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RESEARCH

Clinical pathological characteristics and prognostic analysis of renal primitive neuroectodermal tumours: a multicentre retrospective study of 16 cases in Northwest China

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Abstract

Objective Renal primitive neuroectodermal tumours (rPNETs) are extremely rare and highly aggressive malignancy, posing significant diagnostic and therapeutic challenges. This study aims to describe the clinicopathological characteristics, treatment strategies, and survival outcomes of 16 cases of rPNET from multiple centers in Northwest China, and to explore potential prognostic factors.

Methods A multicenter retrospective study was conducted, including 16 patients diagnosed with rPNET across five hospitals in Northwest China. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) were employed to assess the expression of molecular markers, including P53, BCL-2, Ki-67, and EWSR1 gene rearrangements. Survival analysis was performed using the Kaplan-Meier method, and prognostic factors were evaluated using univariate and multivariate Cox regression models.

Results The median age of the patients was 39 years, with a median Ki-67 proliferation index of 50%. P53 mutations were detected in 87.0% of cases, and BCL-2 positive expression was observed in 56.25% of cases. The median overall survival (OS) was 14 months. Univariate analysis revealed that age, tumor stage, BCL-2 expression, and Ki-67 index were significantly associated with OS. Multivariate analysis identified high Ki-67 expression (HR = 1.100, 95% CI: 1.030–1.174, $p = 0.004$) and negative BCL-2 expression (HR = 0.151, 95% CI: 0.026–0.888, $p = 0.037$) as independent risk factors for poor prognosis. Kaplan-Meier survival curves demonstrated that the median OS was significantly shorter in patients with high Ki-67 expression (12 months) compared to those with low Ki-67 expression (20 months) (Log-rank test, $P < 0.01$). Similarly, the median OS was significantly shorter in the BCL-2 negative group (10 months) compared to the BCL-2 positive group (24 months) (Log-rank test, $P < 0.05$).

Conclusion The absence of rosette structures does not exclude the diagnosis of rPNET. BCL-2 and Ki-67 expression are significant prognostic factors, with high Ki-67 expression and negative BCL-2 expression associated with worse outcomes. These findings highlight the importance of molecular markers in risk stratification and treatment planning for rPNET.

Keywords rPNET · Ki-67 · BCL-2 · Prognostic factors

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Abbreviations

PNET	Primitive neuroectodermal tumor
EWS	Extraskeletal Ewing's sarcoma
cPNET	Center primitive neuroectodermal tumor
pPNET	Peripheral primitive neuroectodermal tumor
rPNET	Renal primitive neuroectodermal tumor
FISH	Fluorescence In Situ Hybridization
OS	Overall survival
PFS	Progression-free survival
H&E	Hematoxylin and Eosin
FISH	Fluorescence in situ hybridization
1R	1 Red signal
1G	1 Green signal
1F	1 Fusion signal
CD99	Cluster of Differentiation 99
FLi-1	Friend Leukemia Integration 1
Syn	Synaptophysin
CgA	ChromograninA
NSE	Neuron-Specific Enolase
Cytokeratin	CK
P53	Tumor Protein 53
BCL-2	B-cell leukemia 2
Ki-6	Kiel 67
EWSR1	Ewing's sarcoma breakpoint 1 gene
AJCC	American Joint Committee on Cancer
VAC	Vincristine + Adriamycin + Cyclophosphamide
IE	Ifosfamide + Etoposide

Introduction

Primitive neuroectodermal tumours (PNETs) are rare and highly aggressive malignancies originating from neural crest-derived embryonic remnants. Based on the site of occurrence, PNETs are classified into two main types: central PNETs (cPNETs), which typically arise in the central nervous system, and peripheral PNETs (pPNETs), which predominantly occur in the extremities, chest wall, retroperitoneum, pelvis, and solid organs. Peripheral PNETs share significant clinical, cellular, and molecular genetic similarities with extraskeletal Ewing's sarcoma (EWS), often making it difficult to distinguish between the two entities. In clinical practice, the terms EWS and pPNET are frequently used interchangeably (Ludwig et al. 2021).

Renal primitive neuroectodermal tumours (rPNETs) are an exceptionally rare subtype of pPNETs, accounting for less than 1% of all renal malignancies (Thyavihally et al. 2008). Clinically, rPNETs present with nonspecific symptoms, including abdominal pain, haematuria, and palpable abdominal masses. Radiological findings are often indistinguishable from those of other renal malignancies, do pathological examination is needed for definitive diagnosis. Histologically, rPNETs are characterized by small round blue cells, and accurate diagnosis typically requires a combination of immunohistochemical staining and molecular genetic testing (Ellison 2002). However, owing to its rarity and the limited availability of advanced diagnostic tools, rPNETs are frequently misdiagnosed as other renal tumours, leading to delays in appropriate treatment. The current standard treatment for rPNETs involves surgical resection, often supplemented with chemotherapy and radiotherapy. However, the lack of standardized treatment protocols results in significant variability in patient outcomes, particularly in advanced cases, which are associated with high rates of local recurrence and distant metastasis. Consequently, the diagnosis and treatment of rPNETs remain substantial clinical challenges.

In China, research on rPNETs has been limited primarily to isolated case reports and small-scale case series, with a notable absence of large-scale, multicentre studies. Existing studies are often limited by small sample sizes, inconsistent diagnostic criteria, nonspecific treatment regimens, and limited exploration of prognostic factors. There is an urgent need for high-quality, multicentre studies to provide robust evidence to guide clinical practice. This study aims to address these gaps by analysing the clinicopathological characteristics and prognostic factors of 16 patients with rPNETs from multiple centres in Northwest China. By integrating the latest advancements in rPNET research, this study aims to enhance the understanding of the diagnosis and treatment of this rare tumour, offering valuable insights for future research and clinical decision-making.

