Protocol for the Examination of Resection Specimens From Patients With Wilms and Other Pediatric Renal Tumors

Version: 4.2.0.0
Protocol Posting Date: March 2022
CAP Laboratory Accreditation Program Protocol Required Use Date: December 2022
The changes included in this current protocol version affect accreditation requirements. The new deadline for implementing this protocol version is reflected in the above accreditation date.

For accreditation purposes, this protocol should be used for the following procedures AND tumor types:

Procedure	Description
Resection	Includes specimens labeled partial nephrectomy and radical nephrectomy
Tumor Type	**Description**
Wilms tumor	Includes pediatric patients with Wilms and other renal tumors

This protocol is NOT required for accreditation purposes for the following:

Procedure
Additional excision performed after the definitive resection (eg, re-excision of surgical margins)
Cytologic specimens

The following should NOT be reported using this protocol:

Procedure
Biopsy (consider Wilms Tumor Biopsy protocol)
Tumor Type
Renal cell carcinoma (consider the Kidney protocol)
Lymphoma (consider the Hodgkin or non-Hodgkin Lymphoma protocols)

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
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Accreditation Requirements

This protocol can be utilized for a variety of procedures and tumor types for clinical care purposes. For accreditation purposes, only the definitive primary cancer resection specimen is required to have the core and conditional data elements reported in a synoptic format.

- Core data elements are required in reports to adequately describe appropriate malignancies. For accreditation purposes, essential data elements must be reported in all instances, even if the response is "not applicable" or "cannot be determined."
- Conditional data elements are only required to be reported if applicable as delineated in the protocol. For instance, the total number of lymph nodes examined must be reported, but only if nodes are present in the specimen.
- Optional data elements are identified with "+" and although not required for CAP accreditation purposes, may be considered for reporting as determined by local practice standards.

The use of this protocol is not required for recurrent tumors or for metastatic tumors that are resected at a different time than the primary tumor. Use of this protocol is also not required for pathology reviews performed at a second institution (ie, secondary consultation, second opinion, or review of outside case at second institution).

Synoptic Reporting

All core and conditionally required data elements outlined on the surgical case summary from this cancer protocol must be displayed in synoptic report format. Synoptic format is defined as:

- Data element: followed by its answer (response), outline format without the paired Data element: Response format is NOT considered synoptic.
- The data element should be represented in the report as it is listed in the case summary. The response for any data element may be modified from those listed in the case summary, including "Cannot be determined" if appropriate.
- Each diagnostic parameter pair (Data element: Response) is listed on a separate line or in a tabular format to achieve visual separation. The following exceptions are allowed to be listed on one line:
    - Anatomic site or specimen, laterality, and procedure
    - Pathologic Stage Classification (pTNM) elements
    - Negative margins, as long as all negative margins are specifically enumerated where applicable
- The synoptic portion of the report can appear in the diagnosis section of the pathology report, at the end of the report or in a separate section, but all Data element: Responses must be listed together in one location

Organizations and pathologists may choose to list the required elements in any order, use additional methods in order to enhance or achieve visual separation, or add optional items within the synoptic report. The report may have required elements in a summary format elsewhere in the report IN ADDITION TO but not as replacement for the synoptic report ie, all required elements must be in the synoptic portion of the report in the format defined above.

Summary of Changes

v 4.2.0.0

- Added Expert Consultation question
- Deprecated the Distance from Tumor to Vascular, Ureteral, and Soft Tissue Margin Questions

Reporting Template

Protocol Posting Date: March 2022

Select a single response unless otherwise indicated.

CASE SUMMARY: (KIDNEY, PEDIATRIC RENAL TUMORS: Resection)

For bilateral tumors, complete a separate checklist for each kidney.
First priority should always be given to formalin-fixed tissues for morphologic evaluation. The second priority for tissue processing may include snap-freezing up to 1 g (minimum of 100 mg) of tumor for molecular studies. (Note A)
For more information, contact: The Children's Oncology Group Biopathology Center. Phone: (614) 722-2890 or (800) 347-2486.

