

EDITOR'S
CHOICE

The cytological diagnosis of paediatric renal tumours

T Shet, S Viswanathan

Department of Pathology, Tata Memorial Hospital, Parel, Mumbai, India

Correspondence to:
Dr T Shet, 309/31,
Prabhudharshan, S.S. Nagar,
Amboli, Andheri (W), Mumbai
400058, India; tanujshet@
yahoo.com; tanujshet5@gmail.
com

Accepted 30 June 2009
Published Online First
20 August 2009

ABSTRACT

Fine needle aspiration cytology (FNAC) is used for preoperative diagnosis of paediatric renal tumours, especially in centres where preoperative chemotherapy is advocated in Wilms' tumour. This review focuses on salient cytological features in specific paediatric renal tumours, the approach to resolving a differential diagnosis and the role of ancillary methods in diagnosis of paediatric renal tumours. Crucial differential diagnoses include distinguishing: Wilms' tumour from benign tumours in the kidney like multicystic nephroma or congenital mesoblastic nephroma; aggressive non-Wilms' tumours of kidney like rhabdoid tumour of kidney; and Wilms' tumour from other paediatric round cell sarcomas like neuroblastoma, non-Hodgkin lymphoma etc. An approach based on classifying smears according to their cellular patterns as triphasic, round cell, spindle cell or epithelioid cell type assists in classifying paediatric renal tumours on cytology. Immunocytochemistry for WT1, cytokeratin, synaptophysin, leucocyte common antigen and MIC2 will aid in evaluating round cell tumours in the renal region, while WT1, bcl2, vimentin and desmin will be useful for spindle cell tumours in that region. Extra material can also be evaluated for demonstration of specific cytogenetic abnormalities in these tumours. A checklist of common tumours in a particular age group, relevant clinical information, awareness of distinctive and overlapping cytological features, and appropriate use of immunocytochemistry with cytogenetics go a long way in ensuring an accurate cytological diagnosis. Used judiciously, FNAC is as effective a tool as a core biopsy for preoperative diagnosis of paediatric renal tumours, and with experience a 92% accuracy rate can be achieved.

Fine needle aspiration cytology (FNAC) in paediatric renal masses is performed for diagnosis of tumours in patients with advanced disease before instituting preoperative chemotherapy and for documentation of contralateral disease/metastases/recurrence.¹

There is a controversy regarding the role of FNAC in the diagnosis of operable paediatric renal tumours. Both the SIOP (International Society of Paediatric Oncology) and NWTS (National Wilms' Tumour Study Group) state that a core biopsy in a paediatric renal tumour increases the risk of flank relapse; hence tumours biopsied are upstaged to stage III.² For operable renal tumours the NWTS approach relies on primary nephrectomy with further therapy based on histological evaluation of specimen, while the SIOP protocol advocates a radiological or FNAC diagnosis permitting preoperative chemotherapy first, followed by surgery, and further therapy based on stage and histology.^{2,3} Recently however the UKCCSG (United Kingdom Children Cancer Study Group) confirmed that a percutaneous needle biopsy offered an

accurate preoperative diagnosis in 85% of Wilms' tumours with minimal risk for complications and local recurrence or upstaging.⁴

Although paediatric renal tumours have well defined radiology, 5–10% of tumours radiologically diagnosed as Wilms' tumour proved to be benign lesions⁴; hence FNAC is used for diagnosis in centres following the SIOP protocol. FNAC is inherently a simple technique as compared to a core biopsy which requires more expertise. The accuracy of diagnosis in various studies on paediatric renal tumours is difficult to extrapolate, however in a prior study at our institute an awareness of cytomorphology of the various paediatric renal tumours improved the accuracy rate of diagnosis from 65% to 92%.⁵

STEPS IN THE CYTOLOGICAL EVALUATION OF PAEDIATRIC RENAL TUMOURS

Preliminary considerations

- ▶ *Clinical information.* A cytopathologist evaluating an aspirate from a renal mass or abdominal mass in a child should always confirm the relevant clinical and radiological information before making a diagnosis. Though exceptions occur, most paediatric renal tumours conform to the outlines of age given in table 1.
- ▶ All aspirations should preferably be performed under radiological guidance (most commonly ultrasound guided) for appropriate sampling. At our institute a long spinal needle (22 or 23 gauge) is usually used for guided aspiration without any untoward event.
- ▶ *Technical considerations.* Aspirations should be performed according to the practice for guided aspirations in a given institution. All aspirations should undergo an on-site evaluation for adequacy. In our department after brief fixation, we stain the smears with 1% toluidine blue stain as this stain gives excellent nuclear details. Some centres prefer a Diff Quick stain for the same purpose. On-site evaluation also assists in collecting material for ancillary studies. If there is sufficient material it should be collected for cell block preparation to bring out the architectural details in haemorrhagic aspirates. The nuclear chromatin in malignant round cell tumours is best appreciated in a Papanicolaou stained smear. Always collect one or two air dried smears for the Giemsa stain as some additional valuable features can be seen in them.
- ▶ *Ancillary studies.* Needle washes or material should be collected in relevant fixatives for conventional cytogenetics, reverse transcriptase PCR or electron microscopy. Extra smears could be made and collected for immunocytochemistry (ICC) or fluorescence in situ hybridisation (FISH).

