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ORIGINAL ARTICLE

Primary Ewing Sarcoma / Primitive Neuroectodermal Tumor of the Kidney: A Clinicopathologic Study of 23 Cases

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Abstract Primary Ewing sarcoma / primitive neuroectodermal tumor (ES) of the kidney is a rare neoplasm with limited clinicopathologic data. We report 23 such cases with no history of ES elsewhere in the body. The patients included 13 male and 10 female, aged 8–70 years (mean, 31 years). The average tumor size was 11.7 cm (range, 5–20 cm). Microscopic analysis showed predominantly lobular growth (n = 14), with focal papillary (n = 3), alveolar (n = 1), and hemangiopericytoma-like (n = 1) patterns. Several tumors (n = 11) exhibited robust mitotic activity (>10 mitoses/10 high-power fields). Necrosis (n = 13) and lymphovascular invasion (n = 14) were common. Homer Wright rosettes (n = 6) and perivascular pseudorosettes (n = 1) were also identified. The tumors invaded the renal sinus or perinephric fat (n = 11), renal vein (n = 13), and adrenal gland (n = 2). Molecular and fluorescence in situ hybridization analysis showed rearrangement of *EWSR1* gene (10/10), associated with *EWSR1-FLI1* gene fusion (7/10). All patients with follow-up information (n = 18) had metastasis, commonly in the lungs (n = 12) and bone (n = 6). Twelve patients died of disease in a mean of 21 months; 6 patients were alive at a mean of 49 months after diagnosis. Primary kidney ES usually present at an advanced stage with extrarenal spread and metastasis. Although renal ES share histologic, immunohistochemical, and molecular features with their bone and soft tissue counterparts, they appear to be more aggressive tumors with poorer clinical outcome.

Keywords Kidney · Ewing sarcoma · Histology · Immunohistochemistry · Cytogenetics · Clinical outcome

Introduction

The Ewing family of tumors, which extend along a spectrum from Ewing sarcoma to the primitive neuroectodermal tumor (PNET) are rare malignancies, usually encountered in the bone and soft tissue of young adults and children. The clinical presentation of these lesions, including pathological features, molecular signatures, prognostic variables, and therapeutic strategies are fairly well defined in recent literature. However, the occurrence of ES in visceral locations, including the kidney, is uncommon and is limited to case reports and small case series. We evaluated a series of 23 cases of Ewing sarcoma of the kidney and elucidated their characteristics in this unique location.

Materials and Methods

We retrospectively searched the MD Anderson Cancer Center pathology database from 1995 to 2010 and found 23 cases of Ewing sarcoma/PNET of the kidney after obtaining approval from the institutional review board. No patient had prior history of Ewing sarcoma elsewhere in the body. The pathology specimens included resections (n = 21) and biopsies (n = 2). The histologic slides, including the immunohistochemical stains where available, were reviewed. Clinical information was collected from the electronic medical records. This

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included patient age, gender, race, clinical symptoms, tumor location, stage, gross features, therapy, and last known clinical status. In addition, molecular data on the *EWSR1-FLI1* gene fusion and FISH analysis on the rearrangement of the *EWSR1* gene was collected where available. The case material included in-house specimens and slides and/or blocks sent from other institutions as referrals or for expert consultation.

Results

Demographics and Clinical Features

The patients included 13 male and 10 female, with a mean age of 31 years (range, 8–70 years). Two patients were below 15 years of age. Seventeen patients were Caucasians while 5 were Hispanic and 1 Asian. The most common presenting symptoms were flank pain (n = 13) and hematuria (n = 8). Constitutional symptoms, including fever, weight loss, night sweats, nausea and vomiting were present in 6 patients. Two patients presented with a palpable mass alone.

Gross Features (Fig. 1)

The tumors were unilateral in all cases (right =11; left =12). Most tumors were large and were located in the upper and upper/mid poles (n = 10). The average tumor size was 12 cm (range 6–20 cm). Extension into the renal vein was identified in 13 cases (in addition, 6 cases had evidence of inferior vena caval extension). Local extension through the Gerota fascia was present in 2 cases, where the tumors showed invasion into the adrenal gland and beyond. Extension into the renal sinus/perinephric fat was frequently identified (n = 12). The tumors were usually solid with necrosis, hemorrhage and focal cystic changes.

