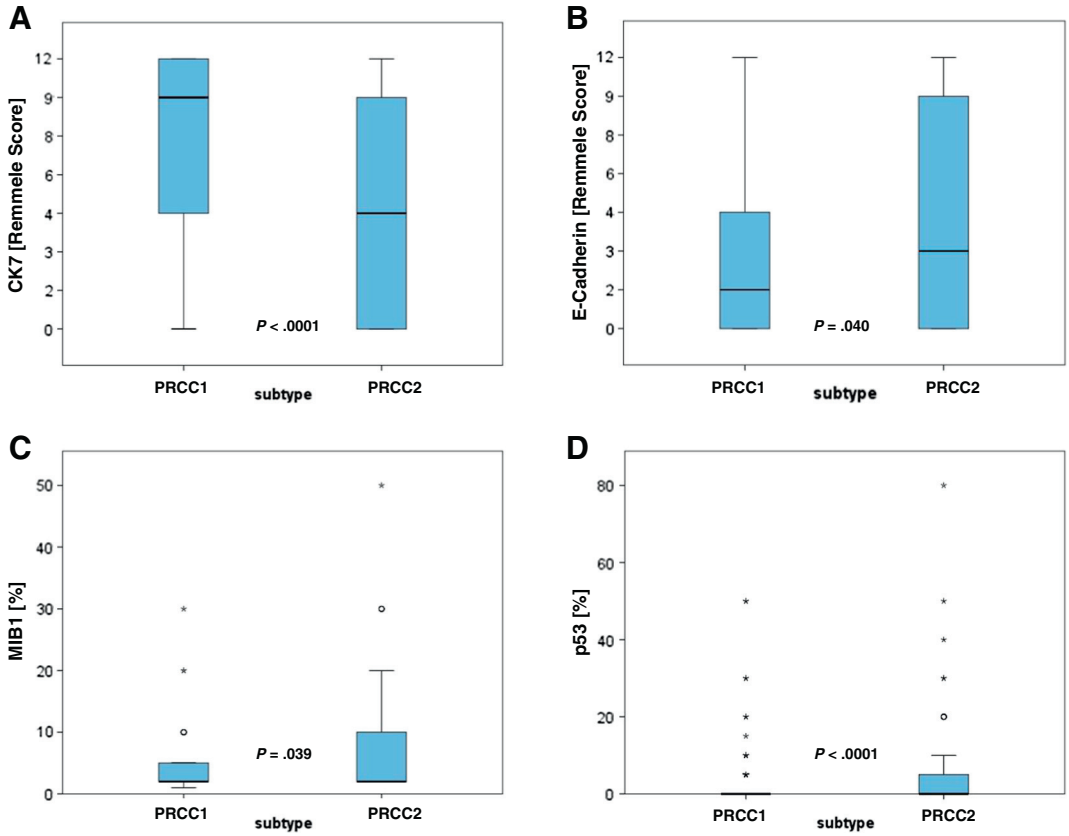

Fig. 4 Staining distribution between papillary renal cell carcinoma type 1 (PRCC1) and type 2 (PRCC2). A, CK7 expression. B, E-Cadherin expression. C, MIB-1 expression. D, p53 expression.

We repeated the univariate Cox regression analyses of OS for PRCC subtypes with stratifications for the different stages or grades, respectively. PRCC2 was a negative predictor of survival only when stratifying for 2016 WHO/ISUP grade 2 or for a combination of 2016 WHO/ISUP grades 2, 3 and 4. Stratifying for 2016 WHO/ISUP grade 1 or tumor stage T4 was not possible due to a lack of PRCC1 cases in these categories (data not shown).

In PRCC2 presence of foamy macrophages (50/102) was associated with good OS in log-rank test (P = .005) and in univariate Cox regression (P = .008; hazard ratio 2.970; CI 1.337–6.597). In few cases in PRCC2 (3/103) presence of psammoma bodies was significant associated with poor OS in log-rank test (P = .037) and in univariate Cox regression (P = .037; hazard ratio 4.256; CI 0.959–18.883).