EXPERT CONSULTATION

Expert Consultation

___ Pending - Completion of this CAP Cancer Protocol is awaiting expert consultation
___ Completed - This CAP Cancer Protocol or some elements have been performed following expert consultation
___ Not applicable (expert consultation not required)

SPECIMEN

Procedure (Note B)

___ Partial nephrectomy
___ Radical nephrectomy
___ Other (specify): _____
___ Not specified

Nephrectomy Weight

___ Specify in Grams (g): _____ g
___ Cannot be determined (explain): _____

Specimen Laterality

___ Not applicable (not a bilateral tumor)
___ Right
___ Left
___ Not specified

TUMOR

Histologic Type (Note C)

___ Wilms tumor, favorable histology
___ Wilms tumor, focal anaplasia
___ Wilms tumor, diffuse anaplasia
___ Nephrogenic rest only
___ Congenital mesoblastic nephroma (cellular, classic, or mixed)
___ Clear cell sarcoma of kidney
___ Rhabdoid tumor
___ Other (specify): _____
___ Malignant neoplasm, type cannot be determined (explain): _____

+Histologic Type Comment: _____

Tumor Size
___ Greatest dimension in Centimeters (cm): _____ cm
+Additional Dimension in Centimeters (cm): ____ x ____ cm
___ Cannot be determined (explain): _____
For specimens with multiple tumors, give greatest dimension of each additional tumor(s)
Greatest Dimension Tumor #2 in Centimeters (cm): _____ cm
Greatest Dimension Tumor #3 in Centimeters (cm): _____ cm
Other (specify): _____

Tumor Focality
___ Unifocal
___ Multifocal
Number of Tumors in Specimen
___ Specify exact number: _____
___ Other (specify): _____
___ Cannot be determined
___ Cannot be determined (explain): _____
Tumor Extent (Note D)

Integrity of Gerota's Fascia
___ Intact
___ Disrupted
___ Cannot be determined: _____

Renal Sinus Involvement
___ Not identified
___ Minimal extension into renal sinus soft tissue
___ More than minimal extension into renal sinus soft tissue
___ Extension into renal sinus lymphovascular spaces
___ Cannot be determined (explain): _____

Renal Vein Involvement
___ Not identified
___ Present
___ Cannot be determined (explain): _____

Extension Beyond Renal Capsule
___ Not identified
___ Present
___ Cannot be determined (explain): _____

Direct Extension into Adjacent Organs
___ Not identified
___ Present (specify sites): _____
___ Cannot be determined (explain): _____

+Nephrogenic Rests (Note E)
___ Not identified
___ Intralobar
___ Perilobar, diffuse and hyperplastic
___ Perilobar, multifocal
___ Perilobar, focal
___ Perilobar
___ Present, unclassified
___ Cannot be determined: _____

+Posttherapy Histologic Classification
The histologic evidence of response to therapy may be used to guide further therapy. Therefore tumors that have previously undergone therapy should be given a posttherapy classification. (Note D)
___ No known preoperative therapy (not applicable)
___ Low risk (no viable Wilms tumor present other than scattered nephroblastic tubules that may represent residual nephrogenic rest)
___ Intermediate risk, with viable tumor present comprising less than 33% of mass, regardless of histology
___ Intermediate risk, with viable tumor present comprising greater than 33% of mass and blastemal histology present in less than 66% of viable tumor
___ Intermediate risk (not otherwise specified)
___ High risk (viable tumor greater than 33% of mass with blastemal histology present in greater than 66% of viable tumor)
___ Cannot be determined: _____

+Tumor Comment: _____

MARGINS

Margin Status
___ All margins negative for tumor
Closest Margin(s) to Tumor (select all that apply)
___ Vascular: _____
___ Ureteral: _____
___ Soft tissue: _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
Distance from Tumor to Closest Margin
Specify in Centimeters (cm).
___ Exact distance: _____ cm
___ Greater than: _____ cm
___ At least: _____ cm
___ Less than: _____ cm
___ Less than 0.1 cm
___ Other (specify): _____
___ Cannot be determined: _____
___ Tumor present at margin

Margin(s) Involved by Tumor (select all that apply)
___ Vascular: _____
___ Ureteral: _____
___ Soft tissue: _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
___ Not applicable

+Margin Comment: _____

REGIONAL LYMPH NODES

Regional Lymph Node Status
___ Not applicable (no regional lymph nodes submitted or found)
___ Regional lymph nodes present
___ All regional lymph nodes negative for tumor
___ Tumor present in regional lymph node(s)
Number of Lymph Nodes with Tumor
___ Exact number (specify): _____
___ At least (specify): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____
Nodal Site(s) with Tumor, if known
___ Specify nodal site(s): _____
___ Cannot be determined
___ Not known
___ Other (specify): _____
___ Cannot be determined (explain): _____
Number of Lymph Nodes Examined
___ Exact number (specify): _____
___ At least (specify): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

+Regional Lymph Node Comment: _____

DISTANT METASTASIS

Distant Site(s) Involved, if applicable (select all that apply)
Distant metastasis includes both hematogenous metastasis or lymph node metastasis outside the abdomen-pelvic region (beyond the renal drainage system).
___ Not applicable
___ Lymph node(s) outside of the abdomino-pelvic region: _____
___ Lung: _____
___ Liver: _____

___ Other (specify): _____
___ Cannot be determined: _____

PATHOLOGIC STAGE

Children's Oncology Group Staging System for Pediatric Renal Tumors Other Than Renal Cell Carcinoma (Note F)

Local stage must be assigned by the pathologist with the caveat that he or she may not be aware of clinical or radiographic information important in assigning the clinical or overall stage (i.e., presence of metastatic disease).