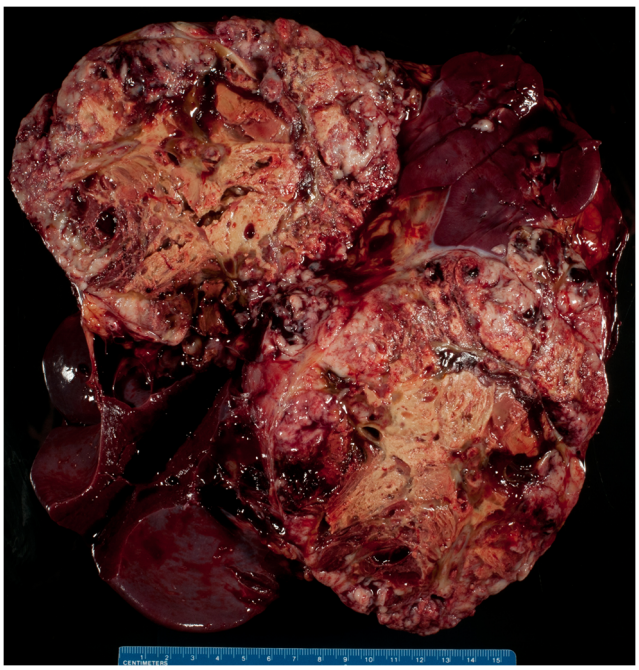

Fig. 1 Gross appearance of renal Ewing sarcoma. The tumor measures 15 cm and replaces most of the kidney. It is poorly circumscribed, solid, gray- tan with areas of necrosis, hemorrhage and cystic degeneration

Microscopic Features (Fig. 2)

Microscopic analysis showed predominantly lobular growth patterns (n = 14), with focal papillary (n = 3), alveolar (n = 1), and hemangiopericytoma-like (n = 1) patterns. All tumors except one were poorly circumscribed. Most tumors (n = 11) showed robust mitotic activity with mitotic figures of >10/10 high-power fields. Necrosis (n = 13) and lymphovascular invasion (n = 14) were common. Homer Wright rosettes (n = 6) and perivascular pseudo rosettes (n = 1) were also identified. Spindled nuclei were identified in one case. Most of the nuclei were high grade with neuroendocrine features (n = 13) with few cases exhibiting moderate grade nuclei (n = 4). The nucleoli were absent or inconspicuous, even in the higher grade nuclei. The cytoplasm was scanty in three cases. Most cases exhibited moderate cytoplasm (n = 14) with cytoplasmic clearing identified in eleven cases.

Molecular and Immunohistochemical Features

Molecular and fluorescence in situ hybridization analysis showed rearrangement of the *EWSR1* gene (10/10), which was associated with *EWSR1-FLI1* gene fusion (7/10). Immunohistochemical analysis showed the tumor cells were positive for CD99 (21/23), vimentin (7/8), neuron-specific enolase (6/6), synaptophysin (5/13), and CD56 (3/7) and negative for cytokeratin (0/16) and desmin (0/9).

Treatment and Follow-Up Data (Table 1)

The follow up period ranged from 3 months to 156 months. Complete follow-up data were available for 18 patients. In brief, fourteen patients had metastasis at presentation and 5 additional patients developed metastasis in an average of 11.6 months after diagnosis. The metastases were commonly found in the lungs (n = 12), bone (n = 6), lymph nodes (n = 4), and liver (n = 2). Five patients received neoadjuvant chemotherapy followed by surgical resection and additional adjuvant chemotherapy. One of these patients had radiation to the tumor bed as well. Fourteen patients received adjuvant chemotherapy following surgery. Two patients received chemotherapy alone. Radiation therapy was employed for local control in 5 patients. Significantly, one of the patients who had complete response following chemotherapy relapsed with metastasis to the lung and bone in 21 months. Twelve patients died

