

SECTION 5

PATHOLOGY PROTOCOL

All renal tumours diagnosed in children up to 18 years of age as well as typical renal tumours of childhood found in older adolescents should be registered. Typical renal tumours of childhood are nephroblastoma, clear cell sarcoma of kidney, rhabdoid tumour of kidney, and mesoblastic nephroma. Consultation for all cases will be provided without charge, and any use of material for teaching purposes or publication will credit the contributing pathologist.

5.1 ROLE OF THE PATHOLOGIST IN A PARTICIPATING CENTRE

The local pathologist has an essential role in both the clinical trial and the prospective study, as follows:

1. **DIAGNOSIS:** He/she makes the diagnosis of the renal tumour.
2. **HISTOLOGICAL SUBTYPE AND RISK GROUP.** He/she assigns the tumour to a histological subtype, i.e. low risk, intermediate risk or high risk.
3. **STAGING:** He/she makes a precise evaluation of the abdominal stage of the tumour (even in children with stage IV disease, local staging is critical to determine the utilisation of radiotherapy). The pathologist should have information regarding pre- or intraoperative tumour rupture (from the surgeon) and clinical information regarding distant metastases. For the purpose of the Trial, please use the SIOP staging system (see 5.5) (the pTNM staging system can be of additional support but should not be used instead of the SIOP staging system).

Patients will be treated according to different therapeutic protocols **depending on tumour histology and stage**. As outlined elsewhere in the Trial protocol (Sections 6 and 7), low risk tumours, stage I, will be treated with no post-operative chemotherapy while high risk tumours will be treated with more intensive chemotherapy after surgery. Therefore, it is of the utmost importance for these tumours to be classified correctly - **in order to confirm the diagnosis prior to post-operative treatment all low and high risk tumours should be sent for rapid review immediately after the operation**. Please submit a full set of H&E slides and one paraffin block from an area of viable tumour, accompanied by the SIOP Institutional Pathology Form and a copy of your report, to the Referring Pathologists (see 5.4). *(Consider asking technician to cut another set of H&E slides at the same time he/she is preparing one for you ~ it is much easier and quicker to do it at that time than later. Please do not send unstained slides only as it will delay the answer).* In case of local relapse(s) or surgically removed metastases, please also send a full set of slides with the appropriate forms.

Do not delay sending the sections for pathology review for whatever reasons, even if you are not sure whether the patient will be entered into the Trial. Although registration of the patient might have been delayed and no trial number yet allocated, please send your slides and you will still have the panel pathologists' opinion in time for making a decision on further postoperative treatment.

5.2 THE REVISED S.I.O.P. WORKING CLASSIFICATION OF RENAL TUMOURS OF CHILDHOOD (2001)

A. FOR PRE-TREATED CASES

LOW RISK TUMOURS

- *Mesoblastic nephroma*
- ~ Cystic partially differentiated nephroblastoma
- Completely necrotic nephroblastoma

INTERMEDIATE RISK TUMOURS

- Nephroblastoma - epithelial type
- Nephroblastoma - stromal type
- Nephroblastoma - mixed type
- Nephroblastoma - regressive type
- Nephroblastoma - focal anaplasia

HIGH RISK TUMOURS

- Nephroblastoma - blastemal type
- Nephroblastoma - diffuse anaplasia
- Clear cell sarcoma of the kidney*
- *Rhabdoid tumour of the kidney*

B. FOR PRIMARY NEPHRECTOMY CASES

LOW RISK TUMOURS

- *Mesoblastic nephroma*
- Cystic partially differentiated nephroblastoma

INTERMEDIATE RISK TUMOURS

- Non-anaplastic nephroblastoma and its variants
- Nephroblastoma - focal anaplasia

HIGH RISK TUMOURS

- Nephroblastoma - diffuse anaplasia
- *Clear cell sarcoma of the kidney*
- *Rhabdoid tumour of the kidney*

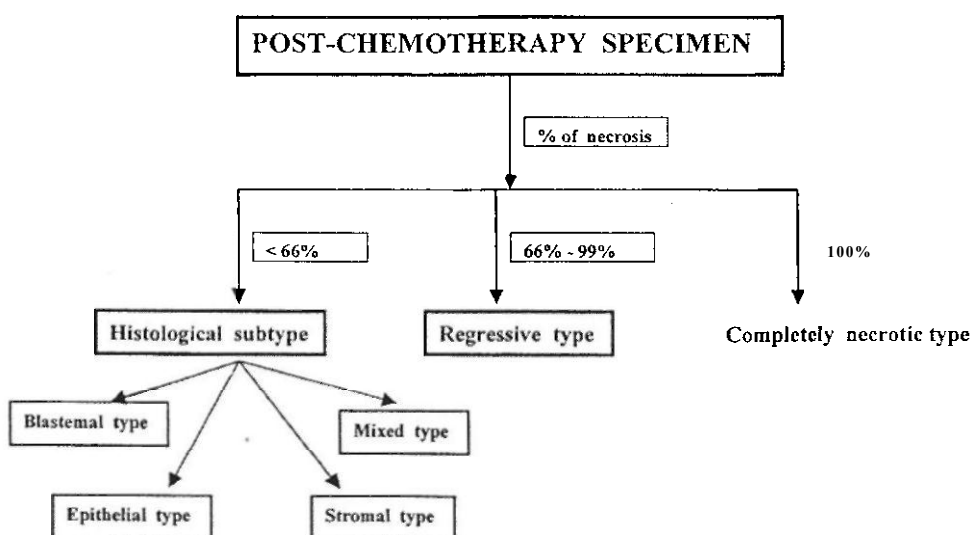
5.3 DEFINITONS OF NEPHROBLASTOMA AND ITS SUBTYPES, AND OTHER TYPICAL RENAL TUMOURS OF CHILDHOOD

Based on the correlation between the histological features and survival, three prognostic groups of typical renal tumours of childhood were discerned in the previous SIOP Trials and Studies: low risk, intermediate risk and high risk tumours.