___ Not applicable (nephrogenic rests only)

 # Local Stage I requires all of the following to be true: No penetration of renal capsule by tumor identified, and; No tumor involvement of extrarenal or renal sinus lymph-vascular spaces identified, and; No tumor metastasis to lymph nodes identified

___ Local Stage I: Tumor limited to kidney and completely resected#

___ Local Stage II: Tumor extends beyond kidney but is completely resected, with negative surgical margins and negative regional lymph nodes

- ___ Tumor extends through the renal capsule
- ___ Tumor involvement of extrarenal or renal sinus lymph-vascular spaces present
- ___ Tumor involves renal vein but has not been transected and is not attached to vein wall at resection margin
- ___ Tumor more than minimally involves the renal sinus soft tissue

___ Local Stage III: Residual tumor is suspected

- ___ Tumor present at margin(s) of resection
- ___ Tumor rupture identified
- ___ Tumor spill before or during surgery identified
- ___ Piecemeal excision of tumor (removal of tumor in more than 1 piece)
- ___ Metastatic tumor in regional lymph nodes identified
- ___ History of renal tumor biopsy before definitive surgery

 # Stage IV requires hematogenous metastases or lymph node metastases outside the abdomino-pelvic region (beyond renal drainage system, e.g., lung, liver)

___ Stage IV: Metastatic disease#

___ Stage V: Bilateral renal involvement at diagnosis

Each side should be staged separately in separate case summaries, according to above criteria, as stage I through IV)
Specify (both):

Right Kidney Stage

- ___ I
- ___ II
- ___ III
- ___ IV

Left Kidney Stage

- ___ I
- ___ II
- ___ III
- ___ IV

ADDITIONAL FINDINGS

+Additional Findings (specify) (Notes G,H): _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Frozen Section

Because of the high number of false-positives, intraoperative frozen sections should be avoided unless the operative procedure will be altered by the result. Biopsies of pediatric renal tumors present significant potential for diagnostic error, even on permanent section. However, frozen sections from the bivalved nephrectomy specimen—to ensure tumor viability or to prompt other differential diagnostic studies—may be of value.

For future potential molecular studies, viable tumor (1 gram or more) should be snap-frozen (liquid nitrogen or cold isopentane) in 2 or more vials, along with a separate portion of nonneoplastic kidney (at least 1 vial).[1] The latter serves as a useful control in molecular genetic studies and helps determine whether any detected genomic abnormalities are germline or intratumoral mutations. Nephrogenic rests may also be sampled and frozen for the same reasons.

References

1. Knezevich SR, Garnett MJ, Pysher TJ, et al. ETV6-NTRK3 gene fusion and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. *Cancer Res.* 1998;58(22):5046-5048.

B. Handling of Renal Specimens

With pediatric renal tumors, there are many issues that can interfere with making accurate diagnostic and staging decisions. The following guidelines are recommended to ensure that the necessary diagnostic features are preserved and properly examined[1]:

- *Nephrectomy specimens should be submitted intact by the surgeon.* The surface of the specimen should be photographed and inked before bivalving to facilitate the recognition of displacement artifacts from the smearing of tumor cells over the specimen surface during sectioning, as well as to evaluate margins. Bivalving will cause the capsule in a fresh kidney to retract, possibly altering the relationship between the tumor and the capsule or surgical margin.
- The capsule from nephrectomy specimens must *never* be stripped. Invasion of the tumor into the capsule is a criterion in staging. In addition, nephrogenic rests are often subcapsular in location. The medial sinus margin is defined as the medial end of soft tissues surrounding the renal artery and vein.
- Inspect the renal vein for tumor thrombus because this is a common route by which Wilms tumor exits the kidney (see Microscopic Examination Note). Care should be taken to not over-interpret the renal vein margin (see Extent of Tumor Note).
- The exact site from which each section or paraffin block is obtained may be documented by photograph, photocopy, or drawing. Often, this documentation is critical for recognizing staging problems and for the evaluation of focal versus diffuse anaplasia.
- Take at least 1 microscopic section per centimeter of maximal tumor diameter, with additional sampling of any suspicious lesions. The majority of random tumor sections should be taken from the periphery of the tumor, because this is where the invasive pattern of the tumor can be identified and its interface with the capsule and native kidney can be evaluated. Peripheral sections also demonstrate invasion of vessels within the intrarenal extension of the renal sinus. The renal sinus is composed of fat containing hilar vessels; the renal sinus is largely located within the hilum of the kidney, but may extend deep into the kidney. Involvement of the intrarenal

renal sinus remains a criteria for local stage 2 disease. The renal cortex at the sinus lacks a capsule. The most important sections are those taken from regions of the sinus adjacent to the tumor to demonstrate involvement (or lack of involvement) of sinus vessels (see Microscopic Examination Note).