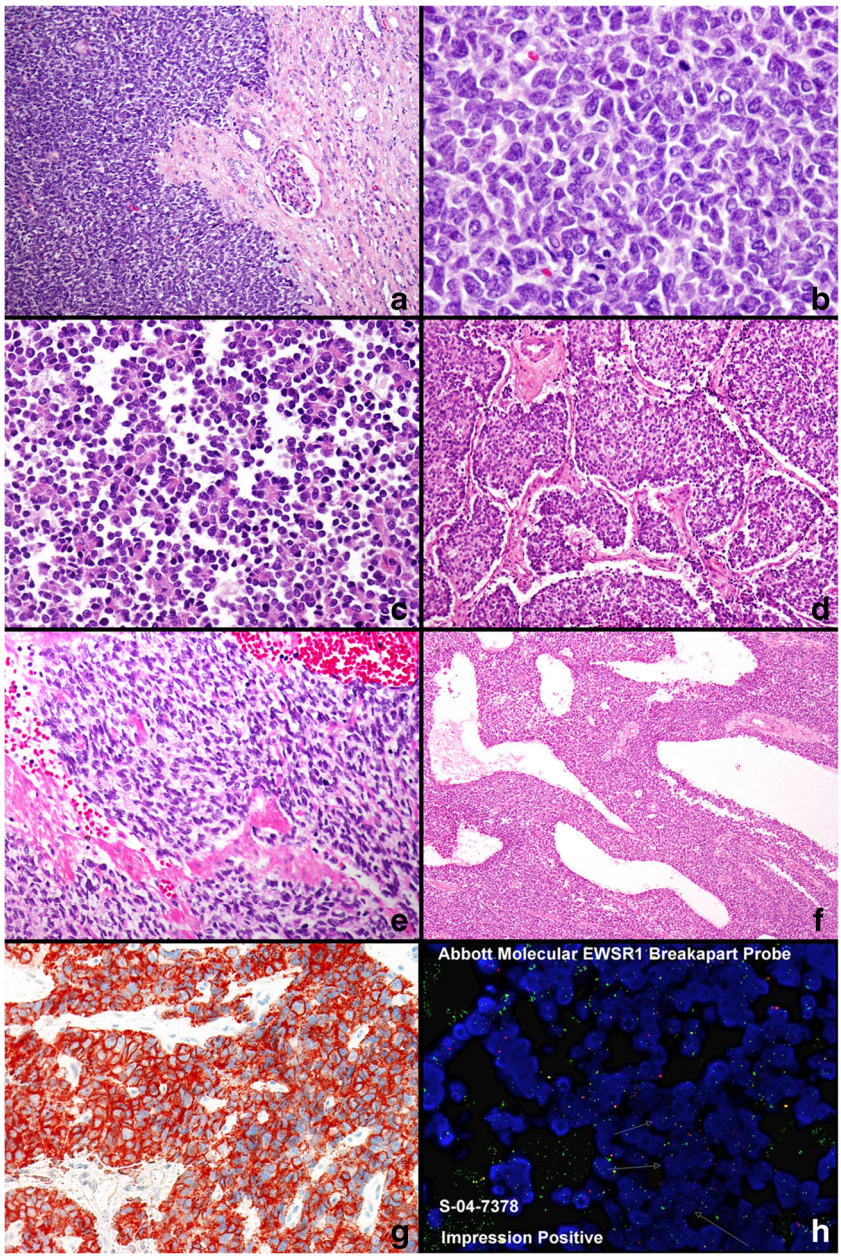

Fig. 2 Tumors showed an ill-defined pattern of invasion into the renal parenchyma **a**. The cells were arranged in sheets with high N/C ratio, speckled chromatin and inconspicuous nucleoli **b**. Homer Wright rosettes were seen in few cases **c**. Most cases had a lobular architecture **d**. Cellular spindling **e** and infrequent patterns such as "hemangiopericytoma-like" were also present **f**. CD 99 was strongly and diffusely positive in a majority of cases **g**. An example of a case with positive FISH study **h**

from the disease in a mean of 21 months after diagnosis; 6 patients were alive at a mean of 49 months after diagnosis.

Discussion

The Ewing family of tumors (EFT), variously described as Ewing's sarcoma of the bone, extraosseus/extraskeletal Ewing's sarcoma, primitive neuroectodermal tumor (PNET), peripheral neuroepithelioma, Askin's tumor (Ewing's sarcoma of the chest wall) and atypical Ewing's sarcoma are rare malignancies, usually encountered in young adults and children. Although James Ewing initially described the entity in the early 1920s [1], it was not until 1975 that Angervall and Enzinger reported the first case of extraskeletal Ewing sarcoma [2], subsequently confirmed by other reports. Related malignancies arising in the soft tissue, originally regarded as distinct from Ewing sarcoma of the bone are now recognized to be part of the same family of tumors that share a balanced translocation (11;22) (q24;q12) [3]. The extraskeletal EFT are usually located in the deep soft tissue of the extremities, but any anatomic site can be involved, including the genitourinary tract [4]. EFT presenting as renal primary is very uncommon, first reported by Mor et al. in 1994 [4]. Over the past decade and half, knowledge on this entity has been accumulating steadily with over 100 cases reported in literature [5].

