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Case report

Role of immunohistochemistry and fluorescence in-situ hybridization (FISH) in the diagnosis of spindle and round cell tumors of the kidney

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Abstract Spindle cell/mesenchymal tumors of the kidney are rare. The diagnosis is supported mainly by the application of ancillary techniques such as immunohistochemistry (IH) and in-situ hybridization (FISH). An accurate diagnosis is essential because early management by complete resection and adjuvant chemotherapy improves the prognosis dramatically. Synovial sarcoma and primitive neuroectodermal tumor/Ewing sarcoma are infrequent malignancies which usually present in soft tissues but rarely in the kidney. The challenge for the pathologists is to histologically differentiate between different types of sarcomas such as PNET/Ewing's sarcoma, sarcomatous dedifferentiated renal cell carcinoma, metastasis, non-Hodgkin's lymphoma, nephroblastoma and angiomyolipoma.

Methods: We report from our experience six exemplary rare cases that presented in the kidney as spindle/round cell tumors.

Results: We have arrived at the accurate diagnosis after performing a large panel of IH and FISH.

Conclusion: In summary we advise an immunohistochemical panel for round/spindle cell tumors of the kidney and for unclear cases we advise to add (FISH) to get the correct diagnosis, as they are completely different regarding surgical approach and post-operative adjuvant therapy.

Introduction

Soft tissue sarcomas (STSs) have remained a therapeutic challenge over the last decades and can be mistaken for a variety of other round cell tumors, including blastema-predominant

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Wilms' tumor (WT, adult-type), lymphoma, small cell carcinoma, rhabdomyosarcoma, and poorly differentiated synovial sarcoma, in large part due to their aggressive biological behavior and lack of consistent literature data. It is important to recognize each of these entities, because each carries unique therapeutic and prognostic implications [1]. Primitive neuroectodermal tumor (PNET) of the kidney is also a rare tumor with only six published reports [1]. It commonly occurs in bones and is microscopically, immunohistochemically, and genetically similar to Ewing's sarcoma. Rarely, it can also occur as primary renal mass like other extra-skeletal sites [2]. Synovial sarcoma (SS) is a STS of uncertain histogenesis and most commonly affects the proximal lower limb of young adults [3]. In this manuscript, we report on six consecutive spindle/round cell tumors of the kidney that have been able to be identified using immunohistochemistry and FISH over the past 7 years.

Case 1

A 33-year-old male presented with about a 2-month history of right flank pain, which recurrently occurred in the last year. A 12 cm mass was detected on ultrasonography in the middle of the kidney at the corticomedullary junction. Clinically, xanthogranulomatous nephritis was suspected. Low density mass was described in CT-images. CT-guided biopsy and frozen section were made, which revealed pyelonephritis with a spindle cell tumor of uncertain malignancy. The patient was scheduled for open exploration of the right kidney. Intraoperatively, the kidney demonstrated with macroscopic necrosis that shined through the renal capsule. At two locations frozen sections were taken, demonstrating inflammation and necrotic tissue. Necrosis was evacuated in order to drain the kidney, but finally the corresponding abdominal CT scan revealed the central part of the kidney was necrotic and the renal pelvis was open, the preoperatively implanted DJ-ureteral catheter laid in the necrotic central part of the kidney. Consecutively, right Nephrectomy was performed, which showed a $12 \times 7 \times 5$ cm tumor with vessel invasion. The tumor showed grayish white tissues with multiple blood spaces, necrosis and cysts. Pathologic confirmation was performed by immunohistochemical methods and in-situ-hybridization (FISH). In histologic examination, solid cellular islets were observed on cross-sections of the tumoral tissue stained with hematoxylin-eosin. Histologic examination of tumoral tissue was composed of solid cellular nests of monomorphic spindle cells with nonuniformly bounded cytoplasm in large areas and fascicles with cystic structures settling among them. Immunohistochemistry analysis, using the streptavidin biotin peroxidase method stained positive for Vimentin, CD99, BCL-2 and focally positive for AE1/AE3 and EMA [4–6]. There was no reaction to CEA, Desmin, Chromogranin, Synaptophysin, S-100, CD10, CD117, BCL-2, β-Catenin, FLI-1, and WT-1. In the molecular study of the case, a rearrangement in the SYT-gene was found. The final pathologic diagnosis in this case resulting from the immunohistochemical and immunofluorescence studies was primary synovial sarcoma of the kidney.