Table 1 Age distribution of renal tumours in children

Age group range	Renal tumours to be expected
<1 year	Congenital mesoblastic nephroma Wilms' tumour Rhabdoid tumour
1–4 years	Wilms' tumour Rhabdoid tumour of kidney Clear cell sarcoma
>4–5 years	Wilms' tumour
>5–10 years	Wilms' tumour Clear cell sarcoma Renal cell carcinoma
>10–19 years	Renal cell carcinoma Primitive neuroectodermal tumour/Ewing sarcoma

Cytological parameters to be analysed

A cytopathologist should note the following cytological parameters while evaluating an aspirate from a paediatric renal tumour.

- ▶ *Background.* Features to watch for are presence of a neurofibrillary matrix in neuroblastoma, metachromatic matrix in clear cell sarcoma of kidney (CCSK), and lymphoglandular bodies in non-Hodgkin lymphoma.
- ▶ *Cohesiveness of cells.* The most discohesive paediatric renal tumour is non-Hodgkin lymphoma, followed by neuroblastoma, primitive neuroectodermal tumour/Ewing sarcoma (PNET/ES) and blastemal dominant Wilms' tumour.
- ▶ *Arrangement.* Features to be observed include rosettes in Wilms' tumour/PNET; acini or tubules, papillary fronds and intracytoplasmic globules in renal cell carcinoma, etc. Arborising vasculature is seen in CCSK, while in renal cell carcinoma (RCC) transgressing vasculature is seen.
- ▶ *Cytoplasmic details.* A clear vacuolated cytoplasm is observed in PNET/ES or CCSK, while blastemal cells in Wilms' tumour lack a discernible cytoplasm.

- ▶ *Nuclei.* While grooved nuclei are pointers towards a CCSK, prominent nucleoli indicate a rhabdoid tumour of kidney (RTK) and lack of nucleoli favour a blastemal Wilms' tumour. If a smear shows variable presence of nucleoli among the cells, a neuroblastoma should be considered.
- ▶ *Chromatin pattern.* Coarse nuclear chromatin indicates a round cell tumour like neuroblastoma or embryonal rhabdomyosarcoma. Paler finely dispersed chromatin in a round cell tumour hints at a blastemal Wilms' tumour or PNET/ES.
- ▶ *Cytological pattern analysis based on cellular subtype.* The cellular patterns in an aspirate can effectively be divided as triphasic population, round cell pattern, spindle cell and epithelioid cell pattern. Figure 1 summarises the diagnosis in paediatric renal tumour based on this approach.

Cytological features in specific paediatric renal tumours
Wilms' tumour

Just as on histology, all three components—blastema, epithelium and mesenchyme—are seen in aspirates from a triphasic Wilms' tumour. In our experience⁵ and that of others⁶ the blastema is more often represented in an aspirate even if a tumour is classically triphasic due to the relative looser cohesiveness of this element compared with the other two elements.

The blastemal component forms loose clusters or sheets of widely dispersed malignant small round cells twice the size of a lymphocyte in a Giemsa stain, with an insignificant amount of deep blue ill defined cytoplasm.⁷ The nuclei are round with finely dispersed nuclear chromatin (fig 2A). The epithelial component shows tightly cohesive cells with a fair degree of cytoplasm forming small cords or acini (fig 2B). Most often the so called "rosettes" in Wilms' tumour are transversely cut tubules rather than true rosettes. The immature glomeruli are seen as tight three-dimensional clusters or crescents with a well defined semi lunar basal lamina (fig 2B). The mesenchymal