Materials and methods

Case selection

This retrospective study involved analysis of 16 cases of pathologically confirmed rPNETs with complete clinical data from five major hospitals in Northwest China between January 2005 and January 2025. The case distribution was as follows: Shaanxi Provincial People's Hospital ($n=5$), Xijing Hospital of Air Force Medical University ($n=4$), Shaanxi Provincial Cancer Hospital ($n=3$), Xinjiang Uygur Autonomous Region People's Hospital ($n=3$), and Lanzhou First Hospital ($n=1$). Data collected from hospital medical records and pathological reports included patient demographics (age, sex), clinical presentation, tumour characteristics (size, location), clinical stage, presence of caval thrombus and lymph node metastasis, histological diagnosis, and expression of molecular markers (including cluster of differentiation 99 (CD99), Friend leukaemia integration

1 (FLi-1-1), synaptophysin (SYN), neuron-specific enolase (NSE), chromogranin A (CgA), tumour protein 53 (P53), B-cell leukaemia 2 (BCL-2), Kiel 67 (Ki-67), and Ewing's sarcoma breakpoint gene 1 (EWSR1), gene rearrangement status, and treatment details (surgical approach, margin status, chemotherapy, radiotherapy).

The PNET staging criteria for this study were adapted from the 8th edition AJCC (American Joint Committee on Cancer) staging system for adult sarcomas; tumours were categorized into three groups: local lesions (stage I and II), tumours with local progression (stage III, involving adjacent organs or regional lymph nodes), or tumours with distant metastases (stage IV, with distant organ involvement).

This study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital (Approval No. 2024 K-388).

Molecular testing and interpretation

For cases with incomplete or ambiguous molecular marker results, additional testing was performed. The panel of molecular markers included at least CD99, FLi-1-1, SYN, NSE, CgA, P53, BCL-2, Ki-67, cytokeratin (CK), vimentin, and leukocyte common antigen (LCA). All of the information concerning the antibodies is presented in Table 1. The tissue samples were fixed in 10% formalin, embedded in paraffin, and sectioned at a thickness of 4 μm. Immunohistochemistry (IHC) was performed using the Elivision two-step method. All cases were independently reviewed by two pathologists (Jing Du and Jiayan Liu) with over ten years of clinical experience in pathology, and differences were resolved by consensus. For each slide, five high-power fields were selected, and 500 tumour cells were counted to determine the percentage of positive cells. Positive expression was defined as brown–yellow staining in the cytoplasm, cell membrane, or nucleus. Although the 20–30% Ki-67 index serves as the conventional cut-off for most tumours, higher thresholds are well established for PNET because of its aggressive nature. Considering our limited samples and to maintain balanced group allocation, we adopted 40% as the proliferation index cut-off, which was considered to have low (≤40% positive cells) or high (>40% positive cells) proliferative activity. Mutant p53 manifested as either (1) diffuse strong nuclear staining (3+, >50% cells) or (2) complete absence (0%). Wild-type p53 was indicated by negative or focal weak staining (1+, <10% cells) with a heterogeneous distribution (McCluggage et al. 2011). BCL-2 positivity was defined as >1% of tumour cells with cytoplasmic or membranous staining of BCL-2 (Lin et al. 2021).

EWSR1 gene rearrangements were detected using fluorescence in situ hybridization (FISH) with probes provided by Wuhan Kanglu Biotechnology Co., Ltd. The FISH procedure included prehybridization treatment, denaturation, hybridization, washing, and counterstaining. The signals were visualized under a fluorescence microscope, with orange signals indicating the 3' end of EWSR1 and green signals indicating the 5' end. A positive result was defined as at least 15% of the tumour cells showing a 1F1R1G signal pattern.

Treatment and Follow-Up

Surgical treatment included radical nephrectomy or partial nephrectomy, with or without caval thrombectomy. Adjuvant chemotherapy regimens included VAC (vincristine, doxorubicin, and cyclophosphamide) and IE (ifosfamide and etoposide). Postoperative radiotherapy was administered to selected patients at doses of 45–50 Gy, delivered in 25–28 fractions. Anti-angiogenic targeted therapy with anlotinib (12 mg daily, administered orally before breakfast for 2 weeks followed by a 1-week break, repeated every 21 days) was used in some cases. Patients were followed every 3 months via outpatient visits, medical records, or telephone interviews. The follow-up data included survival status and recurrence/metastasis. One patient was lost to follow-up, and their last follow-up date was used for analysis.

Table 1 The details of all antibodies

Antibodies	Manufacturer	Positive controls
CD99	MXB Biotechnologies (Fuzhou, China)	Tonsil
FLi-1	Zhongshan Goldenbridge (Beijing, China)	Ewing's sarcoma
P53	Zhongshan Goldenbridge (Beijing, China)	Tonsil
NSE	MXB Biotechnologies (Fuzhou, China)	Tonsil
SYN	MXB Biotechnologies (Fuzhou, China)	Pancreatic islet cells
CgA	MXB Biotechnologies (Fuzhou, China)	Tonsil
CK	MXB Biotechnologies (Fuzhou, Chin)	Cuticle
Vim	MXB Biotechnologies (Fuzhou, China)	Interstitial appendices
LCA	MXB Biotechnologies (Fuzhou, China)	Lymph node
BCL-2	F. Hoffmann-La Roche Ltd (Basel, Switzerland)	Lymph node
Ki-67	F. Hoffmann-La Roche Ltd (Basel, Switzerland)	Tonsil

All reagents: ready-to-use (no dilution required)

Statistical analysis

Overall survival (OS) was the primary endpoint. Survival curves were generated using the Kaplan–Meier method, and differences between groups were assessed using the log-rank test. Univariate Cox regression analysis was performed to identify potential prognostic factors; variables with $p<0.05$ were included in the multivariate Cox regression model. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. All the statistical analyses were performed using SPSS 27.0 (IBM, USA); $p<0.05$ was considered to indicate statistical significance.