Case 2

A 67-year-old female presented with a history of left flank pain and mass in the thyroid gland. A 8.5 cm mass was detected by ultrasonography in the center of the kidney. Clinically, it was suspected as angiomyolipoma. Low density mass is described in CT-images. CT-guided biopsy and frozen section were made, which revealed a spindle cell tumor. The gross appearance of the tumor was gray white with multiple cysts and hemorrhagic foci. Histologic examination of tumoral tissues composed of solid cellular nests of monomorphic spindle cells with nonuniformly bounded cytoplasm in large areas and fascicles with interstitial fibrosis. Immunohistochemistry analysis, using the streptavidin biotin peroxidase method, stained positive for CD117, Vimentin, and CD99 with negativity for AE1/AE3, CK7, CK8/18, CD34, CD45, Desmin, Myogenin, GATA-3, Melan-A, HMB-45 and Racemase. In the molecular study of the case, there is a rearrangement in EWSR-gene. The final histopathological diagnosis of this case depending on the immunohistochemical and immunofluorescence investigation was primary PNET of the kidney.

Case 3

A 24-year-old female presented with a history of right flank pain and jaundice. A 23 cm mass was detected by ultrasonography which revealed a large mass involving all parts of the kidney. Frozen section was done, which partially revealed a papillary and a blastomatous tumor. The gross pathology revealed inhomogeneity in the cut section with a dark brown color. Cystic and hemorrhagic areas are recorded. There was a massive infiltration of the ureter and renal pelvis with involvement of renal vein. Another mass was detected synchronously in the liver. Immunohistochemical studies revealed positivity for PAX-2, CD56, CK8/18, WT-1, and CD99 and focally for CK7. According to immunohistochemical studies, the histopathological diagnosis was nephroblastoma (Wilm's tumor) of the adult type.

Case 4

A 29-year-old female presented with pain and mass in the right kidney. Ultrasonography detected a mass of about 7 cm in the right kidney. Frozen section was performed which showed a mass of 6 cm, ill-defined with yellow color. Histologically, it showed spindle cells intermixed with little fat cells and blood vessels. Immunohistochemically there was positivity for HMB-45 and Melan-A. The proliferative activity was 5% in Ki-67. The histopathological diagnosis depending on the immunohistochemical results was angiomyolipoma of the kidney.

Case 5

A 50-year old female presented with pain and mass in the left kidney as well as synchronously multiple tumors in the vertebral column. Ultrasonography revealed a mass in the kidney. Nephrectomy was performed. The tumor located in the upper pole was around 6 cm in diameter. The cut-section surface was partially yellow and partially brown. Cystic areas were noted. Histologically there were spindle cells with desmoplasia and infiltration of the perirenal fat tissue as well as with infiltration of the renal pelvis. Immunohistochemistry was performed from both the primary in the kidney and the metastasis in the vertebral column. There was positivity for CD10, PAX-2,

Table 1 Differentiation of spindle/round cell tumors by immunohistochemical panel.

Immunomarker/ renal tumor	Primary synovial sarcoma	Nephroblastoma of the adult type	Primary neuroectodermal tumor (PNET)	Angiomyolipoma of the kidney	Sarcomatous renal cell carcinoma	Post-transplant lymphoproliferative disorder (PTLD), monomorphic type
AE1/AE3	Focal +	–	–	–	+	–
BCL-2	+	–	–	–	–	+
PAX-2	+/–	+	+/–	+/–	+	–
RCC	+/–	+	–	+/–	+	–
Racemase	–	–	–	–	+	–
GATA-3	–	–	–	–	–	–
CK 7	–	Focal +	–	–	+	–
Vimentin	+	+	+	+	+	–
Desmin	–	–	–	+	–	–
CD 117	–	–	+	–	–	–
CD 99	+	+	+	–	–	–
S-100	–	–	–	–	–	–
Melan-A	–	–	–	+	–	–
HMB-45	–	–	–	+	–	–
WT-1	–	+	–	–	–	–
Chromogranin	–	–	–	–	–	–
Synaptophysin	–	–	–	–	–	–
CD 45	–	–	–	–	–	+

RCC and a higher proliferative activity (Ki-67). According to immunohistochemical investigation, the diagnosis of sarcomatous dedifferentiated renal cell carcinoma was done.