- For Wilms tumors that are multicentric, sample each nodule. More than 30% of Wilms nephrectomy specimens contain nephrogenic rests. Nephrogenic rests often appear paler than the typical nonneoplastic kidney parenchyma. These areas should be sampled. Nephrogenic rests have important implications concerning the risk of contralateral Wilms tumor development and may have other syndromatic implications. At least 1 random section of normal kidney and possibly more may be taken to detect nephrogenic rests microscopically (see Nephrogenic Rests Note).
- Nephrectomy weight may be an eligibility factor for some clinical trial protocols. Hence, this measurement is critical.
- In addition to the capsular, vascular, and sinus sampling already described, routine sections taken for margins should include sampling of the distal ureter.

References

1. Zuppan CW. Handling and evaluation of pediatric renal tumors. *Am J Clin Pathol.* 1998;109(4 suppl 1):S31-S37.

C. Microscopic Examination

Favorable Histology Wilms Tumor

Classic Wilms tumors present with a mixture of blastemic, stromal, and epithelial cell types. A common difficulty faced by pathologists interpreting a pediatric renal mass is the distinction between a hyperplastic perilobar nephrogenic rest and a Wilms tumor because these may be cytologically identical. The most helpful histologic feature is the absence of a peritumoral fibrous capsule in perilobar nephrogenic rests.

Many other neoplasms may have a histologic appearance similar to blastemal-predominant Wilms tumors. The most common tumors misdiagnosed as Wilms tumors are undifferentiated neuroblastoma, primitive neuroectodermal tumor, and synovial sarcoma. The most helpful feature that favors the diagnosis of Wilms tumor is the presence of overlapping nuclei with finely dispersed chromatin. Similarly, epithelial-predominant Wilms tumors show considerable histologic overlap with papillary renal cell carcinoma and metanephric adenoma. A more detailed differential diagnosis of pediatric renal tumors is provided elsewhere.[1,2,3]

Anaplastic Wilms Tumor

Once a tumor has been diagnosed as Wilms tumor, it is necessary to determine whether it is of favorable histology or if anaplasia is present. Although anaplasia is present in only 5% of all cases,[4,5] it is the major prognostic indicator and will place a tumor in an unfavorable histologic category.

The presence of anaplasia is a significant prognostic factor in Wilms tumor and places the tumor in an unfavorable category. Although the mechanism for unfavorable prognosis is unclear, anaplasia may be a marker of chemotherapy resistance. A diagnosis of anaplasia requires both (1) gigantic polyploid nuclei with increased chromatin content and major diameters at least 3 times those of adjacent cells and (2) the presence of multipolar or otherwise recognizably polyploid mitotic figures. On a small biopsy, a single multipolar mitotic figure or an unequivocally gigantic tumor cell nucleus may be sufficient criteria for diagnosis. *Severe nuclear unrest* is defined as nuclear pleomorphism or atypia approaching the criteria of

anaplasia. Care should be taken in the assessment of anaplasia in cells exhibiting rhabdomyoblastic differentiation, as these cells may show nuclear enlargement, pleomorphism, and hyperchromasia akin to regenerating skeletal muscle. Such areas of "pseudoanaplasia" will have increased cytoplasmic volume and will lack atypical mitoses, as described above.

Criteria for focal versus diffuse anaplasia have been defined topographically and are rigorous.[5] This topographic definition of focal anaplasia makes it mandatory that pathologists carefully document the exact site from which every section is obtained (eg, on a diagram, specimen photocopy, and/or photograph of the gross specimen).

Focal Anaplasia
Diagnosis of focal anaplasia is warranted if *all* of the following are true:

- No anaplasia should be present in tumor within renal vessels or outside the kidney.
- Anaplasia must be confined to 1 or a few sharply localized regions within the primary intrarenal tumor site.
- Each focus of anaplasia must be surrounded on all sides by nonanaplastic tissue. This may require mapping of the tumor during submission.
- The remaining nonanaplastic tumor must not show severe nuclear unrest.

(The same criteria apply to posttreatment nephrectomies. There is no evidence to suggest that either chemotherapy or radiation therapy result in anaplasia.)