Results

Clinical characteristics

The study included 16 patients who were diagnosed with rPNETs, including 10 males (10/16) and 6 females (37.5%), with a mean age of 35.4 years (range: 15–56 years) and a median age of 39 years. The tumours were located in the left kidney in 9 patients (9/16) and in the right kidney in 7 patients (7/16). The maximum tumour diameter ranged from 5 to 14 cm, with an average diameter of 8.5 cm. The most common presenting symptoms included flank pain (9/16, 56.3%), an abdominal mass (8/16, 50.0%), abdominal distension (5/16, 31.3%), and haematuria (1/16, 6.3%). One patient was asymptomatic, with the tumour incidentally detected during a routine physical examination. Imaging and pathological examinations revealed caval thrombus in 8 patients (8/16, 50.0%) and lymph node metastasis in 4 patients (4/16, 25.0%). According to tumour stage, 3 tumours (3/16, 18.8%) were classified as localized disease, 8 tumours (8/16, 50.0%) were classified as locally advanced disease, and 5 tumours (5/16, 31.3%) were classified as metastatic disease at initial diagnosis. During follow-up, recurrence or metastasis occurred in 9 patients (9/16, 56.3%); 3 patients had bone metastasis, 2 patients had concurrent lung and liver metastases, and 1 patient each had isolated liver metastasis, isolated lung metastasis, and pancreatic/splenic metastasis. One patient presented with recurrence with lung metastasis within one year, whereas the remaining 7 patients developed distant organ metastasis within two years (Table 2).

Pathological features and molecular marker expression

Gross examination revealed that all the tumours were dark brown or tan in colour and exhibited areas of cystic degeneration, multifocal haemorrhage, and necrosis. None of the

Table 2 Clinical and pathological characteristics of renal primitive neuroectodermal tumor (rPNET)

Variable	*N*	%
Age(y)		
≤39	8	50.00%
>39	8	50.00%
Gender		
Male	10	62.50%
Famale	6	37.50%
Tumor size(cm)		
≥8	11	68.75%
<8	5	31.25%
Location		
Left sided	9	56.25%
Right sided	7	43.75%
Initial symptom		
Low back pain	9	56.25%
Abdominal mass	8	50.00%
Abdominal distension	5	31.25%
Hematuria	1	6.25%
Physical examination	1	6.25%
Renal venous tumor thrombus		
Yes	8	50.00%
No	8	50.00%
Chrysanthemum-shaped mass structure		
Yes	9	56.25%
No	7	43.75%
Staging		
Local lesions	3	18.75%
Local progression	8	50.00%
Distant metastasis	5	31.25%
Lymph node metastasis		
Positive	4	25.00%
Negative	12	75.00%
CD99		
Positive	16	100%
Negative	0	0%
FLi-1		
Positive	15	93.75%
Negative	1	6.25%
P53		
mt	10	87.00%
wt	6	13.00%
NSE/SYN/CgA		
Positive	12	75.00%
Negative	4	25.00%
BCL-2		
Positive	9	56.25%
Negative	7	43.75%
Ki-67		
≤40%	7	20.40%
>40%	9	57.40%
CK		
Positive	3	18.75%
Negative	13	81.25%
EWSR1 gene rearrangement		
Yes	13	100%

Table 2 (continued)

Variable	*N*	%
No	0	0
Treatment Method		
Surgery	13	81.25%
Surgery + Chemotherapy/Radiotherapy	9	56.25%
Anti-angiogenic targeting drugs		
Yes	3	23.10%
No	13	81.25%

Abbreviations as defined in text

tumours were encapsulated or had incomplete capsules. Microscopically, all 16 patients presented diffuse sheets or nests of small round blue cells separated by fibrous septa. The nuclei were hyperchromatic, irregular, and pleomorphic, with frequent mitotic figures. Pseudorosettes or Homer-Wright rosettes were observed in 9 cases (3/16, 56.3%) (Fig. 1).

IHC analysis revealed diffuse membranous positivity for CD99 in all cases (16/16, 100%). FLi-1 nuclear expression was detected in 15 cases (15/16, 93.8%), whereas SYN, CgA, or NSE expression was detected in 12 cases (12/16, 75.0%). Vimentin expression was observed in 9 cases (56.3%), and BCL-2 expression was positive in 9 cases (9/16, 56.3%). CK expression was detected in 3 cases (18.8%), whereas LCA expression was negative in all cases. P53 expression was positive in 10 cases (10/16, 62.5%), with 6 cases (6/16, 37.5%) showing wild-type expression and 10 cases (10/16, 62.5%) showing mutant-type expression (either strong overexpression or complete absence). The Ki-67 proliferation index ranged from 20 to 90%, with a median of 50%. On the basis of a cut-off of 40%, 7 cases (7/16, 43.8%) were considered to have low proliferative activity (Ki-67 ≤ 40%), and 9 cases (9/16, 56.3%) were considered to have high proliferative activity (Ki-67 > 40%).

EWSR1 gene rearrangements were detected via FISH in all 16 cases, with a typical 1F1R1G signal pattern observed in 13 cases (81.3%). The percentage of positive cells ranged from 40 to 80% (Fig. 2). One patient exhibited red signal amplification, and one patient exhibited an atypical signal pattern (1F1R2G/1F2R2G/1F2R1G) in approximately 30% of the cells.