Case 6

A 69-year old female presented with pain and mass in the right kidney with history of renal transplantation from about 4 years ago. She has in the follow up an acute cellular rejection in January 2013. Now due to pain, fever, weakness and weight loss, ultrasonography was indicated. This showed a mass in the kidney. Nephrectomy was performed. The kidney was 295 g and $13 \times 8.5 \times 5.5$ cm. The tumor located in the lower pole was around 7.5 cm in diameter and the cut-section was partially yellow and partially white. Cystic areas were noted. Histologically there were spindle and discohesive round cells. Immunohistochemistry showed positivity for CD45, CD20, CD5, BCL-2 and negativity for WT-1, Pax-2, CD3, CD30 und CD10 with a higher proliferative activity of about 50% (Ki-67). According to immunohistochemical investigation, the diagnosis of post-transplant lymphoproliferative disorder (PTLD), monomorphic type, was made.

Material and methods

For the immunohistochemistry deparaffinized and rehydrated FFPE tissue sections (1–2 µm) were stained after autoclave pre-treatment. Sections were processed in an automated staining system (Benchmark ULTRA, Ventana Medical Systems, Inc., Tucson, AZ, USA). Mouse monoclonal antibodies were used. FISH analysis was performed on FFPE tissue. Tissue was cut at 5 µm. First and last sections of each analysis were stained with hematoxylin and eosin (H&E) to delineate regions representing tumor and normal tissues. Tissue preparation for dual-probe hybridization was facilitated by ZytoLight FISH-tissue implementation kit according to the manufacturers' instructions (ZytoVision, Bremerhaven, Germany). Nuclei were counterstained with 4,6-diamidino-2 phenylindole (DAPI).