Table 1 Clinical and follow up data

	Age	Gender	Tumor size (cm)	Follow-up (months)/Status	Treatment	Metastasis	IHC CD99	EWSR1 status
1	41	M	12	13/DOD	C	Meninges, bone +LR	Positive	FISH positive
2	35	M	17	5/DOD	N + C	Lung, lymph nodes + LR	Positive	FISH positive
3	43	M	20	7/DOD	C	Bone, lung + LR	Negative	FISH positive
4	33	M	NA	31/DOD	N + C	Lung, brain, liver, skin	Positive	FISH positive
5	70	F	NA	16/DOD	N + C + INF	Lung	Positive	FISH positive
6	21	M	7.2	17/DOD	N + C	Peritoneum	Positive	PCR positive
7	23	M	9	23/DOD	N + C	Lung	Positive	FISH positive
8	45	M	NA	90/DOD	N + C	Adrenal, lymph nodes	Positive	FISH positive
9	31	F	12.5	110/NED	N + C	NED	Positive	FISH negative
10	32	F	12	5/DOD	N + C	Lung, lymph nodes	Positive	FISH negative
11	50	M	NA	NA	NA	NA	Positive	Not done
12	52	F	NA	3/DOD	N + C + INF	Lung, liver	Positive	Not done
13	25	M	11.4	5/DOD	N + C	Lung	Negative	Not done
14	26	F	11	49/NED	N + C	NED	Positive	PCR positive
15	29	M	9.2	2/L	N + C	Lung	Positive	Not done
16	8	M	NA	4/L	N + C	Bone	Positive	Karyotype positive
17	9	F	19	4/L	N + C + R	Bone	Positive	PCR pos
18	18	F	5	34/AWD	N + C + R	Bone, lymph node, brain	Positive	Karyotype positive
19	33	F	8.5	5/L	N + C	Lung	Positive	PCR positive
20	32	M	15	26/NED	N + C	NED	Positive	FISH positive
21	19	M	6	21/DOD	N + C + R	Lung, bone + LR	Positive	PCR positive
22	33	F	NA	24/DOD	N + C	Lung	Positive	Not done
23	24	F	14	156/NED	N + C	NED	Positive	Not done

AWD – Alive With Disease; C – Chemotherapy; DOD – Dead of Disease; F – Female; FISH – Fluorescent in-situ hybridization; INF – Interferon therapy; LR – Local Recurrence; L – Lost to follow up; M – Male; N – Nephrectomy; NA – Data Not Available; NED – No Evidence of Disease; PCR – Polymerase chain reaction; R – Radiotherapy

However, the number of case series has been limited, due to the rarity of the disease and possible under-recognition of EFT in the kidney.

The histogenesis of these tumors, speculated as being derived from endothelial cells by Ewing in his initial description [1], is yet unclear. Currently it is understood that the EWS/FLI oncoprotein, derived from the reciprocal translocation between chromosomes 11 and 22 is necessary to maintain the malignant phenotype of EFT cells. The cell of origin in the kidney, as in other locations is thought to be neuroectodermal cells derived from embryonic migration of neural crest cells [6]. This is evidenced by the presence of neuroectodermal antigens on immunohistochemistry and dense core granules on electron microscopy. More recently, however, a mesenchymal stem cell origin has been proposed, with the neuronal phenotype a result of aberrant transcription due to the presence of the EWS/FLI1 transcript [7].

EFT are tumors of children and young adults, commonly occurring in patients less than 30 years. Studies in the past have postulated an older age of presentation in PNET, but the mean age groups are similar in all the EFT [8]. Ewing sarcoma of the kidney (ESK) presents at a mean age of mid to late 20s, although the tumor may occur across a wide age range from less than 5 years to older than 60 years [9]. This is in concordance to the findings in our series. As observed in the present study, a slight preponderance in tumor incidence has been identified in male patients in some series [9], and the tumor is rarely seen in non-Caucasian races [8, 9].