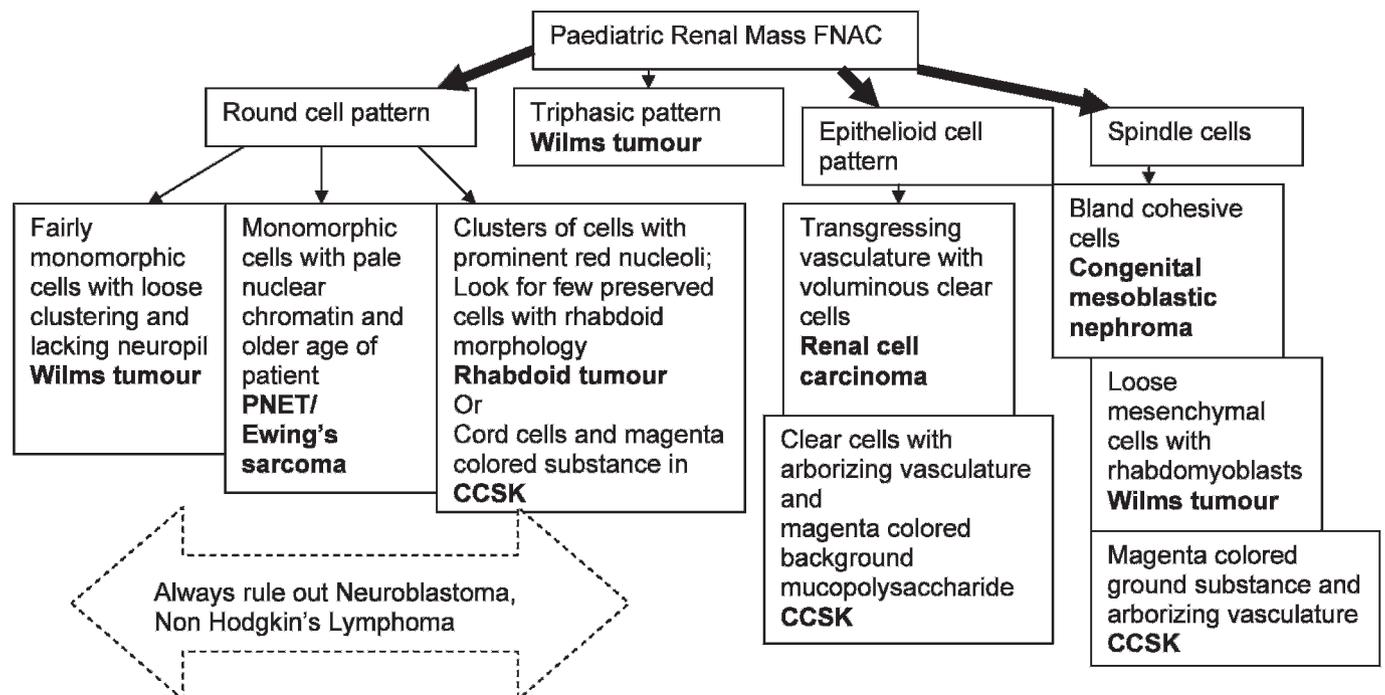


Figure 1 A flow chart depicting the approach to the diagnosis in paediatric renal tumour. CCSK, clear cell sarcoma of kidney; FNAC, fine needle aspiration cytology; PNET, primitive neuroectodermal tumour

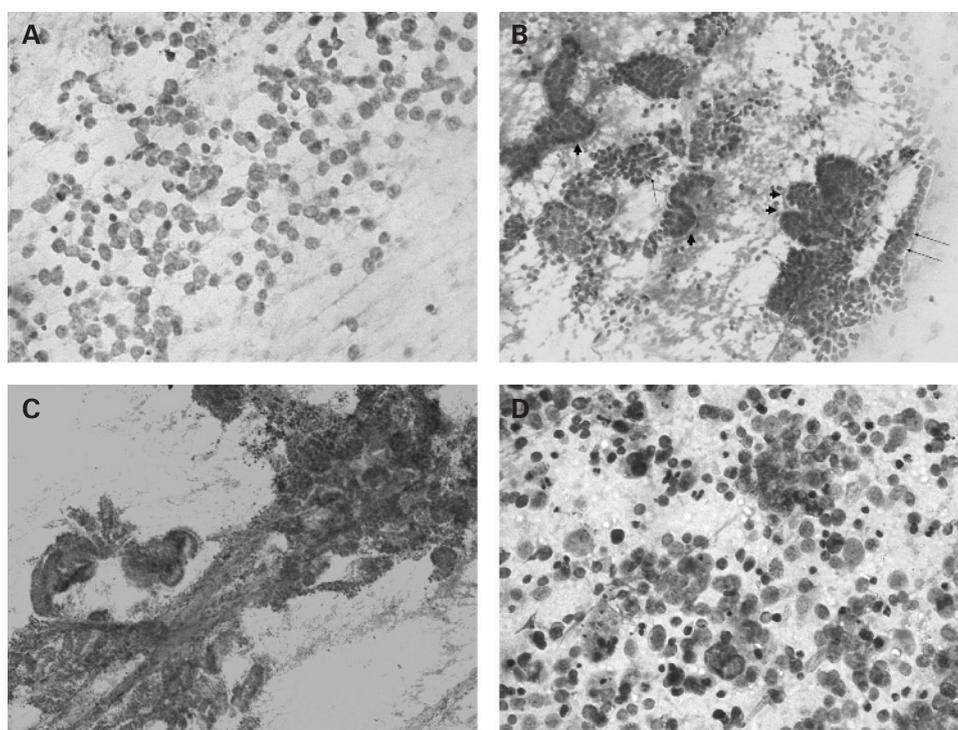
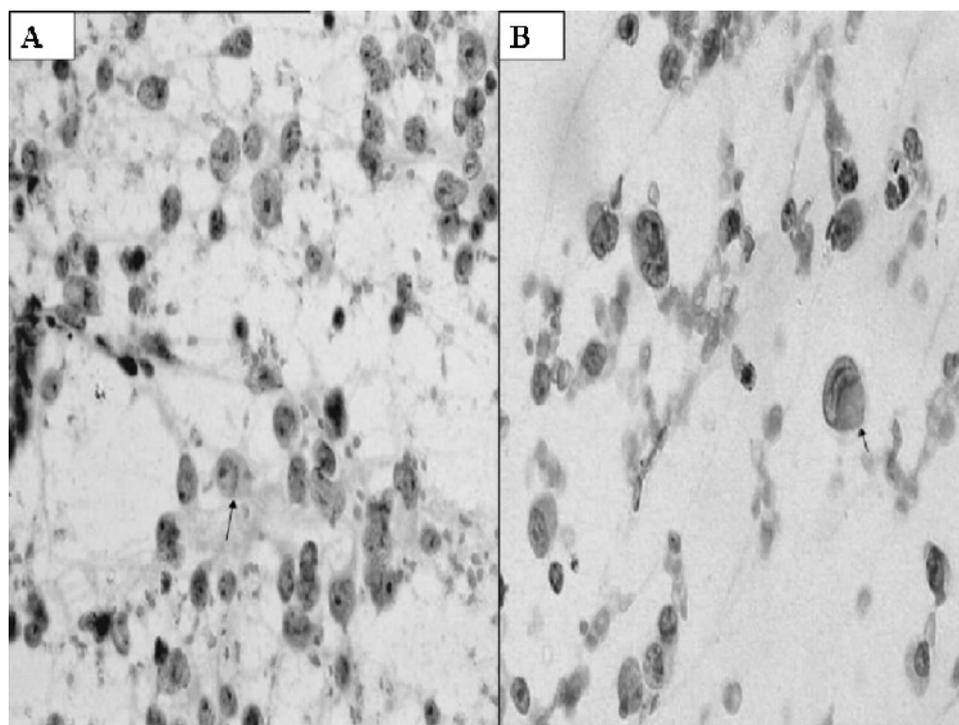