3.3. Immunohistochemistry

Immunohistochemistry of both PRCC subtypes revealed the following results: CK7 protein was expressed in 213 of 304 cases and significantly more frequently in PRCC1 (79.6%, 156/196) than in PRCC2 (52.8%, 57/108; P < .0001).

E-Cadherin protein was expressed in 135 of all evaluable cases (n = 302) and significantly more frequently in PRCC2 (52.8%, 57/108) than in PRCC1 (40.2%, 78/194; P = .040). p53 overexpression also was found in 28 of 303 cases (PRCC2: 15/107, 14% versus PRCC1: 13/196, 6.6%; P = .039).

We used different threshold values (10%, 15%, 20% and 25%) to define high proliferation and tested both PRCC

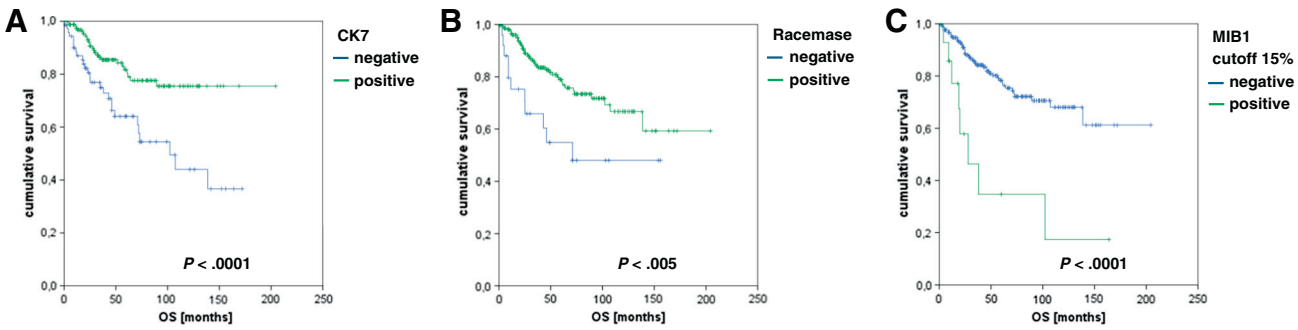

Fig. 5 Overall survival (OS) associated with papillary renal cell carcinoma (PRCC) expression of CK7 (A), racemase (B), and MIB-1 (C).

subtypes for differences in distribution. Interestingly, the distribution of proliferation levels was significantly different using all the thresholds, except for 25% (data not shown). We decided to proceed with a 15% threshold after we had performed univariate Cox regression analyses, where MIB1 was a prognostic marker only at the cutoff values of 10% (data not shown) and 15%. Our approach was conservative; therefore, we chose to continue with the higher value.

A higher proliferation rate (MIB1 >15%) was more frequent in PRCC2 (20/107, 18.7%) than PRCC1 (3/196, 1.5%; $P < .0001$). MIB1 expression in PRCC1 (n = 196) ranged from 1% to 30% (mean 3.26%, median 2%) and from 2% to 50% (mean 7%, median 2%) in PRCC2 (n = 107).

EMA, Racemase and CK20 staining showed no significant differences between the subtypes (summarized in Table 3).

In conclusion, PRCC1 more frequently expressed CK7, whereas PRCC2 more frequently showed expression of E-Cadherin, p53 and higher proliferation index (MIB1), summarized in Fig. 3 (marker expression) and Fig. 4 (distribution boxplots).

Log-rank tests revealed an association of CK7 ($P < .0001$) and Racemase ($P = .005$) expression with better OS. In contrast, high proliferation index (MIB1> 15%) was linked to significantly poorer OS ($P < .0001$). Kaplan Meier curves are shown in Fig. 5.

The results of univariate Cox analysis were in line: CK7 ($P = .001$), Racemase ($P = .007$) and MIB1 ($P < .0001$) expressions were significantly linked to overall survival. EMA, E-Cadherin and CK20 expression were not associated to OS (see Table 2).