Mesoblastic nephroma, clear cell sarcoma of the kidney and rhabdoid tumour of the kidney represent separate entities from nephroblastoma but are typical renal tumours of childhood and are included in the SIOP classification and trial/study. Other, less common renal tumours which may occur at any age including children should be also registered through the SIOP Nephroblastoma Trial Office as they may provide a useful clue in our understanding of renal tumours.

The SIOP (Stockholm) Working Classification of Renal Tumours of Childhood has recently been revised to incorporate the results of the latest SIOP Trials and Studies and it will be followed in this Trial and Study. Some entities that existed in the previous classification, such as nephroblastoma with fibroadenomatous structures and highly differentiated epithelial nephroblastoma, have been either excluded or grouped with other subtypes. Unlike in the previous classification, subtyping of nephroblastomas in the Intermediate risk group is now required (see diagram below).

It is important to emphasise that for treatment purposes, in addition to anaplasia, only three major types of nephroblastoma need to be recognised: completely necrotic nephroblastoma (Low risk tumours), blastemal (high risk tumour) and others (intermediate risk tumours), but pathologists are encouraged to record and enter in their reports a percentage of different components (regressive changes, blastemal, epithelial and stromal) as these features will be analysed prospectively in order to identify those that might have further prognostic significance. (Cystic partially differentiated nephroblastoma should be diagnosed on imaging studies and treated with surgery alone).



5.4 STUDY OF THE NEPHRECTOMY SPECIMENS

The **intact** surgical specimen should be presented to the pathologist **without being opened by the surgeon**. A report of the operation (form 3A) with sufficient information necessary for correct staging should be made available to the pathologist.

Handling the fresh specimen, step by step:

1) **Weigh, measure** and **photograph** the whole specimen. Look carefully for ruptures and fissures and locate any suspicious areas and/or ink it in different colours from the rest of the specimen. Decapsulation makes determination of growth beyond the capsule impossible and therefore should not be done.

2) Look for and dissect the peri-renal and perihilar **lymph nodes**. Block these separately recording the site. (These are rare).

3) **Identify renal vein, artery and ureter** and take transverse section block of each near the resection margin.

4) **Ink** the surface of the whole specimen (or at least areas in which excision margins are dubious) and renal sinus with Indian ink and let it dry **before** opening the specimen. This is a critical step and should always be done **as** otherwise it might be impossible to stage the tumour correctly and give adequate therapy.

5) **Open** by a longitudinal incision to bivalve the **specimen** and reveal the tumour and its relation to the kidney, capsule, and renal sinus.

6) **Photograph** the cut surface, record macroscopic appearance. **Measure** the size of the tumour. It is crucial to **assess the percentage of a necrotic tumour** (this percentage has to be filled in on the Form F4) and also to describe and photograph the multicystic cut surface, if present.

7) **samples required** for biology studies:

- **Tumour:** At least two pieces (0.5 - 1 cm³ each) of morphologically different parts of the tumour should be sampled and snap frozen in liquid nitrogen or at -70°C (Freeze more aliquots if available). If a biopsy is performed prior to commencing pre-operative chemotherapy, then a sample of this should also be frozen, if adequate tissue is available.
- A '**mirror**' **sample** of tumour adjacent to the frozen sample should be fixed in formalin and studied for histology. Please submit this wax block along with the frozen tissue, when requested.
- **Adjacent normal kidney:** two pieces (0.5 - 1 cm³) snap frozen in liquid nitrogen or at -70°C.
- If present, **nephrogenic rests** should be sampled as **above**.
- 10 ml peripheral blood in EDTA (if national procedure for storage available).
- Samples should be stored at -70°C or under liquid nitrogen until transported to the appropriate national research laboratory on dry ice.

The time interval between removal of the tumour and the freezing of the samples should be as short as possible and certainly not exceed a period of 30 - 60 minutes. (See also section 13: 13.5).

8) The specimen should be **fixed** in 4% buffered formalin for 24 to 48 hours, according to the usual procedure of the laboratory. Several additional cuts can be made parallel to the initial cut to divide the specimen into "slabs" for better fixation. *{Alternatively, instead of parallel longitudinal sections, you may find that making horizontal sections and sampling the tumour in this way will give a better view of the renal sinus and a tumour-sinus relationship.}*

9) The samples for histological examination should include:

- a) the macroscopically different areas of the tumour (it is advised to take at least one block per cm of the largest diameter of the tumour, not forgetting to take blocks from grossly necrotic areas, too); mostly from the periphery rather than from the central areas of the tumour;
- a) dubious areas have to be marked by the surgeon and need special attention of the pathologist (they have to be marked with Indian ink or methylen blue);
- b) sinus lymph nodes when present;
- c) other lymph nodes.
- d) renal pelvis and pelvic fat, ureter and sinus vessels; especially the renal vein should be inspected for evidence of tumour thrombus; if present, it is critical to assess whether it is completely resected;
- e) each nodule away from the main mass (in multifocal tumours);
- f) tumour-kidney interface
- g) tumour-kidney capsule
- h) areas of the capsule that are suspected of being invaded by the tumour;
- i) areas of perirenal fat suspected for tumour infiltration (important for assessment whether the tumour is completely resected);
- j) areas of adhesions of the tumour to surrounding tissues;
- k) at least 2 blocks of the normal kidney and blocks from abnormal looking areas in the remaining renal tissue.