Clinically, patients present with symptoms of abdominal pain, hematuria, and palpable mass, with or without constitutional symptoms. Rare patients may present with referred testicular pain, dysuria or varicocele. In cases where the tumor involves the IVC and extends to the atrium, symptoms of dizziness and dyspnea may be encountered [9].

Grossly, ESK are unilateral and replace most of the renal parenchyma with diameters greater than 10 cm in a majority of cases. The tumors are poorly circumscribed and have a solid gray-white cut surface with areas of hemorrhage, necrosis and cystic degeneration. They are generally indistinguishable from other renal tumors on gross examination alone [9, 10]. Local extension into the perinephric fat, Gerota's fascia and renal vein is not infrequent.

On microscopic examination, ESK exhibits features similar to those seen in EFT in other locations. The cells are arranged in solid sheets and lobules with both pushing and finger like infiltration into the surrounding renal parenchyma. Several distinct architectural patterns, frequently in the same tumor, may also be seen. These include papillary, pseudopapillary, perivascular pseudorosettes, alveolar, serpinginous, spindled and hemangiopericytoma-like. Homer Wright rosette formation can also be present in many cases. The cells have characteristic primitive small round blue cell morphology with high nuclear cytoplasmic ratio, rounded nuclei with inconspicuous nucleoli and granular chromatin. Tumors with variant morphology, including those with increased nuclear pleomorphism, moderate amount of clear or eosinophilic cytoplasm can also be encountered. The number of mitotic figures is variable. In the past, the EFT were classified as classic Ewing sarcoma, atypical Ewing sarcoma and PNET based on the presence or absence of some of the morphological features. Current knowledge regarding the underlying molecular signature has shown that these tumors belong to the same family and such distinction is less critical [8]. Regardless, recognition of the various histological features may help avoid misdiagnosis.

The immunohistochemical profile of ESK is identical to ES in other locations. The tumors show diffuse membranous positivity for CD99 in almost all cases. S100 protein and vimentin are positive in 52% to 70% of cases. Rare cases may show aberrant cytokeratin or desmin staining. Neuroendocrine markers such as neuron specific enolase, and synaptophysin may be positive from 48% to 95% of cases [9]. Expression of the FLI1 (Friend leukemia virus integration 1) protein, seen in 71–84% cases may also aid in the diagnosis [11]. Neurosecretory granules may be demonstrated on electron microscopy [9]. Most EFT (85–95%), including ESK have been shown to consistently harbor the reciprocal translocation between chromosomes 11 and 22 involving bands q24 and q12 t(11;22)(q24;q12) [9] which contain the FLI1 and EWS gene loci respectively. The demonstration of the EWS/FLI1 fusion transcript, by cytogenetics or PCR assays is necessary for confirming diagnosis. FISH studies employing break apart probes to detect misplacement of the EWSR1 locus are often utilized for the diagnosis in conjunction with the clinicopathological and immunohistochemical features. In addition to the most commonly encountered translocation partner FLI1, other genes can also be involved, albeit at a much lesser frequency. These include the ERG locus situated in chromosome 21 (q22), seen in 5–10% of cases and other rare partners [9].

ESK needs to be differentiated from other small round blue cell tumors in the kidney namely blastemal Wilms tumor, small cell neuroendocrine carcinoma, neuroblastoma, rhabdomyosarcoma, synovial sarcoma, desmoplastic small round cell tumor, lymphoma and poorly differentiated renal cell carcinoma. This is critical since the prognosis of ESK is poor and a delay in diagnosis results in significant morbidity [5]. On a morphological basis, the presence of Homer Wright rosettes favors EFT compared to other tumors excluding neuroblastomas, but these may not be present in all cases. None of the immunohistochemical markers are specific to the diagnosis. Although CD99 is positive in most EFT, it is non specific and several other small round cell tumors including Wilms tumor, synovial sarcoma, desmoplastic small round cell tumors, lymphomas, rhabdomyosarcomas can also stain positive [8]. A panel of other immunomarkers, for instance CD45 to rule out a majority of lymphomas and WT1 to rule out Wilms tumor may be required. The diagnosis therefore has to be confirmed by molecular studies. However, molecular tests may produce false negative results due to sampling errors or variant fusions, and in the case of FISH break apart probe studies, false positive results, since the EWS gene arrangement may also be present in desmoplastic small round cell tumor, clear cell sarcoma, and neuroblastoma among others. Correlation with clinical findings, morphological and immunohistochemical features is therefore important. Recently recognized entities such as the CIC-DUX4 and BCOR-CCNB3 translocated sarcomas that closely resemble EFT also need to be considered in the differential diagnosis.