Figure 2 (A) Loosely clustered blastemal cells in Wilms' tumour with scanty cytoplasm and round nuclei with finely dispersed chromatin. Few apoptotic cells are noted (Papanicolaou, original magnification $\times 400$). (B) Aspirate from a Wilms' tumour reveals cords and rosette formation by the epithelial element (thin arrows) and immature glomeruli with a semi lunar refractive basal lamina (arrowheads) (Papanicolaou, original magnification $\times 400$). (C) Aspirate from a triphasic Wilms' tumour shows stromal fragments with entrapped glomeruli, tubules and blastema (Papanicolaou, original magnification $\times 100$). (D) Wilms' tumour with anaplasia on cytology shows a very large nucleus (lower half) with brisk mitosis and apoptosis (Papanicolaou, original magnification $\times 400$).

element is the binding component, and in triphasic Wilms' tumour fragments of stroma with entrapped glomeruli, tubules and blastema are diagnostic (fig 2C). Variable amounts of inflammatory cells, apoptosis and necrosis may be seen.⁸ The

term "unfavourable cytology" is used by some authors when a combination of severe pleomorphism, very large nucleoli and atypical mitosis is seen in FNAC smears,⁹ all of which reflect anaplasia in a Wilms' tumour. Patients with anaplastic Wilms'

Figure 3 (A) Rhabdoid tumour of kidney, showing cells with stripped cytoplasm; the prominent nucleolus is seen in most cells (Papanicolaou, original magnification $\times 400$). (B) Aspirate from a rhabdoid tumour of kidney with the typical eccentrically placed cytoplasmic inclusion (thin arrows) (Papanicolaou, original magnification $\times 400$).



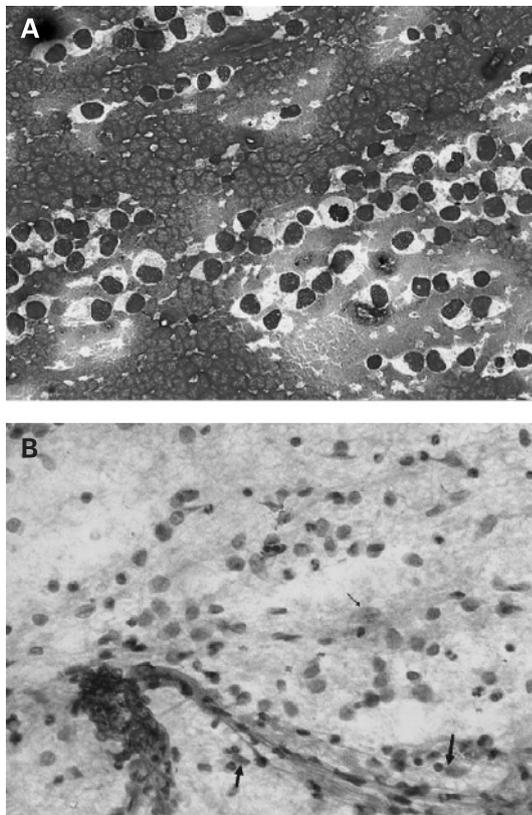


Figure 4 (A) Smear from a clear cell sarcoma of kidney reveals the metachromatic background substance with entrapped large clear polygonal cord cells in the Giemsa stain (original magnification $\times 400$). (B) Aspirate from a clear cell sarcoma of kidney shows the grooved nuclei of cord cells (thin arrow) and septal cells around blood vessels (Papanicolaou, original magnification $\times 400$).

tumour show resistance to chemotherapy and a reduced recurrence free survival rate, even after intensive chemotherapy.¹⁰ A diagnosis of anaplasia on cytology is made when a smear shows extremely large hyperchromatic blastemal cells three times the size of the surrounding blastemal cells coupled with brisk atypical mitosis (fig 2D). A potential limitation of FNAC of Wilms' tumour is the inability to extensively sample a large mass and thereby to detect anaplasia¹ and to distinguish focal from diffuse anaplasia.