All the samples should be numbered and their sites recorded as well as all other samples taken at the time of operation, i.e. adrenals, lymph nodes and various biopsies. **Please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or a photograph.**

5.5 STAGING

Stage is one of the most important therapeutic and prognostic criteria for renal tumours. It has been shown in all multicentre trials that accuracy of staging still represents a major problem. This is partly because of the fact that renal tumours are usually very large at nephrectomy and often it is very difficult to assess their relationship with normal renal anatomical structures such as the renal capsule and the renal sinus. It is absolutely critical to take blocks from all sites that are important for staging and to carefully document the site from which each block is taken (**please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or, preferably, a photograph and mark the sites from which blocks have been taken**).

Please remember that local (abdominal) staging of primary tumour is done following pre-nephrectomy chemotherapy and it is very important even in stage IV cases. The presence/absence of metastases is evaluated at presentation, on the basis of imaging studies.

Criteria for staging:

Stage I

- a) The tumour is limited to kidney or surrounded with a fibrous pseudocapsule if outside of the normal contours of the kidney. The renal capsule or pseudocapsule may be infiltrated with the tumour but it does not reach the outer surface, and it is completely resected (resection margins 'clear')
- b) The tumour may be protruding ('bulging') into the pelvic system and 'dipping' into the ureter (but it is not infiltrating their walls)

- c) The vessels of the renal sinus are not involved
- d) Intrarenal vessel involvement may be present

Fine needle aspiration or percutaneous core needle biopsy ('tru-cut') does not upstage the tumour but the size of the needle gauge should be mentioned to the pathologist.

The presence of necrotic tumour or chemotherapy-induced change in the renal sinus and/or within the perirenal fat should not be regarded as a reason for upstaging a tumour providing it is completely excised and does not reach the resection margins.

Stage II

- a) The tumour extends beyond kidney or penetrates through the renal capsule and/or fibrous pseudocapsule into peri-renal fat but is completely resected (resection margins 'clear')
- b) Tumour infiltrates the renal sinus and/or invades blood and lymphatic vessels outside the renal parenchyma but it is completely resected
- c) Tumour infiltrates adjacent organs or vena cava but is completely resected

Stage III

- a) Incomplete excision of the tumour which extends beyond resection margins (gross or microscopical tumour remains post-operatively)
- b) Any abdominal lymph nodes are involved
- c) Tumour rupture before or intra-operatively (irrespective of other criteria for staging)
- d) The tumour has penetrated through the peritoneal surface
- e) Tumour implants are found on the peritoneal surface
- f) The tumour thrombi present at resection margins of vessels or ureter, transected or removed piecemeal by surgeon
- g) The tumour has been surgically biopsied (wedge biopsy) prior to pre-operative chemotherapy or surgery.

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 microscopic residue and therefore the tumour is assigned stage III (because a possibility that some viable tumour is left behind in the adjacent lymph node or beyond resection margins.)

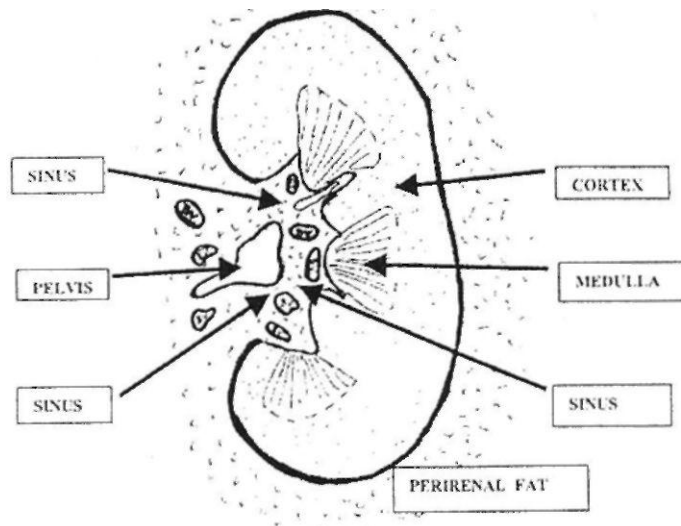
Stage IV

Haematogeneous metastases (lung, liver, bone, brain, etc.) or lymph node metastases outside the abdomino-pelvic region.

Stage V

Bilateral renal tumours at diagnosis. Each side should be substaged according to above classifications.

If in any doubt about a tumour's stage or for its confirmation, please send it for urgent review to the referring pathologist.



HISTOLOGICAL DESCRIPTION OF THE SUBTYPES OF NEPHROBLASTOMA AND OTHER RENAL TUMOURS OF CHILDHOOD

5.6 LOW RISK TUMOURS

MESOBLASTIC NEPHROMA

Mesoblastic nephroma is a renal tumour that usually occurs in the first year of life. The oldest child with confirmed mesoblastic nephroma in the National Wilms' Tumor Study (NWTs) files was diagnosed at age of 29 months. Cases of 'mesoblastic nephromas' in older children have been shown to be Metanephric Stromal Tumours - a recently described new entity . However, for both entities treatment is surgery and prognosis is excellent, so the distinction between them has no important therapeutic implications.