Diffuse Anaplasia
Diagnosis of diffuse anaplasia is warranted if *any* of the following are true:

- Anaplasia is present in tumor in any extrarenal site, including vessels of the renal sinus, extracapsular infiltrates, or nodal or distant metastases. Also, anaplasia is present in intrarenal vascular involvement by tumor.
- Anaplasia is present in a random biopsy.
- Anaplasia is unequivocally identified, but the tumor fails any of the above criteria for focal anaplasia.

Posttherapy Classification of Wilms Tumor: The response of a Wilms tumor to prior therapy may help guide the subsequent therapeutic strategy. For this reason, the Children's Oncology Group is using the overall categories utilized by International Society of Paediatric Oncology (SIOP) when categorizing posttherapy tumors.[6] As outlined above, these categories are based on the proportion of the tumor that is viable and blastemal, and only apply to favourable histology Wilms tumor. It is acknowledged that such quantitative analysis is quite difficult to reproduce and is highly dependent on how representative of the entire tumor the sections submitted are. The overall concept is that tumor that remains highly undifferentiated and proliferative following therapy will require more aggressive therapy going forward. Pathologists should, as always, use their best judgment. Such categorization is likely to change in the future.

The staging for posttherapy nephrectomy specimens differs only in the interpretation of areas of necrosis outside the kidney. The presence of necrotic tumor or chemotherapy-induced change (in the absence of viable tumor) in the renal sinus and/or within the perirenal fat is not regarded as a reason for upstaging following chemotherapy, providing the tumor (either viable or necrotic) is completely excised and does not reach the resection margins. In contrast, the presence of necrotic tumour or chemotherapy-induced

changes in a lymph node or at the resection margins is regarded as proof of previous tumour with potential microscopic residual disease, and therefore the tumour is assigned stage III.

Congenital Mesoblastic Nephroma

There is a growing appreciation that congenital mesoblastic nephroma (CMN), a tumor of infancy, represents 2 genetically distinct tumors: the “classic” CMN (24% of cases), which may correspond to a type of fibromatosis; and “cellular” CMN (66% of cases), which corresponds to infantile fibrosarcoma and often contains the characteristic t(12;15) or other variant translocations, resulting in a fusion product detectable by reverse transcriptase polymerase chain reaction.[1] Absence of this translocation does not exclude the diagnosis of cellular congenital mesoblastic nephroma. Occasional cases (10%) are classified as “mixed” CMN, owing to the presence of both histologic types. Increasingly a subset of CMN, often but not exclusively “mixed” pattern, has been recognized to have *EGFR* activating mutations (most often internal tandem duplications).[7]

Approximately 10% of CMNs recur. Virtually all CMNs that recur are of the cellular subtype. Recurrences occur very rapidly, often within the first month of diagnosis. Virtually all recurrences occur by 1 year of age. More than half are local recurrences; however, pulmonary metastases have been identified in 20% of patients who relapse. However, the primary determinant of outcome is the completeness of excision. Surgeons should be educated and encouraged to secure wide margins, particularly medial margins, when resecting renal tumors in infants. Nonetheless, one can rarely be sure that the medial margin is clear; therefore, all patients should be followed closely. Monthly abdominal ultrasounds should be performed for 1 year, with the hope of catching recurrences early enough to surgically excise them. Adjuvant chemotherapy is required when there is gross residual tumor. Radiation has no demonstrable effect.

Clear Cell Sarcoma of the Kidney

Clear cell sarcoma of the kidney (CCSK) is capable of mimicking, or being mimicked by, every other major neoplastic entity in the pediatric kidney. CCSK is characterized molecularly by *BCOR* internal tandem duplications or *YWHAE-NUTM2B* fusions.[8] Immunohistochemical stains other than vimentin are inconsistent, but these negative results can help rule out other neoplasia in the differential diagnosis. In recent years, CCSK has been shown to be reliably positive for several immunohistochemical markers. In particular, immunohistochemistry for BCOR, nerve growth factor receptor, and cyclin D1 have been shown to positively stain CCSKs, although their specificity is variable and unclear.[9]

The histologic spectrum and clinical outcome of patients with CCSK have recently been reported by the National Wilms Tumor Study Group.[10] Nearly all patients with stage I CCSK survive. Conversely, patients with more advanced disease have a propensity for local recurrence and metastasis. Recurrences can occur from years to decades after initial presentation, sometimes demonstrating a bland histology that differs from the primary tumor. The metastatic pattern tends to be more widespread than that of Wilms tumor and includes bone, brain, and soft tissue. There is a high recurrence rate and death rate even when treated by combination chemotherapy, but survival can be greatly improved after treatment with doxorubicin,[10] which underscores the importance of identifying this neoplasia to facilitate early administration of more effective chemotherapy regimens.