3.4. Multivariate Cox analysis:

In multivariate Cox analysis, independent variables for patients OS were tumor stage (T), metastasis (M), age at surgery (median) and proliferation rate (MIB1 >15%). All other variables, including PRCC subtype or 2016 WHO/ISUP grade, were not of independent prognostic values (summarized in Table 4).

We also did a subgroup analysis of M0 cases and we found comparable results in multivariate Cox analysis: The only independent parameters were tumor stage (T), age at surgery (median) and nodal status (N) – PRCC subtype was not an independent prognostic marker (Supplementary Table 2).

3.5. Distribution of enhanced MIB1 positivity among stage and grade:

Finally, we took a closer look at MIB1 expression and possible associations to tumor characteristics.

In addition to the higher amount of MIB1 overexpression in PRCC2 as described above, we also found a significant association with higher tumor stages (T3 + T4: 15/579, 26.3% vs. T1 + T2: 4/202, 2.0%) and higher 2016 WHO/ISUP

Table 4 Multivariate Cox analysis

Variable	Hazard Ratio	95% CI	P [c]
Multivariate Cox analysis (n = 248) [a]			
PRCC subtype	0.940	0.457–1.932	.865
T1			.036
T2	1.232	0.562–2.699	.603
T3	3.111	1.387–6.975	.006
T4	1.516	0.162–14.173	.715
N	2.223	0.806–6.131	.123
M	4.334	1.400–13.416	.011
2016 WHO/ISUP grade (2 groups)	1.674	0.621–4.512	.309
Age at surgery (median)	2.384	1.301–4.367	.005
Surgical treatment	2.082	0.719–6.034	.177
Multivariate Cox analysis (n = 223) [b]			
PRCC subtype	1.095	0.542–2.211	.800
T1			.013
T2	1.988	0.910–4.341	.085
T3	3.932	1.739–8.890	.001
T4	3.200	0.351–29.156	.302
M	9.401	3.856–22.917	<.0001
MIB1 (cutoff >15%)	2.465	1.075–5.651	.033

Abbreviation: CI, confidence interval.

[a] Multivariable Cox analysis including variables: papillary renal cell carcinoma (PRCC) subtype, tumor-stage (T), nodal status (N), metastasis (M), 2016 Worlds Health Organization (WHO)/International Society of Urological Pathology (ISUP) grade in 2 groups (G1 versus G2-4), age at surgery (median 63 y) and surgical treatment.

[b] Multivariable Cox analysis including variables: PRCC subtype, tumor-stage (T), metastasis (M) and proliferation index (MIB1) with cutoff >15%.

[c] $P < .05$ was considered as statistically significant.

grades (G2 + G3 + G4: 20/232, 8.6% vs. G1: 0/48, 0.0%), characteristics which are typical for more aggressive tumors. This supports the results of our overall survival analyses. A summary of MIB1 overexpression distribution can be found in Supplementary Table 3.

4. Discussion

The classification of epithelial renal neoplasms has been the subject of a dynamic evolution during the last decades. PRCC is not exempted from these recent developments and conceptual reappraisal. Our results in this large multi-institutional collaborative study highlight the well-known clinicopathological differences between the 2 major subtypes of PRCC. The frequency of PRCC1 and PRCC2 among PRCC is not entirely clear. There are reports of PRCC1 predominance (2:1), as well as balanced frequencies or PRCC2 predominance [17,18]. Our study confirms the distinctness of the immunohistochemical profiles of PRCC subtypes with CK7 being more frequently expressed in PRCC1 whereas E-Cadherin, p53 and MIB1 are more frequently expressed in PRCC2, a finding that fits the common classification of PRCC and is in line with other publications [4,5]. In our study, PRCC2 showed a higher proliferation rate (MIB1) than PRCC1 which correlated with worse overall survival as published before [19,20]. Multivariate Cox regression analysis showed that expression of immunohistochemical markers other than MIB1 (Racemase, CK7, CK20, EMA, E-Cadherin) do not correlate with survival.