Treatment and survival outcomes

Among the 16 patients, 2 declined treatment, and 2 underwent palliative nephrectomy due to extensive tumour adhesion to surrounding tissues or organs. A single patient was treated with pharmacological therapy alone. The remaining 11 patients (11/16, 68.75%) underwent radical nephrectomy with/without caval thrombectomy, all of whom demonstrated negative margins at the ureteral stump, renal vasculature, and surgical resection sites. Postoperative adjuvant chemotherapy was administered to 9 patients (9/16, 56.3%) via either the VAC or IE regimen for 4–6 cycles. Disease progression occurred in 2 patients (2/16, 12.5%) following chemotherapy. Three patients (3/16, 18.8%) received anti-angiogenic targeted therapy with anlotinib (12 mg daily, administered orally for 2 weeks followed by a 1-week break, repeated every 21 days), achieving a 100% disease control rate (2 complete responses and 1 partial response). Postoperative radiotherapy was administered to 4 patients (4/16, 25.0%), with a median dose of 48 Gy delivered in 25–28 fractions).

The 1-year, 3-year, and 5-year OS rates were 56.3%, 31.3%, and 0%, respectively, with a median OS of 26 months. The median follow-up duration was 14 months (range: 1–56 months). At the time of analysis, 14 patients had died, and 2 patients remained alive; the longest follow-up duration was 56 months.

Analysis of survival and prognostic factors

Kaplan–Meier survival analysis revealed that the median OS was significantly shorter in the high Ki-67 expression group (12 months) than in the low-expression group (20 months) (log-rank test, $P < 0.01$). Similarly, the median OS was significantly shorter in the BCL-2-negative group (10 months) than in the BCL-2-positive group (24 months) (log-rank test, $P < 0.05$). Univariate analysis revealed that patient age, tumour stage, the Ki-67 index, BCL-2 expression, and the use of antiangiogenic drugs were significantly associated with OS ($P < 0.05$). Multivariate Cox regression analysis confirmed that high Ki-67 expression (HR = 1.100, 95% CI: 1.030–1.174, $p = 0.004$) and negative BCL-2 expression (HR = 0.151, 95% CI: 0.026–0.888, $p = 0.037$) were independent risk factors for a poor rPNET prognosis (Tables 3 and 4, Fig. 3 and 4).

Discussion

In this study, the male-to-female ratio among rPNET patients was 1.8:1; the proportion of males was greater, but sex did not significantly impact survival outcomes. The average age at diagnosis was 39 years, and 7 patients (43.8%) were aged 40 years or older. This contrasts with studies from the United States, where the average age of rPNET patients was reported to be 26 years, with 38% of cases occurring in patients aged 20 to 29 years (Ellinger et al. 2006). The higher median age in our study may reflect regional demographic characteristics or the limited sample size. Univariate analysis revealed a significant correlation between age and OS ($p < 0.05$). This finding aligns with those of previous studies, such as those by Grohar et al., which indicated

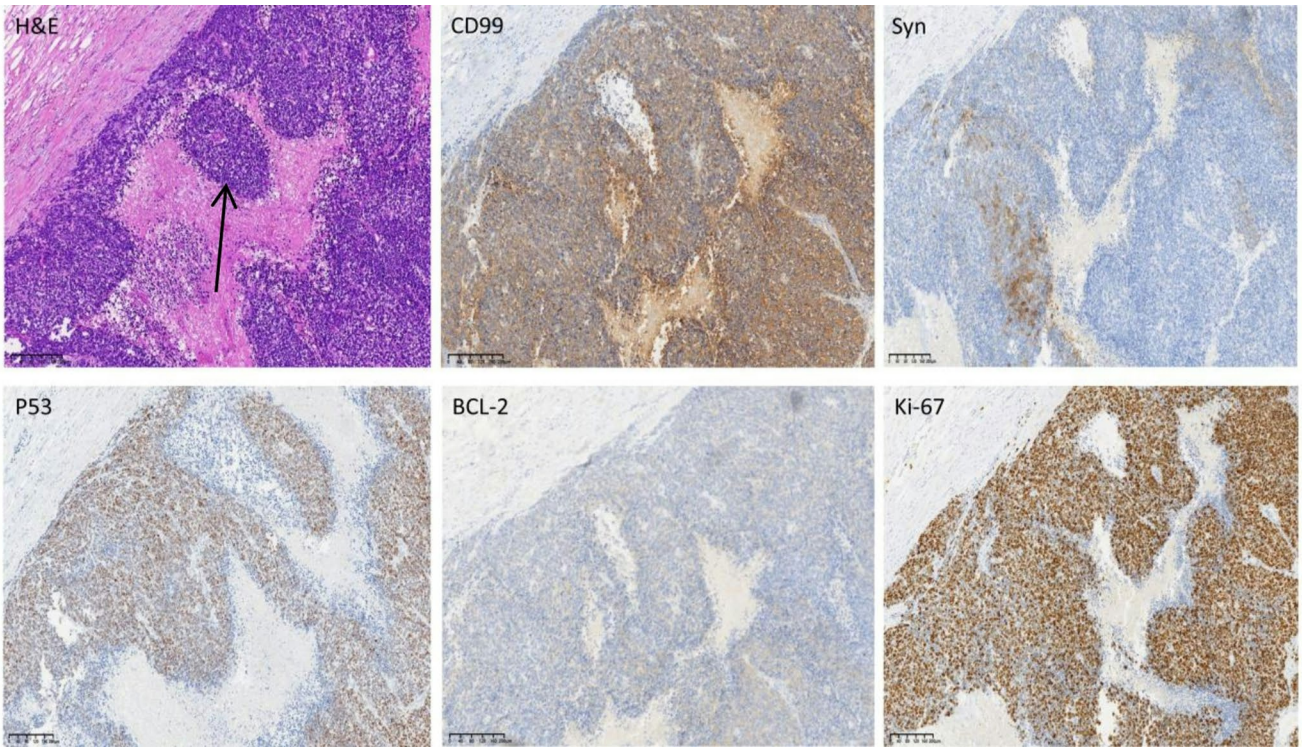

Fig. 1 Hematoxylin and eosin (H&E) and Immunohistochemical (IHC) Staining Images of Renal primitive neuroectodermal tumor (rPNET). Arranged from left to right and top to bottom, are as follows: H&E staining(The small round blue cells are arranged in a nest-like pattern. The arrow indicates a rosette-like structure.), CD99, Syn, P53, BCL-2, and Ki-67 All show positive protein expression. (Light microscope, 40x magnification)