Rhabdomyoblastic differentiation in a Wilms' tumour can assume different proportions. Most commonly seen are the plasmacytoid rhabdomyoblasts that resemble the ganglion cells in a neuroblastoma.⁵ The fetal rhabdomyomatous variant of nephroblastoma shows long rhabdomyoblasts with cross striations similar to an embryonal rhabdomyosarcoma.¹¹

Cystic variants of Wilms' tumour include a benign counterpart multicystic nephroma and cystic partially differentiated nephroblastoma, which is a well differentiated cystic Wilms' tumour. Aspirates from multicystic nephroma are of low cellularity and reveal a cystic background with the differentiated bland orderly arranged epithelial component.¹²⁻¹⁴ Tumours showing nucleolated epithelial cells or round cell pattern due to stripping off of the cytoplasm mimic a Wilms' tumour.^{14 15} In a cystic partially differentiated nephroblastoma, in addition to the above features, scanty blastemal element may also be noted.¹⁶

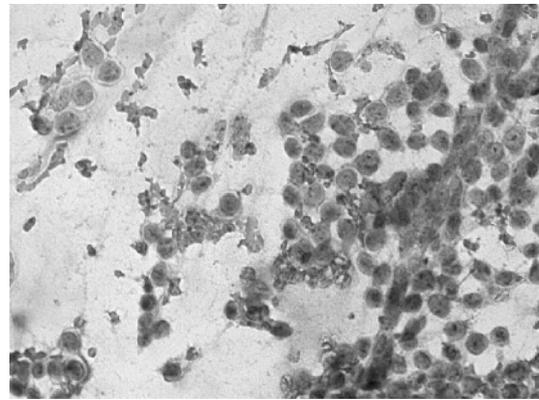


Figure 5 A primitive neuroectodermal tumour of kidney on cytology shows round cells with distinct clear cytoplasm and pale nuclear chromatin (Papanicolaou, original magnification $\times 400$).

Rhabdoid tumour of the kidney

Besides an aggressive behaviour, RTKs are associated with CNS tumours in 13.5% of cases and hence accurate cytological recognition is important. Aspirates from RTK show a dispersed population of small cells with focal clustering or sheets and stripped bare nuclei in the background. RTK never has a rhabdoid an appearance as some of the other tumours with rhabdoid like phenotype, as the "rhabdoid" cytoplasm is fragile and easily stripped during smearing, becoming less obvious in some aspirates.^{5 17} The presence of irregular nuclei with prominent red nucleolus is often the first clue to the diagnosis. Few cells with eccentrically placed "rhabdoid" cytoplasm/eosinophilic cytoplasmic inclusion are always seen (fig 3).

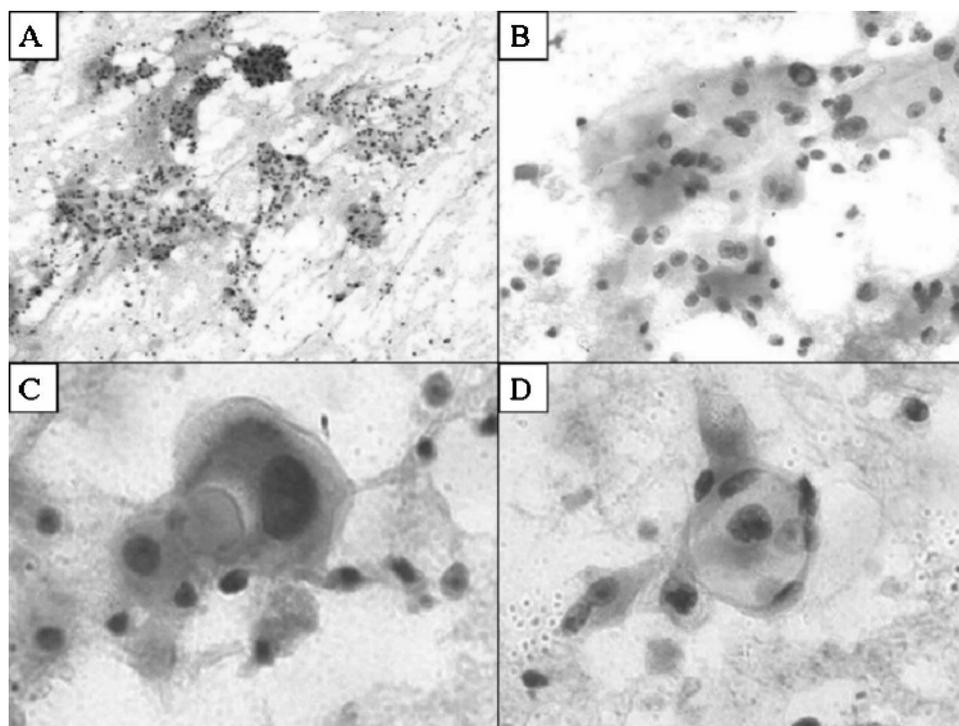
Clear cell sarcoma of kidney

On cytology CCSK shows varying proportions of cord cells, septal cells, arborising vasculature and mucopolysaccharide substance.^{12 18 19} Aspirates typically reveal polygonal cord cells with eccentrically placed grooved nuclei and a wispy clear cytoplasm on the backdrop of the magenta coloured mucopolysaccharide ground substance (fig 4). A second useful clue is the prominent arborising blood vessels with the septal spindle-shaped cells adjacent to endothelium (fig 4B). In addition, "dark cells" or pyknotic degenerative apoptotic cells are also described.¹⁸ CCSK also has various less common histological variants which have further deviations in their cytomorphology.¹² One known pitfall in the cytological diagnosis of CCSK is the aspiration of normal renal tubules, simulating the immature epithelial cells of a Wilms' tumour.¹⁹