Furthermore we investigated several morphological aspects of PRCC architecture and cytological features. We could show that PRCC2 was associated with a higher TNM stage and a higher grade (2016 WHO/ISUP grading system) than PRCC1. Although there was some overlap, morphological features showed a distinct distribution between the subtypes: Foamy macrophages were more frequent in (but not restricted to) PRCC1 whereas abundant cytoplasm was a more common finding in PRCC2. These findings are in line with other studies, although presence of psammoma bodies, fibrous stroma and necrosis in our study showed no significant difference between the subtypes [18].

In log-rank test and univariate Cox analysis, presence of foamy macrophages in PRCC was associated with a better outcome, whereas tumors with abundant cytoplasm and psammoma bodies showed poorer OS, as described before [12,21].

In PRCC2 presence of foamy macrophages was associated with better OS in log-rank test and univariate Cox regression. The three cases in PRCC2 with presence of psammoma bodies showed poorer OS in log-rank test and univariate Cox regression.

PRCC2 was associated with worse OS than PRCC1 in log-rank test and univariate Cox analysis by significance which might suggest, PRCC subtype is a prognostic factor. However, multivariate Cox analysis showed it was not an independent predictor for outcome, when age, TNM, Grading (2016 WHO/ISUP grading system) are included [18,22,23]. The only independent prognostic factors for OS were T, M, age at surgery and proliferation rate (MIB1). Therefore the better OS in PRCC1 is mainly a reflection of its encapsulated nature associated with lower TNM stage [24], while enhanced proliferation might add to the aggressive nature of high grade and high stage tumors independently from PRCC subtype. Protein expression of Racemase, E-Cadherin, EMA, CK7, CK20 and p53 are not of prognostic value but in addition to morphological features may be useful for correct PRCC subtyping. With our cohort of 376 PRCC including 130 PRCC2 the aim of our study was to better characterize PRCC by describing histomorphological features and protein expression. As we have shown, current subtyping seems not helpful for estimating patient survival but correctly staging and grading according to TNM and 2016 WHO/ISUP grading system. In order to expand our understanding of PRCC tumor biology, more information about genome profiles and cell signaling in large cohorts of PRCC is necessary [25]. Especially PRCC2 needs further examination, as the existence of highly malignant tumors in the heterogeneous spectrum of PRCC2 has been postulated. [5-7,26,27].

Currently, there is increasing controversy regarding the definition criteria and classification of what used to be considered PRCC2. This is mainly based on the significant overlapping between this PRCC subtype and other types of high-grade distal nephron adenocarcinoma with prominent papillary or tubulopapillary pattern [28]. Recent studies have identified fumarate hydratase-deficient RCC as a specific genetically defined subtype of RCC in the historical spectrum of PRCC2 [29-31]. In addition, it became apparent that several RCC in the PRCC2 category likely represent collecting duct carcinoma with prominent tubulopapillary features [29]. According to these recent developments, PRCC2 seems to be a diagnosis of exclusion. Whether PRCC2 (after exclusion of other overlapping entities such as collecting duct carcinoma and fumarate-hydratase-deficient RCC) should be considered a high-grade RCC unclassified/NOS or as genuine PRCC2 remains unclear at the moment and deserves further morphological studies combined with genetic profiling.

Author's contributions

I. Polifka participated in the pathological data management, constructed the tissue microarrays and took part in pathological data acquisition and staining evaluation. She merged, managed and summarized the analyzed data of the paper. She did literature search and compared the project of the paper with the available other studies. She wrote the manuscript and revised it by the co-authors suggestions. C. Stöhr PhD participated in the pathological data management, performed the statistical analysis, participated in the data interpretation and drafting of the manuscript. A. Hartmann MD carried out pathological data acquisition, performed the staining evaluation, participated in the

data interpretation and drafting of the manuscript. A. Agaimy MD took part in pathological data acquisition, participated in the data interpretation and drafting of the manuscript. V. Spath MD took part in pathological data acquisition. C. I. Geppert MD performed the scanning procedure and took part in creating the composite pictures of the manuscript. E. Herrmann MD took great part in starting the collective material for this study and clinical data acquisition. All others participated in collecting the material and clinical data acquisition. All authors read and approved the final manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.08.006.

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