There are two histological subtypes of mesoblastic nephroma: the classical and the cellular type. The distinction between the two types has no implication for therapy so far. Classical mesoblastic nephroma is a monomorphous tumour composed of spindle cells with large, vesicular nuclei, noticeable nucleoli and abundant cytoplasm. The cells are arranged in interlacing bundles and mitotic figures are usually present. The tumour-kidney border is irregular and long radial extensions (finger-like extensions) of tumour tissue into the adjacent renal tissue are a characteristic finding. Also, within the tumour small rests of connective tissue with entrapped tubules are usually seen. Cellular mesoblastic nephroma has a sharper, pushing tumour-kidney border, increased cellularity and numerous mitoses. Both types show infiltrative growth and may infiltrate the adjacent perirenal fat and spread into the renal sinus. Complete, wide surgical resection is the only recommended treatment for localized disease. Local recurrences and metastases have been described in a few cases, especially in children older than six months of age, although some children were < 1 month old at diagnosis. The vast majority of relapses occur within 12 months of nephrectomy and in about 70% of relapsed cases the tumour is of the cellular type.

In the differential diagnosis, metanephric stromal tumour, blastemal and stromal nephroblastoma, clear cell sarcoma and rhabdoid tumour of kidney must be considered (in difficult cases, please consult excellent tables in 3rd series of AFIP Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994).

Recently, cytogenetic abnormalities of chromosome 11 and a translocation involving chromosome 15 have been reported in cellular mesoblastic nephroma. The finding of ETV6-NTRK3 gene fusions and trisomy 11 has established a histogenetic link between cellular mesoblastic nephroma and congenital fibrosarcoma.

CYSTIC PARTIALLY DIFFERENTIATED NEPHROBLASTOMA (CPDN)

CPDN is a distinct variant of nephroblastoma that usually occurs in children less than 2 years of age. The histological criteria for making a diagnosis of CPDN are as follows:

- a) it is composed entirely of cysts and their thin septa;
- b) the thin septa are the only 'solid' portion of the tumour;
- c) the tumour forms a discrete mass, well demarcated from the non-cystic renal parenchyma;
- d) the cysts are lined by flattened, cuboidal or hobnail epithelium; and
- e) the septa contain blastemal cells in any amount, with or without other embryonal stromal or epithelial cell types.

Thus, variable differentiated glomeruli, tubules, mesenchyme, striated muscle, cartilage, fibrous tissue, and fat may be admixed with blastemal cells in septa. The presence of well-differentiated tubules only is not enough to make a diagnosis of this tumour and separate it from cystic nephroma. However, from a therapeutic and prognostic point of view there is no need to distinguish between CPDN and cystic nephroma as they are both treated with surgery alone and both share the same, excellent prognosis. However, intermediate risk nephroblastomas may present with numerous cysts but they also contain solid areas and septa are usually thicker and show chemotherapy-induced changes. Be aware that other renal tumours, such as clear cell sarcoma and rhabdoid tumour, may have a predominantly cystic appearance.

COMPLETELY NECROTIC NEPHROBLASTOMA

Pre-operative chemotherapy given in SIOP trial patients results in so-called 'chemotherapy-induced change' in many nephroblastomas. Depending on their initial histological pattern, some nephroblastomas are completely or almost completely necrotic, while others show less marked or minimal/moderate changes. The relationship between the percentage of these chemotherapy-induced changes and prognosis has been shown in other tumours such as osteosarcoma as well as in a recent SIOP study on nephroblastoma in which completely necrotic nephroblastomas had an excellent prognosis with 100% survival in all stages.

The histological criteria for making a diagnosis of completely necrotic nephroblastoma are:

- a) the absence of any viable tumour tissue on gross and microscopic examination of multiple blocks taken from different areas of a tumour, according to the recommended protocol (ie at least one block per cm of tumour); the presence of scattered mature tubules is allowed as they

may represent remnants of nephrogenic rests.

b) the presence of regressive and/or necrotic changes caused by chemotherapy.

Although complete tumour necrosis makes histological sub-typing of nephroblastoma impossible, 'ghost' tumour structures (mainly blastema, occasionally epithelial elements) can be recognised, and are helpful in distinguishing nephroblastoma from other renal tumours. In addition, the presence of nephrogenic rests, which are virtually never associated with non-Wilms tumour and are generally not affected by chemotherapy, is a very reliable clue that the tumour has been a nephroblastoma before chemotherapy. Finally, it is well known that regression of other renal tumors such as clear cell sarcoma, rhabdoid tumor or renal cell carcinoma, is minimal to moderate under the actinomycin D - vincristine protocol, and their histological features can be easily recognised even in treated cases.

The typical histological appearance of treated nephroblastoma is a mixture of necrosis, fibromyxomatous stroma containing lipid- and/or haemosiderin-laden macrophages, and haemorrhage. In some cases scattered mature tubules may be seen within necrotic areas - this may represent remnants of pre-existing rests and should not be regarded as viable tumour tissue. The main pattern of the necrotic area is coagulative-type necrosis of small round cells or tubules, with the majority of 'ghost' structures consisting of large sheets of small, pink, necrotic nuclei, consistent with coagulative necrosis of blastemal cells or tubules. (*If in doubt whether the necrotic tumour is a nephroblastoma, the reticulin staining may help to identify scarce epithelial or mesenchymal 'ghost' structures.*) The presence of identical changes in a lymph node is regarded as a proof of its involvement with a tumour and it is, therefore, very important to sample and microscopically examine all lymph nodes removed. Beware of Tamm Horsfall protein which is sometimes accompanied by discrete epithelium in a lymph node - this must not be interpreted as a metastasis (for other lesions and changes which may mimic lymph node metastases, see paper by Weeks et al. 1990).