Primitive neuroectodermal tumour/Ewing sarcoma

In the last decade this tumour has been documented with increasing frequency in the kidney within a wide age group from 6 to 35 years.²⁰ PNET/ES of kidney are clinically aggressive and require chemotherapy regimens that are more intensive than a Wilms' tumour. PNET/ES are frequently misdiagnosed as Wilms' tumour, both being monotonous round cell tumours.^{20 21} PNET/ES on cytology reveals variably cohesive clusters of small round cells with irregular nuclei and the typical "Ewingoid" or pale nuclear chromatin (fig 5). Apoptotic cells, mitosis and necrosis are easily identified. The intracytoplasmic glycogen also produces a tigroid background in the air dried smears. Few cells always show a preserved clear vacuolated cytoplasm better appreciated in the Giemsa stained smear (fig 5).

Figure 6 Aspirate from a translocation associated renal cell carcinoma shows: (A) sheets of large cells and some papillae (Papanicolaou, original magnification $\times 100$); (B) large cells with eosinophilic or clear cytoplasm and intranuclear inclusion (Papanicolaou, original magnification $\times 200$); (C) large cell with intracytoplasmic inclusion (Papanicolaou, original magnification $\times 400$); (D) cell within cell appearance (Papanicolaou, original magnification $\times 400$).



Renal cell carcinoma

Renal cell carcinomas (RCC) in children are now recognised as a unique group of translocation associated carcinomas. Most of them have Xp11.2 t translocations/TFE3 gene fusions with two subtypes: RCC with t(X; 17) (p11.2; q21) and RCC with t(X; 1) (p11.2; p34). As opposed to adult RCC they do not show immunoreactivity to vimentin, or epithelial markers but are immunoreactive to CD10 and TFE3 protein. Though indolent these neoplasms often present in advanced stages. Though earlier reports reported similar cytological findings as in adult RCC,¹² our experience (unpublished observations) and reports by some authors indicate that translocation associated RCC have unique cytomorphology.²² Compared to adult RCC they show larger mostly polygonal, eosinophilic or clear cells with an eccentrically placed nucleus with prominent nucleoli, sometimes intranuclear inclusions and intracytoplasmic hyaline eosinophilic inclusions. Curled up three-dimensional papillae

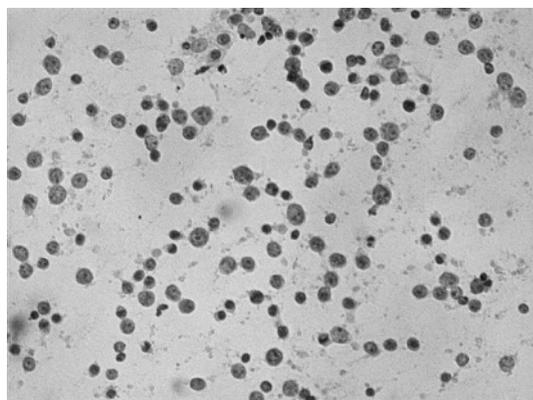


Figure 7 Non-Hodgkin lymphoma of Burkitt subtype involving the kidney reveals dispersed cells with lymphoglandular bodies in background and intermediate size cells with peripheral nucleoli (Papanicolaou, original magnification $\times 400$).

lined by cells with clear cytoplasm, psammoma bodies and a cell in cell appearance are also noted (fig 6).

Congenital mesoblastic nephroma

Congenital mesoblastic nephroma (CMN) is an uncommon benign spindle cell tumour of the kidney in infant children. The typical CMN is a hypocellular tumour just like a fibromatosis with no specific cytogenetic aberration, while the cellular CMN is equivalent to an infantile fibrosarcoma occurring in the kidney with a similar t(12;15)(p13,q25). While the former behaves in a benign fashion, some of the latter show metastases. Most of the typical CMN, being cohesive, yield non-diagnostic aspirates or cohesive clusters of bland spindle cells embedded in a fibrillary ill defined matrix with a few stripped nuclei.^{23, 24} The nuclei are oval to elongated, smooth in contour, with evenly dispersed chromatin and indistinct nucleoli.²⁴ Conversely a cellular CMN shows cellular smears with groups of and isolated spindle ζ ovoid cells in a necrotic background. Tumour cells are elongated and have irregular nuclei with coarse chromatin. Some cells with nucleoli and mitosis can be seen.¹⁹

Non-Hodgkin lymphoma

Renal involvement in non-Hodgkin lymphoma (NHL) is usually secondary, but some cases of primary renal NHL have been described.²⁵ The common NHLs to involve the kidney in children are diffuse large B cell lymphomas or Burkitt lymphomas. The most important diagnostic clue in NHL is the presence of predominantly dispersed population of round cells with typical lymphoglandular bodies which represent the stripped of cytoplasm of the lymphoid cells in smears (fig 7).