There are several variants of CCSK, among which the following are most important:

Classical Pattern
The classical pattern of CCSK presents an evenly dispersed network of fine, arborizing vessels accompanied by a variable amount of spindle-cell stroma, subdividing the tumor into nests or cords of regular size, usually about 8 to 12 cells in width. The tumor cells are of regular size, usually with stellate cytoplasm, which often surrounds clear vacuoles. The nuclei are notably regular in size, with finely dispersed chromatin and usually inconspicuous nucleoli. Mitotic activity may be sparse. Scattered preexistent tubules or glomeruli often are dispersed through the peripheral regions of the tumor. This pattern of growth, which isolates and separates individual nephronic units or collecting tubules, is an important clue that one is not dealing with a Wilms tumor. The latter almost always has a sharply defined, "pushing" border.

Hyalinizing Pattern
The hyalinizing pattern of CCSK often has an osteoid-like, nonbirefringent matrix that separates tumor cells, giving an appearance reminiscent of osteosarcoma. A similar change may be seen in rhabdoid tumor of the kidney (RTK).

Epithelioid Pattern
The epithelioid pattern is the most deceptive of the patterns of CCSK, in which the tumor cells align themselves along vessels in a manner mimicking the tubules of Wilms tumor. Often these cells form filigree-like strands.

Rhabdoid Tumor of the Kidney
This distinctive renal neoplasm most commonly is encountered in infants younger than 1 year of age and is extremely uncommon in patients older than 5 years. It is extremely aggressive and is the most prognostically unfavorable neoplasm of the kidney in early life. Rhabdoid tumors continue to present significant diagnostic challenges, particularly when they do not show overt rhabdoid features. However, the growing appreciation that this tumor arises in sites other than the kidney and the central nervous system, and the increased appreciation of the wide histologic spectrum of rhabdoid tumors, have contributed to a marked increase in their correct diagnosis. Rhabdoid tumor of the kidney should not be confused with the true myogenic cells, which are often found in Wilms tumors.

The most distinctive features of rhabdoid tumor of the kidney (RTK) are rather large cells with large vesicular nuclei, a prominent single nucleolus, and the presence in at least some cells of globular eosinophilic cytoplasmic inclusions composed of whorled masses of intermediate filaments. Another distinctive feature is the extremely aggressive, invasive pattern of this lesion. RTK has a diverse immunohistochemical profile. Tumors may be positive for many supposedly incompatible epitopes for epithelial, myogenous, neural, and mesenchymal cell types. Epithelial membrane antigen (EMA) should be included in the routine panel applied to small blue cell tumors, largely because of the typical focal strong positivity for EMA (as well as a multitude of other markers) that rhabdoid tumors demonstrate.

Rapid advances in our understanding of the genetic events leading to the development of rhabdoid tumors have been made recently. It now is clear that the vast majority of both renal and extrarenal rhabdoid tumors carry homozygous deletions and/or mutations of the *hSNF5/INI1* gene located at 22q11.2.[11] Furthermore, germline mutations have been identified in individuals with both renal and central nervous system rhabdoid tumors. The *INI1* gene causes conformational changes in the nucleosome, thereby altering histone-DNA binding and facilitating transcription factor access. The *INI1* deletion can be evaluated with immunohistochemistry using the BAF47 antibody.[2] This antibody shows strong nuclear

staining in virtually all cell types except rhabdoid tumor cells. Important exceptions are renal medullary carcinoma and epithelioid sarcoma, which also often show loss of INI-1 protein.