5.7 INTERMEDIATE RISK TUMOURS

Beckwith and Palmer's criteria for histological subtyping of nephroblastomas state that one component has to comprise at least 2/3 (66%) of a tumour mass for the tumour to be subclassified accordingly. However, pre-operative chemotherapy alters the original histological features of nephroblastomas and often results in areas of necrosis and regression. Therefore the criteria applicable to subclassification of primarily operated tumours have to be modified to take these changes into account. The reason that only viable tumour is taken into account when subclassifying nephroblastomas which are not completely necrotic is based on previous studies which have shown that chemotherapy-induced changes are a prognostically favourable effect of treatment (Zuppan et al, 1991; Boccon-Gibod et al., 2000). On the other hand, the presence of blastema after pre-operative chemotherapy clearly indicates its non-responsiveness to chemotherapy and has been shown to be associated with poorer outcome (Weirich et al., 2001). For all these reasons, we believe modification of the criteria for certain subtypes of nephroblastoma is justified. We are aware that the assessment of percentage of necrosis/regression is subjective, but since it is very important for subclassification of nephroblastomas, it should be done on both gross and histological examination.

Histological types of nephroblastoma from this group are described below, but a simple approach can be the following (please, also see a diagram in section 5.3):

1) Assess the percentage of necrosis/regressive changes caused by chemotherapy

- 2) If regressive changes comprise more than 2/3 of a tumour mass - it is a regressive type
- 3) If regressive changes comprise less than 2/3 of a tumour mass - look for a predominant histological component and subclassify a tumour accordingly (blastemal, epithelial or stromal type). If no component is predominant, it is a mixed type.
- 4) Even if focal anaplasia is found, try to subclassify the tumour as below.

In the group of intermediate risk tumours, five subtypes of nephroblastoma have been recognised as follows;

NEPHROBLASTOMA- EPITHELIAL TYPE

The histological criteria for making a diagnosis of epithelial type nephroblastoma are as follows:

- a) only the viable part of a tumour is assessed and it has to comprise more than 1/3 of a tumour mass;
- b) at least 2/3 of the viable tumour consists of epithelial structures
- c) the stromal component may comprise the rest of the viable tumour; and
- d) scattered small foci of blastema comprising less than 10% of the tumour may occur (*the finding of larger nodules of blastema comprising about 10% of the viable tumour mass is not acceptable and such tumours should be subclassified as mixed subtype*).

The epithelial elements are regarded as follows:

- a) *tubules* - spaces lined by columnar epithelial cells arranged in a fairly regular manner radially around the central space; celi margins are sharp, they have basal, crowded nuclei, and mitotic activity may be marked; tubules are usually back-to-back, with virtually no supporting stroma;
- b) *rosettes* - circular arranged tumour cells with elongated ovoid nuclei, but no central lumen is present;
- c) *papillary structures* - finger-like projections of a stroma covered with epithelial cells;
- d) *glomerular structures* - tuft-like masses of malignant cells surrounded by a well-formed capsule or rather flattened tumour cells.

The stromal elements are regarded as follows: undifferentiated stromal cells, myxoid, fibroblastic, smooth muscle, skeletal muscle, adipose cells, cartilage and osteoid formations.

The presence of genuine anaplasia classifies the tumour as anaplastic nephroblastoma even if otherwise completely epithelial (see 5.8 for criteria anaplasia). Epithelial nephroblastoma usually occurs in younger children (median age 9 months in a SIOP series), and about 80% of cases are in stage I. Beware of epithelial nephroblastoma in older children and look carefully for anaplasia.

NEPHROBLASTOMA- STROMAL TYPE

Stromal nephroblastoma represents a subtype in which the stromal elements are a predominant component of the tumour. The fetal rhabdomyomatous nephroblastoma, which in the past was regarded as a nephroblastoma with better prognosis, is also included here.

The **histological criteria for making a diagnosis of stromal type nephroblastoma** are as follows:

- a) only the viable part of a tumour is assessed and it has to comprise more than 1/3 of a tumour mass;
- b) at least 2/3 of the viable tumour consists of stromal elements;
- c) the epithelial component may comprise the rest of the viable tumour; and
- d) scattered small foci of blastema comprising less than 10% of the tumour may occur *{the finding of larger nodules of blastema comprising about 10% of the viable tumour mass is not acceptable and such tumours should be subclassified as mixed subtype}*.

The stromal elements are regarded as follows: undifferentiated, myxoid, fibroblastic, smooth muscle, skeletal muscle, adipose cells, cartilage, bone, and osteoid. Stromal differentiation may be induced by preoperative chemotherapy as a stromal type nephroblastoma is far more common in children who have received pre-operative chemotherapy. It is likely that other tumour components, especially blastema, are destroyed by pre-operative chemotherapy while stromal elements are chemotherapy resistant and may even further differentiate resulting in prominent skeletal muscle component, for example.

Stromal nephroblastoma usually occurs in younger children and usually shows minimal to moderate chemotherapy-induced changes since stromal tissue seems to be resistant to chemotherapy. Fetal rhabdomyomatous nephroblastoma is bilateral in 30% of cases.

NEPHROBLASTOMA - MIXED TYPE

Mixed type nephroblastoma represents a subtype in which none of the viable component is predominant.

The **histological criteria for making a diagnosis of mixed type nephroblastoma** are as follows:

- a) only the viable part of a tumour is assessed and it has to comprise more than 1/3 of a tumour mass;
- b) the viable tumour consists of blastemal and/or epithelial and/or stromal elements but none of them comprise more than 2/3 of the viable tumour.