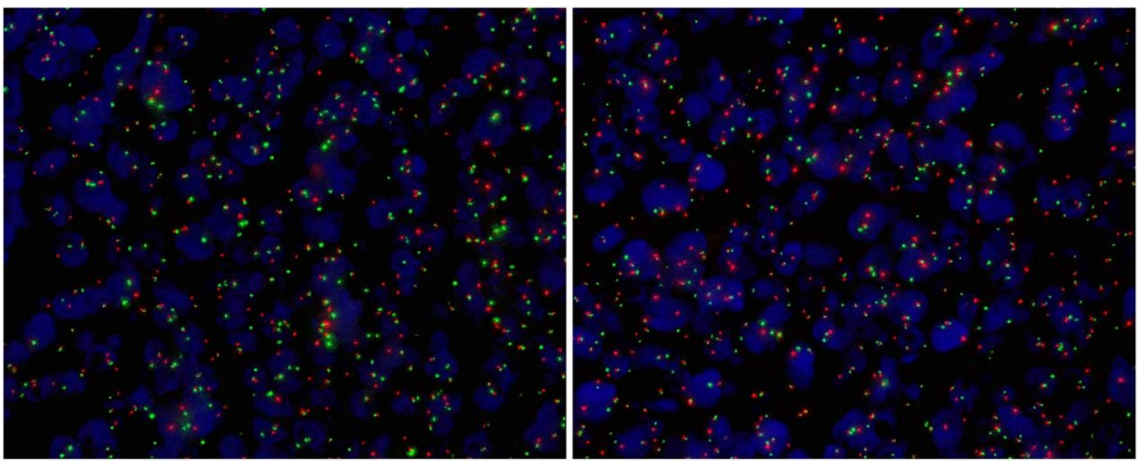

Fig. 2 This indicates that the fluorescence in situ hybridization (FISH) test detected a breakage in the EWSR1 gene, displaying a signal pattern of 1F1R1G. The red signal (R) marks the 3' end of the EWSR1 gene, while the green signal (G) marks the 5' end of the EWSR1 gene. The test result is positive: the EWSR1 gene has undergone a break, and the positive signal pattern is 1F1R1G. The right shows partial cells with a positive signal pattern of 1F2R1G.(Oil immersion, 100x magnification)

poorer survival outcomes for older rPNET patients (Grohar et al. 2011). Several important limitations should be considered when interpreting our findings. The small sample size of this study may limit the statistical power of the analysis. Our data suggest that advanced age is correlated with poorer clinical outcomes in rPNET patients. This observation may be explained through multiple interrelated factors. Older patients are likely to face compounded challenges, including age-related immune dysfunction, a higher prevalence of comorbidities, and potentially more aggressive tumour biology. In contrast, younger patients generally demonstrate better tolerance to intensive therapies such as chemotherapy

Table 3 Univariate analysis of overall survival (OS) for rPNET

Variable	X^2	*p*
Age	35.441	**0.001**
≤39		
>39		
Gender	2.351	0.852
Male		
Famale		
Tumor size	13.390	0.146
≥8		
<8		
Location	0.101	0.751
Left sided		
Right sided		
Renal venous tumor thrombus	2.602	0.107
Yes		
No		
Chrysanthemum-shaped mass structure	7.524	0.089
Yes		
No		
Staging	18.955	**0.021**
Local lesions		
Local progression		
Distant metastasis		
Lymph node metastasis	3.080	0.079
Positive		
Negative		
P53	0.743	0.389
mt		
wt		
BCL-2	4.704	**0.03**
Positive		
Negative		
Ki-67	23.472	**0.001**
≤40%		
>40%		
Treatment Method	0.522	0.213
Surgery		
Surgery + Chemotherapy/Radiotherapy		
Anti-angiogenic targeting drugs	21.154	**0.017**
Yes		
No		

Abbreviations as defined in text

Table 4 Multivariate analysis of OS for rPNET

	p	HR	95.0% CI	
BCL-2	**0.037**	0.151	0.026	0.888
Ki-67	**0.004**	1.100	1.030	1.174
Age	0.892	0.995	0.932	1.063
Staging	0.141	0.231	0.033	1.622
Anti-angiogenic targeting drugs	0.935	0.524	0.853	1.984

Abbreviations: HR, hazards ratio; CI, confidence interval; OS, Overall survival; rPNET, Renal primitive neuroectodermal tumor

and radiation, which may contribute to improved outcomes. In Northwest China, socioeconomic factors and health care access disparities may also influence these patterns. Elderly patients in underserved regions often present with advanced disease at diagnosis, potentially due to atypical symptom presentation or cultural barriers that delay medical consultation. Our results indicate that age may serve as an important prognostic factor in rPNET management, suggesting that older patients could benefit from more aggressive therapeutic approaches and enhanced surveillance protocols; however, these conclusions require cautious interpretation. The preliminary nature of these findings underscores the need for validation through larger, multicentre prospective studies with extended follow-up periods.