Metanephric adenoma

Though metanephric adenoma is uncommon, it may occur in children in the same age group as Wilms' tumour. Essentially it shows bland immature tubules which out of context can be mistaken for a Wilms' tumour. However, these tubules are

Table 2 Immunocytochemical panel in a round cell tumour in the renal region

Tumour	Cytokeratin	WT1	MIC2	Synaptophysin	LCA
Blastemal WT	Positive (focal in 75%)	70% (nuclear and cytoplasmic)	Negative (rare cell may be positive)	Negative	Negative
RTK	Positive in most tumours	Negative	Negative	Negative	Negative
CCSK	Negative	Negative	Negative	Negative	Negative
PNET	Negative	Negative	94–100% positive	Positive	Negative
Neuroblastoma	Negative	Negative or weak positive	Negative	Positive	Negative
Embryonal rhabdomyosarcoma	Negative	Cytoplasmic staining	Negative	Negative	Negative
Non-Hodgkin lymphoma	Negative	Negative (except in lymphoblastic lymphoma)	Negative (except in lymphoblastic lymphoma)	Negative	Positive

evenly spaced and form tight tubules. The lining cells are monomorphic, bland, smaller, and lack mitosis as compared to usual blastema.^{19 26}

Rare renal tumours in children

The spectrum of renal tumours is ever expanding and the cytopathologist can encounter any uncommon tumour in the kidney. The moot point to remember is to concentrate on the accurate diagnosis of clearly defined malignant tumours slotted for chemotherapy and avoid false positive diagnosis.

Role of ancillary methods in diagnosis of paediatric renal tumours

As areas of diagnostic difficulty do occur, the cytopathologist assessing aspirates from a tumour in the renal region in children should take recourse to ancillary methods. The following techniques are useful in evaluating paediatric renal tumours.

Immunocytochemistry

ICC is the most popular method used for resolving difficulties in cytological diagnosis as it is very easy to obtain extra smears for ICC or to destain already stained smears without repeating the aspiration. The panel of ICC to be chosen will depend on the differentials that the pathologist has homed in on. Table 2 shows the general panel of use in round cell tumours in the renal region. The percentages given are based on our personal experience with histological evaluation of paediatric renal tumours (unpublished observations)/cytology⁵ and some histology based studies on renal tumours.^{27 28} The pathologist should be alerted with the expanding immunoprofile of renal tumours to use this methodology to its full potential and avoid errors. For example, the INI1 antibody immunohistochemistry, though not used on cytology to date could be added in order to confirm the histological diagnosis of RTK²⁹; or WT1 has been documented in some patients with CMN.³⁰ ICC helps in the following problem zones:

- ▶ *Wilms' tumour vs other round cell tumours.* A nuclear and cytoplasmic staining with WT1 antigen aids in the diagnosis of Wilms' tumour,⁵ while a PNET/ES of kidney shows membranous staining for the MIC2 antigen and is WT1 negative.
- ▶ *Wilms' tumour vs non-Wilms' tumour of kidney.* Cytoplasmic immunopositivity for cytokeratin will help differentiate a RTK and Wilms' tumour from a CCSK while CCSK marks only with vimentin. Negativity for cytokeratin cannot rule out blastemal Wilms' tumour as some poorly differentiated Wilms' tumours show cytokeratin negativity. The vimentin staining in CCSK is at best moderate and a strong cytoplasmic staining with vimentin actually points away from this diagnosis.¹⁸
- ▶ *Differentiating within the non-Wilms' spectrum.* CCSK marks with vimentin only, while translocation associated carcinomas lack CK/EMA expression and express CD10. Cytokeratin will help identify RTK over other non-Wilms' tumours.
- ▶ *Spindle cell tumours of kidney.* The ICC panel for this group includes WT1, bcl2, vimentin and desmin.³¹ WT1 is uniformly positive in the primitive undifferentiated stromal component in Wilms' tumour and negative in the differentiated stromal elements of Wilms' tumours, cellular mesoblastic nephroma (except for rare cases), CCSK and synovial sarcomas.³¹ Bcl-2 is positive in all stromal Wilms' tumours, all synovial sarcomas and some CCSK, but negative in CMN.³¹

Electron microscopy

Electron microscopy is fast losing ground to the explosion of molecular cytogenetics in the diagnosis of paediatric round cell tumours. Needle aspirates can easily be fixed in Karnosky or Universal fixative and the sediments can be processed for examination of specific ultrastructural features. Electron microscopy is particularly useful in identifying neuroblastoma and differentiating it from a Wilms' tumour.¹ While cell processes,

Table 3 Common cytogenetic alterations in paediatric renal tumours^{32–34}

Tumour	Type of abnormality
Rhabdoid tumour of kidney	70% show mutation or deletion of both copies of the <i>hSNF5/INI1</i> gene that map to chromosome band 22q11.2
Cellular congenital mesoblastic nephroma	t(12; 15) (p13, q25)/ETV6/NTRK3 gene fusion
Neuroblastoma	N myc amplification in 30–40%
Primitive neuroectodermal tumour/Ewing sarcoma	t(11;22) or EWS-FLI1 is the commonest seen in 85%; t(21;22), t(7;22), t(17;22), and t(2;22) are observed in remainder
Synovial sarcoma	90% show t(X;18)(p11.2;q11.2)
Renal cell carcinoma	t(X; 17) (p11.2; q21) and t(X; 1) (p11.2; p34)/Xp11.2 translocations/TFE3 gene fusions