As for the other subtypes, please try to assess the percentage of different (viable) tumour component as well as the percentage of necrosis/regression.

NEPHROBLASTOMA - REGRESSIVE TYPE

Nephroblastoma - regressive type is regarded as a tumour in which chemotherapy-induced changes comprise more than 2/3 of the tumour mass. Please note that assessment of percentage of necrosis/regression is done on both gross and histological examination, so blocks should be taken not only from viable parts of the tumour mass but also from those that show necrotic/regressive changes.

The histological criteria for making a diagnosis of regressive type nephroblastoma are:

- a) the presence of more than 2/3 of non-viable tumour tissue (regressive and/or necrotic changes caused by chemotherapy) on gross and microscopical examination of multiple blocks taken from different areas of a tumour, according to the recommended protocol (see 5.4).
- b) the viable tumour elements are histological components of nephroblastoma including blastemal, epithelial and stromal elements.

The typical histological appearance of treated nephroblastoma is a mixture of necrosis, fibro-myxo-sclerotic stroma containing lipid- and/or haemosiderin-laden macrophages, and haemorrhage. The main pattern of the necrotic area is coagulative-type necrosis of small round cells, with the majority of 'ghost' structures consisting of large sheets of small, pink, necrotic nuclei, consistent with coagulative necrosis of blastemal cells.

NEPHROBLASTOMA WITH FOCAL ANAPLASIA

Nephroblastoma with focal anaplasia has been moved into the Intermediate risk group since both NWTSS and SIOP studies have shown that it has the same prognosis as non-anaplastic nephroblastomas (other than blastemal type, in the SIOP trials). Diagnostic criteria for focal anaplasia have been described with diffuse anaplasia (see below).

5.8 HIGH RISK TUMOURS

NEPHROBLASTOMA - BLASTEMAL TYPE

This nephroblastoma type has been moved into the high risk tumours but only if diagnosed after pre-operative chemotherapy. The reason for this change is based on the results of previous SIOP trials showing that tumours with chemotherapy-resistant blastema had a worse prognosis and require more intensive treatment. In cases diagnosed after primary nephrectomy, blastemal nephroblastoma remains in the Intermediate risk tumours.

The histological criteria for making a diagnosis of blastemal type nephroblastoma are as follows:

- a) only the viable part of a tumour is assessed and it has to comprise more than 1/3 of the tumour mass;
- b) at least 2/3 of the viable tumour consists of blastema
- c) other components of nephroblastoma may be present in varying proportions.

The blastemal elements are regarded as undifferentiated round or elongated cells which are usually closely packed and show no evidence of epithelial and/or stromal differentiation. There are several distinctive patterns in which blastemal cells may occur and it is not uncommon to find more than one pattern in the same tumour. They include the diffuse, serpentine, nodular, and basaloid patterns but they are of no prognostic or therapeutic significance (for detailed criteria for different blastemal patterns, please see 3rd series of AFIP

Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994).

NEPHROBLASTOMA WITH ANAPLASIA

Anaplasia was recognised as an unfavourable histological feature of nephroblastoma in earlier trials. **The histological criteria for making a diagnosis of anaplastic nephroblastoma are the presence of all three criteria for anaplasia** including:

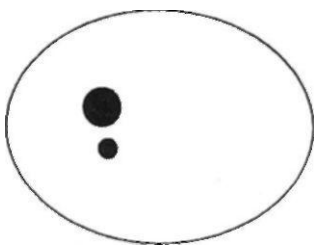
- a) the presence of atypical tri/multipolar mitotic figures;
- b) marked nuclear enlargement, with diameters at least three times those of adjacent cells; and
- c) the presence of hyperchromatic tumour cell nuclei.

Anaplasia may occur in the blastemal, epithelial or stromal component of nephroblastoma and it can be focal or diffuse. The recent (topographic) definition of focal anaplasia emphasizes the distribution of anaplasia which has to be sharply demarcated within the primary tumour. This proved to be of prognostic significance in both primarily operated and prenephrectomy treated cases.

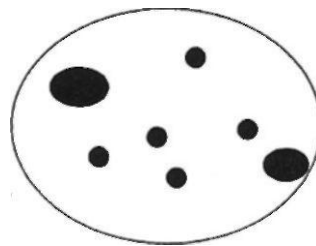
Focal anaplasia has now been defined as the presence of a clearly defined focus within a primary intrarenal tumour, without evidence of anaplasia or prominent nuclear atypia in extrarenal tumour sites.

Diffuse anaplasia is defined if any of the following are present:

- 1) non-localised anaplasia, and/or anaplasia beyond the original tumour capsule;
- 2) anaplastic cells present in intrarenal or extrarenal vessels, renal sinus, extracapsular invasive sites, or metastatic deposits;
- 3) anaplasia is focal, but nuclear atypia approaching the criteria for anaplasia (so-called 'unrest nuclear change') is present elsewhere in the tumour;
- 4) anaplasia that is not clearly demarcated from non-anaplastic tumour; and
- 5) anaplasia is present in a biopsy or other incomplete tumour sample.



Focal anaplasia



Diffuse anaplasia

This topographic definition of focal anaplasia makes it mandatory that pathologists carefully document the exact site from which every section is obtained (e.g. on a diagram, specimen photocopy, and/or photograph of the gross specimen). Please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or a photograph.