As evidenced from the above discussion, ESK shares histological, immunohistochemical, and molecular features with its counterparts in other locations. However, the behavior of these tumors in the kidney appears to be worse in comparison [5]. They are usually locally advanced and often present with regional and distant metastases. In the present study, 67% (14/21) of patients presented with metastasis. Among the rest of the patients, 57% (4/7) developed metastasis in a mean of 14 months despite surgical resection of the primary tumor and multiagent chemotherapy. This is in comparison to a 25% incidence of metastasis at presentation in EFT at non renal locations [12]. In an analysis of 107 published cases of ESK, Rowe et al. [5] identified 44% of patients with metastasis at presentation, which is significantly greater than bone or soft tissue EFT. The overall survival (at 4 years) in patients with metastatic ES in non renal locations is reported to be less than 40% with modern therapy [13]; only 7% (1/14) of treated patients with metastasis in this series were alive at three years. This difference in survival has been noted by other investigators as well, with overall median survival of 15 months reported in literature in concordance with 17 months in the present study [5, 10, 14]. However, localized ESK had survival rate similar to EFT at other locations [10, 14–16] (Table 2).

The most important factors influencing poor prognosis in ESK, given the fact that it is similar to EFT at other locations appear to be the greater size of these tumors and presence of locally advanced and metastatic disease at presentation [5]. In

Table 2 Comparison of data on renal Ewing sarcoma between previous studies (107 cases) and the present study – adapted from Rowe et al. [5]

Variable	Previous studies	Present study
Age at diagnosis (years)		
Median	27	31
Range	3–78	8–70
Patients aged <= 18y	24 (22%)	3 (14%)
Largest tumor dimension (cm)		
Median	12	11
Range	2–35	6–20
Male sex	55 (52%)	13 (57%)
Metastasis at diagnosis	43 (44%)	14 (67%)
CD99 +	72 (100%)	21 (91%)
EWS rearrangement +	33 (89%)	16 (84%)
Median survival		
Localized disease	Up to 60 months	110 months
Metastatic disease	15 months	17 months

turn, these may be attributed to the delay in initial diagnosis owing to relatively unhindered tumor expansion in the retroperitoneal location. Studies have shown 58% of ESK are diagnosed at an advanced stage [5].

The current treatment protocol for EFT includes a combination of surgery, chemotherapy and radiotherapy. The standard chemotherapy regimen includes a three drug combination of vincristine, doxorubicin, d-actinomycin, along with additional cycles of ifosfamide and etoposide [17]. Radiotherapy may be included to treat local recurrence or residual tumor. However, this protocol appears to be of lesser benefit in patients with ESK owing to advanced presentation. These patients may require more aggressive therapies including neoadjuvant and dose intensified regimens to halt disease progression [5, 13]. Given that renal tumors are usually resected without a preoperative diagnosis, presenting features such as large bulky tumors in younger patients should prompt a biopsy diagnosis, since it provides an opportunity for neoadjuvant therapy in patients with ESK [5]. In addition, it is important to differentiate these tumors from other small round cell malignancies of the kidney.

Alternate treatment modalities are being researched for EFT, including insulin-like growth factor receptor antibodies (IGFR-1), antibodies against the CD99 receptor and inhibitory RNA techniques [13]. A protein called GSTM4 has been identified in the EWS/FLI1 molecular pathway and is reportedly present in high levels among patients who do not respond to chemotherapy [18]. This may lead to early identification of such patients and possible treatment with agents targeted against GSTM4. These modalities may provide better therapeutic options for the more advanced renal EFT in the future.

Conclusion

Primary Ewing sarcoma/PNET of the kidney occurs in patients of a wide age range. Tumors usually present at an advanced stage with extra renal spread and metastasis. Although primary ES of the kidney share histologic, immunohistochemical, and molecular features with their counterpart in the bone and soft tissue, the former appear to be more aggressive with poorer clinical outcome.

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