In this study, tumours were located in the left kidney in 9 patients and in the right kidney in 7 patients, but these findings do not suggest a predilection for the left kidney. Larger sample sizes are needed to draw definitive conclusions. No cases of bilateral renal involvement were observed, which is consistent with the findings of most studies, indicating that rPNETs typically present unilaterally (Cheng et al. 2020). The average tumour diameter was 8.5 cm, which is consistent with previous reports, suggesting that rPNETs are often large at diagnosis (Li et al. 2022). Owing to their size, patients commonly present with symptoms such as abdominal masses, flank pain, or haematuria. In this study, the most frequent initial symptoms were abdominal masses, flank pain, abdominal distension, haematuria, and fever, which is consistent with the literature (Thyavihally et al. 2008). Tumour size was not significantly correlated with survival, although this may be due to the small sample size, which limited the statistical power. Additionally, rPNETs may exhibit significant heterogeneity in tumour size across patients. Some studies have also failed to find a correlation between tumour size and survival in rPNET patients (Celli & Cai 2016). Future large-scale multicentre studies are needed to explore the relationship between tumour size and prognosis in rPNETs further.

The presence of caval thrombus and lymph node metastasis did not significantly correlate with OS ($p > 0.05$). These findings may suggest that rPNET prognosis depends more on intrinsic molecular or clinical characteristics rather than solely on the presence of caval tumour thrombus or lymph node metastasis. Furthermore, we observed that patients with caval tumour thrombus or lymph node metastasis might have received more aggressive treatment regimens (e.g., surgical resection combined with chemoradiotherapy), which may have improved their clinical outcomes and affected the observed correlation between these pathological features and survival.

A study by Jawad et al. demonstrated an inverse correlation between tumour stage and survival in patients with