Anaplasia occurs in about 5% of patients with nephroblastoma. Preoperative chemotherapy does not obliterate or produce anaplasia but it makes its recognition easier since non-anaplastic areas are destroyed by chemotherapy while anaplastic foci remain unchanged. This

provides further support to the hypothesis that anaplasia represents a more resistant rather than a more aggressive cell line. The age distribution of anaplastic nephroblastoma differs from non-anaplastic nephroblastoma: anaplasia never occurs in the first six months of life, it is very rare between 6-12 months (1-2%), median age at diagnosis is 61 months and >50% of children are over five years of age (for non-anaplastic nephroblastoma median age is 45 months, and 25% of children are over five years of age).

Although the criteria for anaplasia have been well established, it still represents a diagnostic problem resulting in either missed or 'overdiagnosed' cases, while only in rare instances it is confused with other renal tumours. It is important to bear in mind that all three criteria for the diagnosis of anaplasia have to be met and that some histological changes may mimic anaplasia including calcification, fused or smudged masses of nuclear chromatin due to technical artefact, stain precipitate, circulating megakaryocytes, overlapping cells in thick sections, and bizarre nuclei resulting from chemotherapy with the formation of hyperchromatic multinucleated and bizarre macronucleated skeletal muscle cells in response to injury. However, the diagnosis of anaplasia in the skeletal muscle must be made if atypical mitoses and other histological criteria are present.

CLEAR CELL SARCOMA OF THE KIDNEY (CCSK)

This distinctive tumour comprises 5% of primary renal tumours of childhood. It is extremely rare in the first six months of life and in young adults, and the majority of patients are between 2 and 3 years of age. There is a male predominance, but no association with chromosomal defects, genetic abnormalities or specific malformations and syndromes has been reported. Unlike nephroblastoma, CCSK is always unilateral and unicentric.

Histologically, this tumour has a deceptively bland appearance and many histological subtypes. The classical pattern has a uniform appearance of a diffuse growth of relatively small cells with normochromatic nuclei, inconspicuous nucleoli, pale staining cytoplasm, and ill-defined cell membranes. In only 20% of the cases do the tumour cells have clear cytoplasm. The most characteristic feature is a peculiar vascular pattern consisting of arborising blood vessels that create an alveolar or trabecular pattern (best seen with the reticulin stain).

The classical pattern of CCSK is relatively simple to diagnose, but others including the myxoid, sclerosing, cellular, epithelioid, psammomatous, spindle cell, storiform, and anaplastic pattern can cause problems in reaching the diagnosis. In some CCSKs, there can be extensive hyalinisation and these tumours may be confused with cases of nephroblastoma with sclerosis due to pre-operative treatment, or rhabdoid tumour. In the differential diagnosis blastemal nephroblastoma, mesoblastic nephroma, PNET and rhabdoid tumour must be considered (in difficult cases, please consult excellent tables in 3rd series of AFIP Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994, and the paper by Argani et. al., Am J Surg Pathol 2000).

The histogenesis of the tumour is uncertain. The tumour cells are only positive for vimentin and are generally negative for cytokeratin, factor VIII associated antigen, epithelial membrane antigen, desmin, and S100 protein.

RHABDOID TUMOUR OF THE KIDNEY

Rhabdoid tumour of kidney (RTK) is rare, constituting 2% of paediatric renal tumours. It typically occurs in early childhood, with about 80% of patients younger than 2 years, while it is extremely rare after 5 years of age. Two characteristic associations of RTK are hypercalcaemia and the development of synchronous or metachronous primary brain tumours. On the other hand, it is never associated with conditions predisposing to nephroblastoma or with nephrogenic rests.

Histological criteria for diagnosis of rhabdoid tumour include the finding of its characteristic histological features and unique immunohistochemical profile. Typical histological features comprise non-cohesive sheets of cells with abundant eosinophilic cytoplasm and *large eccentric nuclei with prominent eosinophilic central nucleoli* - these are regarded as the most characteristic feature of the tumour and they are always present at least in some areas of the tumour. Another characteristic feature is the presence of *large oval intracytoplasmic hyaline inclusions* composed of whorled masses of intermediate filaments. Both of these features may only be focal, and should be specifically looked for in any undifferentiated renal tumour of childhood. In addition to the classical pattern of rhabdoid tumour, many other patterns have been described including sclerosing, clear cell sarcoma-like, epithelioid, spindled, lymphomatoid, vascular, pseudopapillary and cystic patterns.

Immunohistochemistry shows consistent positivity of tumour cells for vimentin with frequent co-expression of cytokeratin, while many other markers including epithelial membrane antigen, S-100 protein, neurofilaments, neuron-specific enolase, desmin, myoglobin, alpha-1-antichymotrypsin have been reported but are not found consistently. CD99 (Mic-2) positive staining may be seen too. In some cases abnormalities of chromosome 22 and 11p13 have been described.

5.9 NEPHROGENIC RESTS

Nephrogenic rests are foci of embryonal cells which persist after 36 weeks of gestation and they are considered as potential precursors of nephroblastoma. They have been found not only in 25-40% of patients with nephroblastoma but also in 1% of routinely examined perinatal postmortem kidneys. However, they have not been described associated with other typical renal tumours of childhood and their finding in problematic cases should be regarded as a very useful clue that the tumour is nephroblastoma. Two main types of nephrogenic rests have been recognised: perilobar and intralobar rests. They can be further subclassified as dormant, sclerosing, or hyperplastic, and all these appearances may be present in an individual case.

The rests may regress to fibrous tissue or progress to nephroblastoma. Hyperplastic rests may be difficult to distinguish from a small nephroblastoma but it is usually of no therapeutic significance since both hyperplastic rests and nephroblastoma should be treated (see appendix 5). Perilobar rests occur in hemihypertrophy and Beckwith-Wiedemann syndrome while intralobar rests are associated with WAGR and Denys-Drash syndromes.