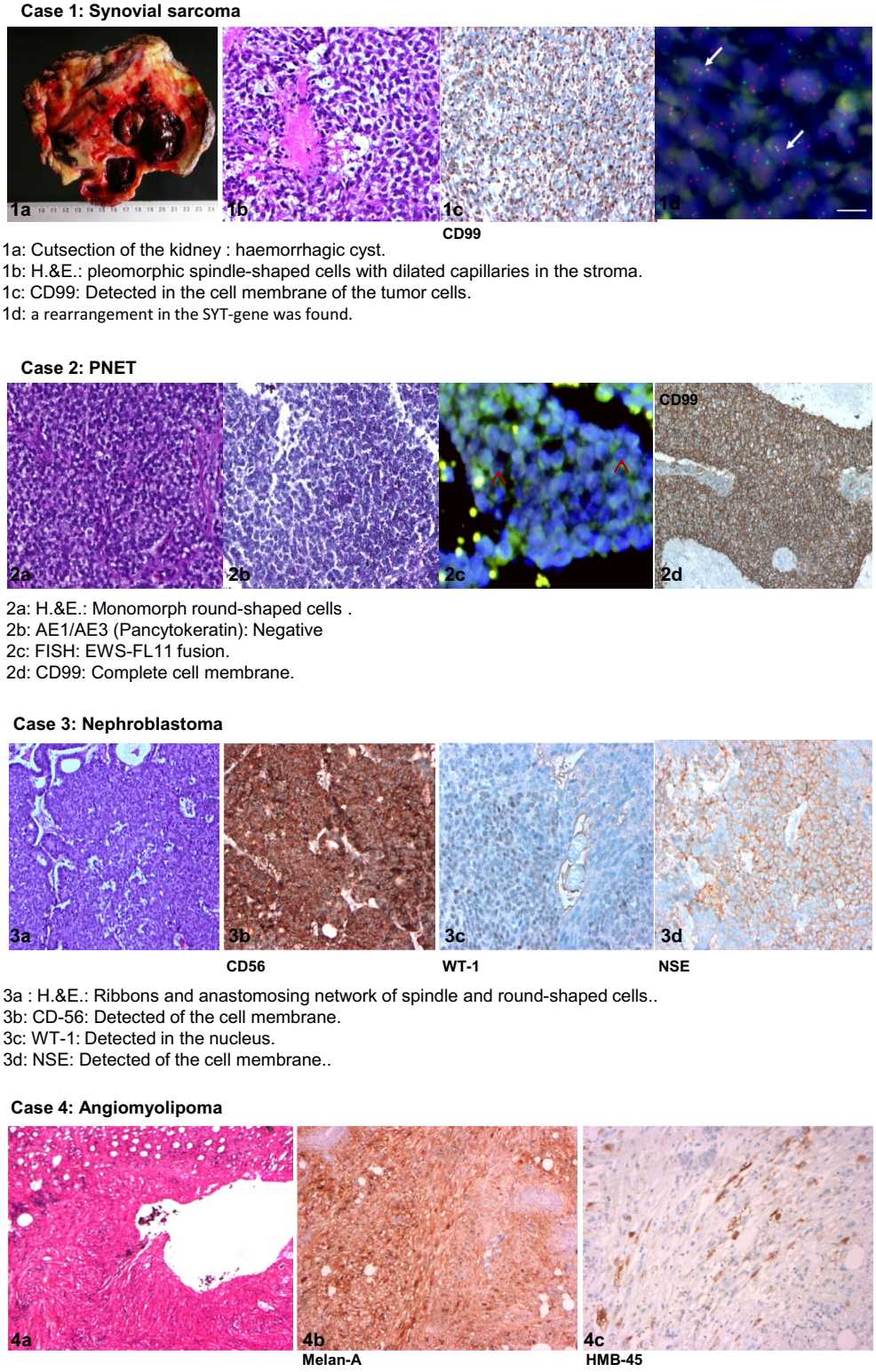
Results

The relevant immunomarkers that could differentiate different round/spindle cell tumors in the kidney are summarized in Table 1.

Discussion

The spindle cell tumors of the kidney include a wide range of unrelated neoplasms with overlapping morphologic features and different prognostic/therapeutic implications. The tumors usually included in this differential diagnosis include blastema-predominant WT, ES/PNET, metastatic neuroblastoma, synovial sarcoma, desmoplastic round cell tumor, small cell carcinoma, clear cell sarcoma, sarcomatous dedifferentiated renal cell carcinoma, and lymphoma. This differential diagnosis is further complicated by the relatively rare occurrence of most of these entities in the kidney.

Synovial sarcoma affects both genders and young individuals in the range of 20–50 years of age [4]. The limited number of cases reported has shown a gender ratio close to one, a mean age at diagnosis of 37 years (ranging between 13 and 67), and mean tumor diameter of 11 cm, ranging from 3 to 21 cm. The rate of metastasis on admission seems to be low [4]. Poorly-differentiated SS is composed of sheets of undifferentiated round cells with hyperchromatic nuclei and frequent mitoses, and shows the poorest outcome [5]. We found multiple immunohistochemical markers that have been investigated in cases of RSS. Markers such as Bcl-2, Vimentin, CD99, EMA, CD56, S100, Desmin, SMA, CD34, AE1/AE3, and WT-1 have all been tested for the use in diagnosis of the tumor from paraffin-embedded sections but the current diagnostic gold standard for synovial sarcoma is to demonstrate the fusion of the SYT (Synonyms: SS18-synovial sarcoma translocation,



1a: Cutsection of the kidney : haemorrhagic cyst.
1b: H.&E.: pleomorphic spindle-shaped cells with dilated capillaries in the stroma.
1c: CD99: Detected in the cell membrane of the tumor cells.
1d: a rearrangement in the SYT-gene was found.

2a: H.&E.: Monomorph round-shaped cells .
2b: AE1/AE3 (Pancytokeratin): Negative
2c: FISH: EWS-FL11 fusion.
2d: CD99: Complete cell membrane.