References

1. Knezevich SR, Garnett MJ, Pysher TJ, et al. ETV6-NTRK3 gene fusion and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. *Cancer Res.* 1998;58(22):5046-5048.
2. Hoot AC, Russo P, Judkins AR, Perlman EJ, Biegel JA. Immunohistochemical analysis of hSNF5/INI1 distinguishes renal and extra-renal malignant rhabdoid tumors from other pediatric soft tissue tumors. *Am J Surg Pathol*. 2004;28(11):1485-1491.
3. Murphy WM, Perlman EJ, Grignon D. Tumors of the kidney, bladder, and related urinary structures. *Atlas of Tumor Pathology*. 4th Series. Washington DC: Armed Forces Institute of Pathology, 2004.
4. Zuppan CW. Handling and evaluation of pediatric renal tumors. *Am J Clin Pathol.* 1998;109(4 suppl 1):S31-S37.
5. Faria P, Beckwith JB, Mishra K, et al. Focal versus diffuse anaplasia in Wilms tumor—new definitions with prognostic significance: a report from the National Wilms Tumor Study Group. *Am J Surg Pathol.* 1996;20(8):909-920.
6. Vujanic GM, Sandstedt B. The pathology of Wilms' tumour (nephroblastoma): the International Society of Paediatric Oncology Approach. *J Clin Pathol.* 2010;63:102-109.
7. Wegert J, Vokuhl C, Collord G, et al. Recurrent intragenic rearrangements of EGFR and BRAF in soft tissue tumors of infants. *Nat Commun*. 2018;9(1):2378.
8. Ueno-Yokohata H, Okita H, Nakasato K, et al. Consistent in-frame internal tandem duplications of BCOR characterize clear cell sarcoma of the kidney. *Nat Genet.* 2015;47(8):861-863.
9. Arva NC, Bonadio J, Perlman EJ**,** Cajaiba MM. Diagnostic utility of Pax8, Pax2, and NGFR immunohistochemical expression in pediatric renal tumors. *Appl Immunohistochem Mol Morphol*. 2017. doi: 10.1097 PMID: 28426529.
10. Argani P, Perlman EJ, Breslow NE, et al. Clear cell sarcoma of the kidney: a review of 351 cases from the National Wilms Tumor Study Group Pathology Center. *Am J Surg Pathol.* 2000;24(1):4-18.
11. Biegel JA, Zhou J-Y, Rorke LB, et al. Germline and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. *Cancer Res.* 1999;59(1):74-79.

D. Extent of Tumor

Evaluation of Renal Sinus Invasion

The most common cause of upstaging upon central review is failure to appreciate renal sinus involvement. Renal sinus vascular involvement is easy to confirm when the tumor fills the lumen or invades the vascular wall. Displacement artifact is also readily identified when it is present in arterial lumina, when it is accompanied by abundant displacement artifact elsewhere, or when ink is present within the aggregates. More difficult are foci of unattached tumor intermingling with fibrin and red cells, or free-floating rounded tumor fragments that are not associated with other displacement artifact. The presence of these foci in children with small, otherwise stage I tumors not treated with adjuvant chemotherapy are biologically significant and should upstage the patient. The other difficulty with the evaluation of the renal sinus is the fact that it extends well into the kidney and is not limited to the hilum. The renal sinus can be identified by the presence of fat and mesenchymal tissue surrounding vascular structures. The involvement of soft tissue confined to the intrarenal portion of the renal sinus is considered to be limited (unless close to a surgical margin) and would not upstage a patient to stage II.

However, the involvement of a vessel within the intrarenal portion of the renal sinus does upstage the patient to stage II. Intrarenal vascular invasion does not upstage a renal tumor.

Evaluation of Renal Vein Invasion

Caution should be used in the evaluation of the margin of the renal vein that contains a thrombus. The vein often retracts after the surgeon sections it, leaving a protruding tumor thrombus, which may erroneously be considered a positive margin. If the thrombus itself is not transected, and if the margin of the vascular wall itself does not contain tumor, this surgical margin is interpreted as being negative.

E. Nephrogenic Rests

Nephrogenic rests[1] are regions of persistent embryonal tissue in the renal parenchyma and can be found in 30%-44% of kidneys removed for Wilms tumor, 4% of kidneys removed for dysplasia or urinary tract malformations, and 0.21%-0.87% of kidneys in pediatric autopsy series (higher incidence in infants less than 3 months of age). The term *nephroblastomatosis* refers to multiple or diffusely distributed nephrogenic rests. The 2 fundamental categories of nephrogenic rests are based on the topography of the lesion. *Perilobar nephrogenic rests* (PLNRs) are located at the periphery of the lobule and are usually subcapsular. They are often multiple and can be diffuse (diffuse perilobar nephrogenic rests or DPLNs).[2] Microscopically, perilobar rests are well demarcated, but not encapsulated. They are typically composed of blastema and tubules with little intervening stroma. Similarly, tumors arising in association with PLNR are more likely to be blastemal or epithelial predominant. PLNRs are associated with higher birthweights and overgrowth syndromes, including Beckwith-Wiedemann syndrome. PLNRs serve as a marker of loss of imprinting or loss of heterozygosity for *IGF-2*. Intralobar nephrogenic rests (ILNRs) are located deep within the lobule and are usually solitary. They have indistinct margins with respect to the normal kidney. ILNRs contain blastemal, tubular, and prominent stromal elements interspersed among normal glomerular and tubular elements. ILNR are also more often associated with early-onset, stromal-predominant Wilms tumor or Wilms tumor showing divergent (teratomatous) differentiation. ILNRs are strongly associated with WAGR (*W*ilms tumor, *a*niridia, *g*enitourinary anomalies, and mental *r*etardation) and Denys-Drash syndromes. It is thought that ILNRs result from an error earlier in nephrogenesis as compared with PLNRs, explaining the typical ILNR location deep within the lobule.