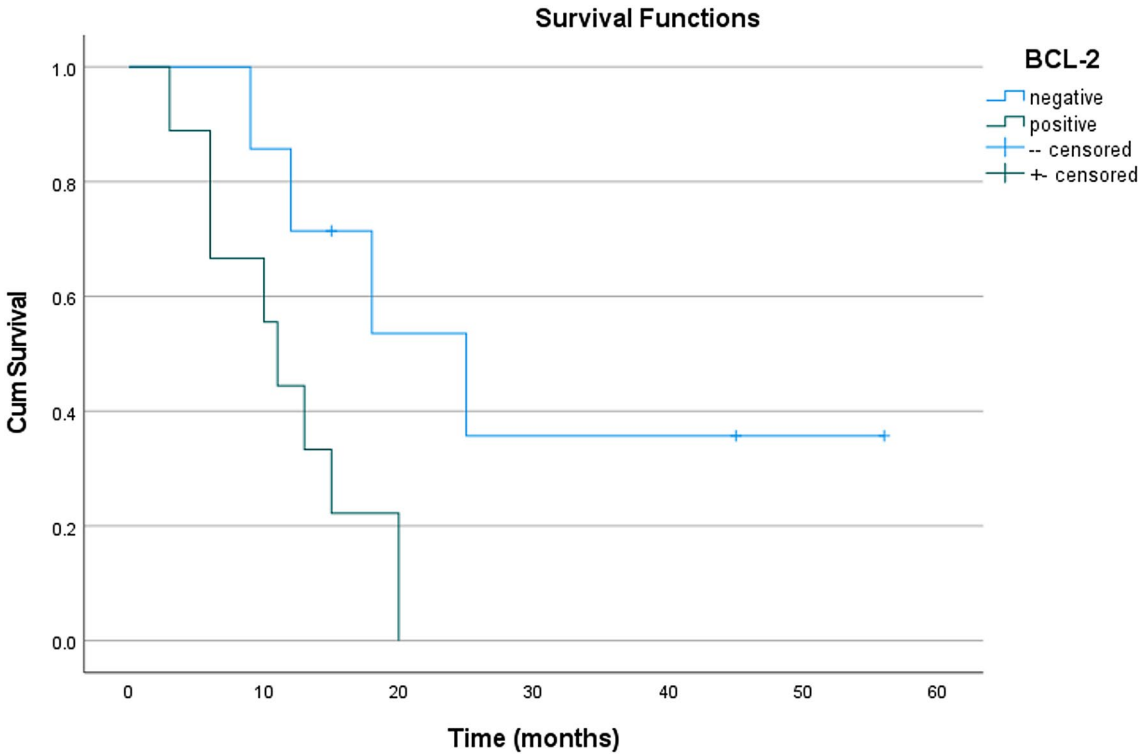

Fig. 3 Functional plot of survival analysis of BCL-2

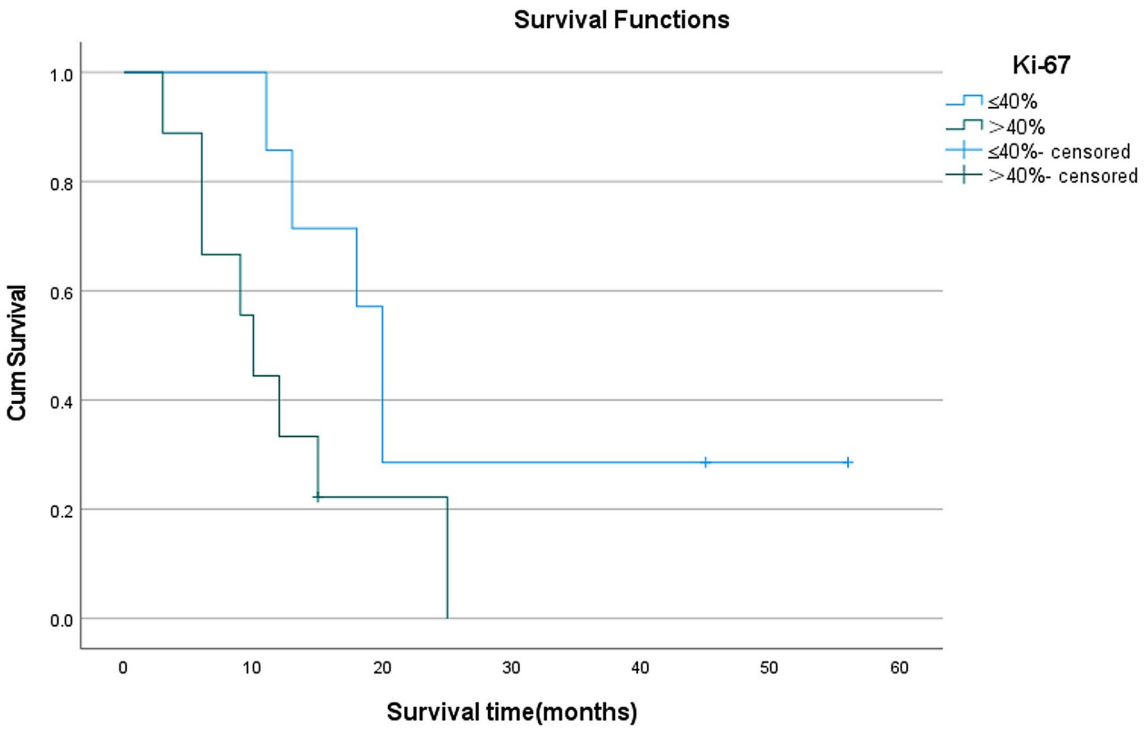

Fig. 4 Functional plot of survival analysis of Ki-67

peripheral Ewing sarcoma (Jawad et al. 2009). In our current study, while univariate analysis revealed a significant association between tumour stage and overall survival (OS, $p<0.05$), this correlation was not maintained in multivariate analysis ($p>0.05$). We propose several potential explanations: First, the prognostic impact of tumour stage might have been overshadowed by stronger confounding factors in the multivariate model; second, and perhaps more importantly, the limited sample size ($n=16$) in our study likely constrained the statistical power of the multivariate analysis. This fundamental limitation underscores the necessity for larger-scale, multicentre studies to better elucidate the relationship between tumour stage and survival outcomes in patients with rPNETs.

Among the 16 patients with rPNETs, 3 received antiangiogenic drug therapy. Univariate analysis revealed that the use of antiangiogenic drugs was significantly associated with improved OS in patients ($P<0.05$). These findings suggest that antiangiogenic therapy may have a positive role in the treatment of rPNETs. Given the highly vascularized nature of rPNETs, antiangiogenic therapy may effectively reduce the tumour blood supply, limit tumour growth, and potentially inhibit metastasis. This mechanism is consistent with the observed improvement in OS among patients treated with antiangiogenic drugs (Lopes-Coelho et al. 2021). Although the results are encouraging, only 3 patients who received antiangiogenic therapy in this study, we definitive conclusions cannot be drawn. Additionally, the retrospective study design may introduce selection bias, as patients receiving antiangiogenic drugs may differ from those who do not in terms of baseline characteristics or disease severity. Future research should focus on randomized controlled trials to evaluate the efficacy and safety of antiangiogenic drugs in rPNET, as well as their potential synergistic effects with chemotherapy and radiotherapy. There is also an urgent need to expand the sample size in future studies.

Pathologically, 9 cases presented characteristic pseudorosettes or Homer-Wright rosettes. While the presence of rosette structures typically supports the diagnosis of PNET, 4 cases (4/16, 25%) in this study did not present these structures, further confirming that rosette structures are a typical but not exclusive feature of rPNET/EWS. The absence of rosette structures may complicate the differential diagnosis of rPNETs from other small round cell tumours (e.g., rhabdomyosarcoma) and may reflect tumour heterogeneity. Owing to the small sample size (16 cases), the statistical significance of the absence of rosette structures may be limited, and their identification may depend on the experience of the pathologist and staining methods. Whether the absence of rosette structures indicates different biological behaviours requires further investigation. However, this study provides limited evidence that the absence of rosette structures may not significantly impact rPNET prognosis.

CD99 was diffusely positive in all cases, and FLi-1 was negative in only 1 case(1/16), supporting the diagnosis of PNET. The positive expression of NSE, Syn, and CgA in 75% (12/16) of cases further confirmed the neuroectodermal origin of PNET and helped differentiate it from non-neuroendocrine renal tumours (e.g., renal cell carcinoma), highlighting its diagnostic value. EWSR1 gene rearrangements were detected in all patients, which is consistent with previous studies of pPNETs, further supporting the importance of EWSR1 in the diagnosis of rPNETs (Ke Ch, Duan et al. 2017). Unfortunately, the specific fusion partners of EWSR1 were not identified, precluding analysis of whether different fusion types affect prognosis. EWSR1 gene rearrangements may provide potential targets for molecular therapies, such as drugs targeting the EWSR1-FLi-11 fusion. Future large-scale multicentre studies are needed to explore the incidence, types, and prognostic significance of different EWSR1 fusion types in rPNETs, as well as their implications for targeted therapies. In summary, the diagnosis of rPNETs should consider tumour morphology, with rosette structures being an important histological feature, although their absence does not exclude a diagnosis. IHC markers such as CD99 and FLi-1-1 should be used to improve diagnostic sensitivity, along with positive expression of neural markers. Molecular genetic testing should also be considered when necessary.

P53 is a tumour suppressor gene that induces cell cycle arrest or apoptosis in response to DNA damage. Mutant P53 loses these functions, potentially contributing to tumorigenesis (Duffy et al. 2022). Many studies have linked P53 mutation or abnormal expression to tumour aggressiveness, drug resistance, and poor prognosis (Thoenen et al. 2019). In this study, P53 expression did not significantly correlate with OS ($p>0.05$). First, these limitations may stem from the study population characteristics, the small sample size, or methodological constraints related to the statistical analyses. Additionally, the complex functions of P53 may result in different biological behaviours in different tumour types or patients, or P53 may interact with other unknown genes, weakening its correlation with survival. Future large-scale multicentre studies are needed to further explore the relationship between P53 expression and prognosis in patients with rPNETs.