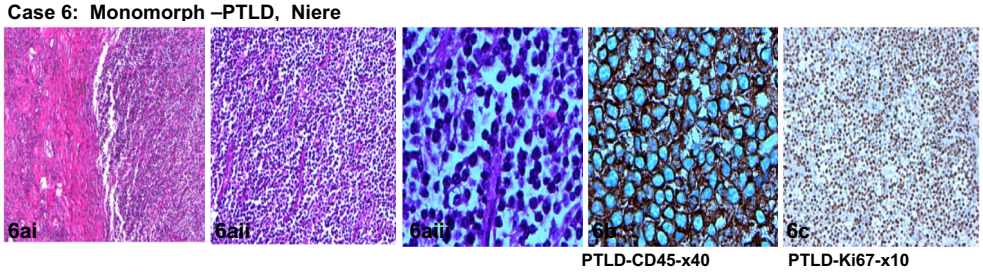
3a : H.&E.: Ribbons and anastomosing network of spindle and round-shaped cells..
3b: CD-56: Detected of the cell membrane.
3c: WT-1: Detected in the nucleus.
3d: NSE: Detected of the cell membrane..

4a: H.&E.: Spindle-shaped cells intermixed with fat cells and dilated capillaries.
4b: Melan-A: Detected in the cytoplasm.
4c: HMB-45: Focal positivity in the cytoplasm.

Figure 1 Conventional H.&E., immunohistochemistry and FISH analysis of some rare spindle cell tumours of the kidney.

chromosome 18) gene on chromosome 18 with either SSX1 (synovial sarcoma, X breakpoint 1) or SSX2 (synovial sarcoma, X breakpoint 2) gene on chromosome Xp11 [6,7].

Renal ES/PNET was first reported by Mor et al., 1994 [8]. Most of these previously reported cases have occurred in young adults (mean age, 28 yr; range, 4–69 yr), with a slight



6ai-iii:H.&E.: Monomorphe descohesive cells. Nuclear pleomorphism and hyperchromasia.
6b: CD-45: Detected in the cell membrane..
6c: Ki-67: High around 85%.

Fig. 1 (*continued*)

male predominance (male:female = 1.5:1). A wide panel of immunohistochemistry was made. Strong positivity was detected for CD99, Vimentin and CD117 but the gold standard of the diagnosis was the identification of the same EWS-FLI-1 gene fusions in renal ES/PNET as in extrarenal tumors, by Parham et al., 2001 [9]. In comparison with findings of Gu et al. [10], we did not notice an expression of cytokeratin. We see that renal ES/PNET is most likely to be confused with blastema-predominant Wilms' tumor of adult type and with the synovial sarcoma of the kidney but the use of immunohistochemistry as well as FISH is important to support the right diagnosis.

Angiomyolipoma of the kidney with sarcomatous transformation with spindle cell features and little fat cells can be confused with other sarcomas. Renal angiomyolipoma usually has a benign course and the treatment is based on lesion size and symptoms. Progressive enlargement and venous thrombosis are rare complications [11]. It occurs both sporadically and in association with tuberous sclerosis [12]. Other sarcomas can occur in the tumor like liposarcoma and fibrosarcoma [13]. Diagnosis of sarcomatous transformation can be only made by immunohistochemistry. A Panel of immunohistochemical markers includes HMB-45, S-100, Vimentin, CD10, Pax-2, Ki-67 and Melan-A was done. By positivity for HMB-45 and Melan-A, we have arrived at the correct diagnosis for angiomyolipoma in the kidney.

Recently it is advised to perform PAX-2, RCC and PAX-8 immunohistochemical markers to detect tumor cells and to differentiate them from other sarcomas [14]. Using these immunomarkers we were able to adequately diagnose the sarcomatous dedifferentiated renal cell carcinoma.

One of the most recent rarities is the Post-transplant lymphoproliferative disorders (PTLD). It comprises a spectrum ranging from usually EBV-driven, mostly B-cell polyclonal proliferations to B- and T-Cell lymphomas [15]. Among these is the monomorphic PTLD after renal transplantation ($<1\%$) [16]. According to the Cincinnati Transplant Tumor Registry, most PTLD are of B cell origin or, more rarely, of T cell proliferations [17]. To our knowledge, there are very few papers that describe monomorphic PTLD in the kidney after renal transplantation. The differential diagnosis includes all round cell tumors of the kidney, which were excluded with the help of immunohistochemical and molecular investigations. In summary we advise to perform a wide panel of immunohistochemical markers for spindle/round cell tumors of the kidney as well as to add FISH to get the correct diagnosis, because of the wide variety of surgical and chemotherapeutic options between entities which have a direct impact on the prognosis and the outcome (Fig. 1).

Conflict of interest

We declare that we have no conflict of interest and we have not a financial relationship with any organization.

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