The presence of nephrogenic rests has clinical implications for their association with genetic syndromes as well as the risk for development of contralateral Wilms tumor, particularly in patients whose tumors are diagnosed in the first year of life.[3]

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F. Staging

The American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) TNM staging systems currently do not apply to Wilms tumor. The Children's Oncology Group staging system for Wilms tumors is recommended and shown below.[1]

Stage I

- Tumor limited to kidney and completely resected
- Renal capsule intact
- Tumor not ruptured or biopsied before removal
- No residual tumor apparent beyond margins of resection
- Renal vein and renal sinus vessels contain no tumor (intrarenal vessel involvement may be present)
- No lymph node involvement or distant metastases

Stage II

- Tumor extends beyond kidney but is completely resected
- Regional extension of tumor (vascular invasion outside the renal parenchyma or within the renal sinus, extensive renal sinus soft tissue invasion, and/or capsular penetration with negative excision margin)

Stage III

- Nonhematogenous metastases confined to the abdomen (eg, tumor in regional lymph nodes), including tumor implants on or penetrating the peritoneum
- Gross or microscopic tumor remains postoperatively (tumor at margins of resection)
- Tumor spill before or during surgery not confined to flank
- Piecemeal excision of the tumor (removal in more than 1 piece)
- Operative tumor rupture
- Tumor biopsy before surgery

Stage IV

- Hematogenous metastases or lymph node metastases outside the abdomino-pelvic region (beyond renal drainage system, eg, lung, liver)

Stage V

- Bilateral renal involvement at diagnosis (each side should also be staged separately, according to above criteria, as I through IV)

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G. Special Studies

The diagnosis of primary renal tumors in children remains largely based on examination of hematoxylin-eosin (H&E)-stained sections. Although some studies suggest that p53 immunostaining may be a more sensitive predictor of poor outcome than histologic assessment of anaplasia,[1] such studies are fraught with difficulties in interpreting the outside limits of “positivity” as well as with interinstitutional variability in immunostaining techniques. Furthermore, some p53 mutations by their nature do not result in abnormal protein accumulation. However, strong, unequivocal abnormal p53 protein accumulation identified in a tumor that is suspicious for anaplasia may contribute to the diagnosis.[2]

Other immunohistochemical stains are often utilized in the diagnosis of Wilms tumor, although it should always be remembered that no single or panel of markers can with 100% confidence either prove or exclude the diagnosis of Wilms tumor. WT1 is commonly positive in blastemal and epithelial elements but may be negative in up to 20% of Wilms tumors. CD56 is a sensitive marker of Wilms tumor but is quite nonspecific. PAX8 positivity is quite useful in excluding small blue cell tumors of the soft tissue that happen to present in the kidney.[3] Almost any other immunohistochemical marker may be found in Wilms tumors in the correct pathologic context.

No single cytogenetic or molecular abnormality has been consistently abnormal in Wilms tumor or its host, but constitutional deletions of the *WT-1* tumor suppressor gene at 11p13 often predispose the patient to development of Wilms tumors. WAGR syndrome and Denys-Drash syndrome are characterized by the deletion or mutation of this gene. ILNRs are associated with WAGR and Denys-Drash syndromes. PLNRs are associated with Beckwith-Wiedemann syndrome, Perlman syndrome, and hemihypertrophy.[4,5]

Genetic tests are often quite useful in the evaluation of several pediatric tumors arising in the kidney that mimic Wilms tumor. These include the characteristic translocation of cellular mesoblastic nephroma, t(12;15); and peripheral primitive neuroectodermal tumor, t(11;22). Molecular evaluation of the *INI1* gene may be useful not only in the diagnosis of rhabdoid tumor, but also in counseling the family in the frequent event that this is constitutional.

Molecular tests such as loss of heterozygosity (LOH) at chromosomes 1p and 16q, 1q gain, and 11p15 loss have been and remain active study questions for augmenting risk stratification and treatment for patients with Wilms tumor. However, the results of therapeutic interventions based on these findings are stage specific and should be interpreted with care until mature data is available.[6,7,8]

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H. Syndromes Associated with Wilms Tumor

The following syndromes are associated with Wilms tumor[1,2]:

- Beckwith-Wiedemann syndrome
- Perlman familial nephroblastomatosis syndrome
- Denys-Drash syndrome
- Trisomy 18
- Neurofibromatosis
- Bloom syndrome
- WAGR syndrome

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