5.10 DIFFERENTIAL DIAGNOSIS OF RENAL TUMOURS OF CHILDHOOD

The results of the SIOP 9 and SIOP 93 01 trials showed that there were a number of cases of both low and high risk tumours that were misdiagnosed, including cystic partially differentiated nephroblastoma, highly differentiated epithelial type nephroblastoma, anaplastic nephroblastoma, clear cell sarcoma and rhabdoid tumour of the kidney. Since many of them were seen by the Panel retrospectively, this resulted in either over-treatment (for low risk tumours) or under-treatment (for high risk tumours). As groups of low and high risk tumours have changed in this Trial, it has become even more important to reach a correct diagnosis before any post-operative treatment is administered.

There are some clinical, macroscopical and histological features of renal tumours of childhood which might be a useful clue in reaching a correct diagnosis.

Age at diagnosis is a rather reliable criterion. Anaplastic nephroblastoma has never been described in the first six months and is extremely rare in the first year of life, but after 5 years of age it comprises 10% of nephroblastomas. Clear cell sarcoma of kidney hardly occurs in the first 6 months of life, while mesoblastic nephroma and rhabdoid tumour of kidney are extremely rare in children over 3 years of age.

Grossly, many renal tumours may show areas with **cysts** but only CPDN and cystic nephroma **are** entirely cystic neoplasms, with no solid areas.

There are some unigue features of nephroblastoma which are very useful in distinguishing it from other renal tumours including:

- a) nephroblastoma **is** the only typical renal tumour of childhood which may **be bilateral** (in 5% of cases) or **multifocal**;
- b) **nephrogenic rests** are commonly present in nephroblastoma but not in other tumours (there is only one report of nephrogenic rests associated with mesoblastic nephroma and CCSK, respectively)
- c) the presence of **skeletal muscle, adipose tissue** and genuine **neoplastic tubules** has only been seen in nephroblastoma (although fat may be present in metanephric stromal tumours, other features should be sufficient to make a correct diagnosis).
- d) nephroblastoma has been diagnosed in a child with a **syndrome predisposing to nephroblastoma** (WAGR, Beckwith-Wiedemann, Denys-Drash syndrome) while mesoblastic nephroma **is** the only other renal tumour that has occasionally **been** described with Beckwith-Wiedemann syndrome.

When in doubt about either the histological type or the stage, please immediately send a full set of histological sections to the national reference pathologist (see 5.11).

Other tumours included in the study:

In addition to the more common renal tumours of childhood discussed above, there are numerous other tumours which may occur at any age. Although these tumours are not entered

in the Trial, they should be registered and submitted as they may provide important information in our understanding of renal tumours in general.

These include:

1. Metanephric tumours
(metanephric stromal tumour, metanephric adenofibroma, metanephric adenoma)
2. Adenomas (all other types)
3. Cystic nephroma
4. Renal cell carcinoma (all variants)
5. Transitional cell carcinoma
6. Neuroepithelial tumours (renal neuroblastoma, renal PNET, renal carcinoid)
7. Miscellaneous sarcomas (without evidence of blastemic cells and/or epithelial component in five different blocks)
8. Renal lymphoma
9. Angiomyolipoma
10. Other 'tumours (adrenal tumours, teratoma) and lesions (xanthogranulomatous pyelonephritis, etc), if preoperative chemotherapy for nephroblastoma has been given
11. Metastases from other sites

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REFERENCES

General references

Beckwith JB.

Renal neoplasms of childhood.

In: Stenberg S ed. Diagnostic Surgical Pathology. 2nd ed, Vol 2. New York, Raven Press Ltd, 1994,p 1741-66

Beckwith JB, Palmer NF.

Histopathology and prognosis of Wilms' tumor. Results from the first National Wilms' Tumor Study.

Cancer 1978; 41: 1937-1948

Schmidt D, Harms D, Leuschner I.

Malignant renal tumors of childhood.

PathResPract 1992; 188: 1-15

Toumade MF, Com-Nougue C, Youte PA, et al.

Results of the Sixth International Society of Pediatric Oncology Wilms' Tumor Trial and Study: A Risk-Adapted Therapeutic Approach in Wilms' Tumor.

J Clin Oncol 1993; 11: 1014-1023

Pritchard J, Imerson J, Bames J et al.

Results of the United Kingdom Children's Cancer Group first Wilms' tumour study.

J Clin Oncol 1995; 13: 124-133

Mitchell C, Morris Jones P, Kelsey A, et al.

The treatment of Wilms' tumour: results of the United Kingdom Children's Cancer Study Group (UKCCSG) second Wilms' tumour study.

Br J Cancer 2000; 83: 602-608

Delemarre JFM, Sandstedt B, Gerard-Marchant R, Toumade MF.

SIOP Nephroblastoma trials and studies, morphological aspects.

Excerpta Medica, Amsterdam, 1982; 261-272

Yujanic GM, Delemarre JFM, Sandstedt B, Harms D, Boccon-Gibod L.

The New SIOP (Stockholm) Working Classification of Renal Tumours of Childhood.

Med Pediatr Oncol 1996; 26: 145-146 (1996; 27: 68)

Zuppan CW.

Handling and evaluation of pediatric renal tumors.

Am J Clin Pathol 1998; 109 (Suppl 1): 531-537

Weeks DA, Beckwith JB, Mierau GW.

Benign nodal lesions mimicking metastases from pediatric renal neoplasms: A report of the National Wilms' Tumor Study Pathology Center.

Hum Pathol 1990; 21: 1239-1244