In this study, a high Ki-67 index (≥40%) was significantly associated with a poor prognosis, which is consistent with findings in previous studies on Ewing sarcoma and suggests that high proliferative activity may drive tumour aggressiveness and poor outcomes (Marino et al. 2014). Multivariate analysis confirmed that the Ki-67 index is an independent prognostic factor ($p<0.05$). However, owing to the limited

sample size, future large-scale studies are needed to validate these findings and explore the potential application of Ki-67 in treatment decision-making.

BCL-2 (B-cell lymphoma 2) is an antiapoptotic protein that plays a central role in cancer development by inhibiting apoptosis, thereby reducing the efficacy of traditional cytotoxic therapies. Several studies have reported that members of the BCL-2 protein family have proapoptotic or antiapoptotic effects, and their role in regulating apoptosis, tumorigenesis, and the response to anticancer therapies has been widely studied (Qian et al. 2022, Delbridge et al. 2015). In this study, BCL-2 was expressed in 56.25% of rPNET cases, reflecting regional characteristics and suggesting that environmental or genetic factors in Northwest China may influence tumour biology. Survival analysis revealed that the median survival time of patients in the BCL-2-positive group was 10 months, which was significantly shorter than that of patients in the negative group ($p < 0.05$). Multivariate analysis further confirmed that BCL-2 positivity is an independent protective factor (HR = 0.151, $p = 0.037$). Studies have demonstrated that the protective effect of BCL-2 on tumours may be associated with its regulation of the tumour microenvironment or the immune response (Lee et al. 2022; Kiraz et al. 2016). BCL-2 may inhibit excessive inflammatory responses or maintain cellular homeostasis, indirectly suppressing tumour progression. Additionally, BCL-2 may form a dynamic balance with other apoptosis-regulating proteins (e.g., BAX and BAK), influencing tumour cell fate. Our findings suggest that patients with high BCL-2 expression may have improved survival outcomes, although this observation should be interpreted with caution. While these results provide preliminary insights, further studies focusing on Northwest China are warranted to investigate whether regional factors influence the biological behaviour of rPNETs. Additionally, our data highlight the need for careful consideration when exploring BCL-2-targeted therapeutic strategies (e.g., BCL-2 inhibitors) for rPNET management, given the potential variability in treatment response. Future studies should expand the sample size and incorporate multiomics analyses (e.g., transcriptomics and proteomics) to explore the specific mechanisms of BCL-2 in rPNETs. In summary, Cox regression analysis suggested that BCL-2 and Ki-67 may serve as potential prognostic factors in rPNET patients. However, the limited sample size limits the robustness of these findings. Future large-scale, multicentre studies are warranted to validate the prognostic significance of BCL-2 and Ki-67 and to explore their potential as therapeutic targets in this rare malignancy.

Limitations

This study has several important limitations. Despite the collection of data from five major hospitals in Northwest China over 20 years, the rarity of rPNET limited the sample size to only 16 cases. The small sample size significantly constrained the statistical power, particularly affecting the reliability of the multivariate analyses. Second, as a retrospective study, it inherently has unavoidable selection bias and issues related to missing data. In this study, 5 patients (5/16) had metastasis at the first diagnosis, so it was difficult to clarify the boundary between the starting point of PFS and disease progression, and some patients relapsed within a short period of time after treatment, which led to bias in the PFS time distribution and made it difficult to conduct a meaningful analysis. Therefore, in the survival analysis, we used OS as a prognostic indicator. PFS was not included, which may miss important clinical information about disease progression, and relying only on OS may affect the comprehensive evaluation of treatment regimens. Future studies should consider including PFS as a secondary endpoint to provide a more comprehensive prognostic assessment, especially when novel treatment options are explored. Moreover, follow-up studies are recommended and should include assessment of detailed clinical parameters at the time of disease progression to enable analysis of the key factors affecting prognosis more accurately. Additionally, variations in treatment strategies across different medical centres may have influenced the accuracy of the prognostic analyses. Most importantly, the findings may reflect region-specific characteristics and inherent limitations. Additional validation through larger, more diverse datasets is needed to confirm the generalizability of the observed patterns to other populations in different demographic and geographic contexts. On the basis of these findings, while we have preliminarily identified several clinical characteristics and potential prognostic factors of rPNETs, these conclusions require validation through larger-scale prospective studies. In particular, there is a need for multicentre collaborative research that integrates clinical data with molecular testing to more comprehensively evaluate the prognostic value of markers such as BCL-2. Simultaneously, considering the health care characteristics of Northwest China, we recommend increasing the capacity for early diagnosis of this disease. Research should also be performed to explore the potential application value of targeted therapies in rPNETs, although this must be based on more robust evidence-based medical data. This study provides first-hand data from Northwest China for understanding this rare disease, but caution should be taken in the application of related conclusions.

Conclusion

This study demonstrated that while rosette structures are frequently observed in rPNET/EWS, their absence does not preclude a diagnosis. Preliminary data suggest that altered expression of BCL-2 and Ki-67 may be associated with disease progression, although their definitive prognostic significance requires further support. We observed that patients in this region tended to be older at diagnosis and present with more advanced disease in terms of clinical stage. These characteristics may correlate with complex regional factors, including geographic environment, dietary patterns, and health care resource allocation, but the precise relationships warrant verification through more rigorous investigations.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval This study was granted ethical approval from the Ethics Committee of the People's Hospital of Shaanxi Province (Approval No. 2024 K-388).

Consent All informed consent was obtained from the subject(s) and/or guardian(s).

Competing interests The authors declare no competing interests.

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