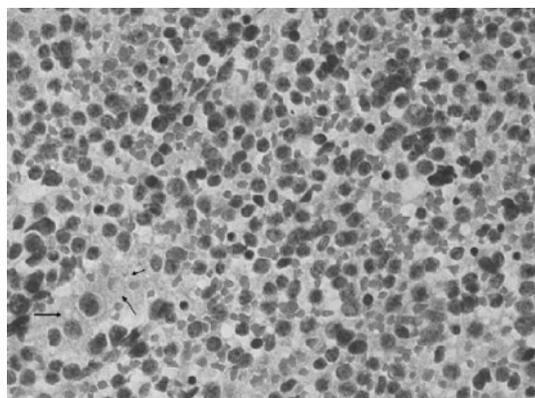


Figure 8 Smears from neuroblastoma reveals cells with variable amount of cytoplasm and background neuropil (arrow) (Papanicolaou, original magnification $\times 400$).

dense-core granules and neurotubules are features that indicate a neuroblastoma, Wilms' tumour reveals cell junctions, microvilli, flocculent basement membrane-like material, cilia and autophagolysosomes.¹ Similarly, RTK, PNET/ES and the newly described translocation associated renal cell carcinomas show unique ultrastructural features that will aid in their diagnosis.

Cytogenetic evaluation of paediatric renal tumours

Immunocytochemistry is not always conclusive for diagnosis of paediatric renal tumours as some of these entities even share antigenicity.³² In such situations unique cytogenetic features of each tumour can help in the correct diagnosis. Table 3 presents the common cytogenetic findings in paediatric renal tumours.^{32–34} FNAC material is generally of suitable quality to perform traditional cytogenetic chromosome analysis, as well as PCR based molecular techniques.³² Interphase FISH can also be done on fixed smears for confirming translocation associated tumours. Often one pass is only required for FISH or cytogenetic studies.³⁵

Box 1: Cytological differences between Wilms' tumour and neuroblastoma

Wilms' tumour

- ▶ Rosettes lack central neuropil and usually show only one layer of cells.
- ▶ Background is clean or haemorrhagic.
- ▶ Minimal polymorphism unless there is skeletal muscle differentiation or anaplasia.
- ▶ Mitotic activity is not very brisk unless there is anaplasia.
- ▶ On immunocytochemistry WT1 and cytokeratin are positive.

Neuroblastoma

- ▶ Rosettes show central neuropil and multilayering of cells around them. The cells lining the rosettes have very scanty cytoplasm.
- ▶ Background shows neuropil that entraps cells.
- ▶ Polymorphism in cell cytoplasm and size is observed.
- ▶ Mitotic activity is very high.
- ▶ Immunocytochemistry reveals chromogranin or synaptophysin positivity in neuroblastic cells.

Box 2: Differences in Wilms' tumour and primitive neuroectodermal tumour/Ewing sarcoma (PNET/ES) on cytology

Blastemal Wilms' tumour

- ▶ Small cells with scanty cytoplasm.
- ▶ Round nuclei with dispersed chromatin.
- ▶ Blastemal cells adhere to vessels through a perivascular cuff of mesenchyme.
- ▶ True rosettes not seen.
- ▶ Cells are WT1 positive, and lack synaptophysin.

PNET/ES

- ▶ Small cells with fair amount of vacuolated cytoplasm.
- ▶ Slightly irregular nuclei with pale chromatin.
- ▶ Direct perivascular arrangement of the small round cells is seen.
- ▶ Homer Wright rosettes are noted.
- ▶ Cells are WT1 negative but MIC2 and synaptophysin are positive.

Resolving the differential diagnosis

Paediatric renal tumours should be differentiated from all round cell tumours, especially non-Hodgkin lymphoma or other tumours in the renal region in view of different management options. It is important to distinguish Wilms' tumour from aggressive non-Wilms' tumour like CCSK, RTK and PNET/ES as the chemotherapeutic regimens in the latter are modified to suit their aggressiveness; for example, actinomycin D and a four-drug regimen are administered in CCSK as opposed to the three-drug regimen in a Wilms' tumour. Under-treating these aggressive types with usual Wilms' tumour chemotherapy leads to progression and decreased survival.

Wilms' tumour vs neuroblastoma (compare fig 2A and fig 8)

A common mistake made by a cytopathologist is interpreting a large abdominal blastemal Wilms' tumour as a neuroblastoma or vice versa.⁶ To add to the problem, rare intrarenal

Box 3: Cytomorphological differences in Wilms' tumour and metanephric adenoma

Wilms' tumour

- ▶ Mildly pleomorphic dispersed tumour cells with nuclear overlapping, crowding and disarray.
- ▶ Frankly malignant nuclei with irregular chromatin clumping and sometimes nucleoli.
- ▶ Mitosis is easily appreciated.
- ▶ Calcification absent in untreated cases.
- ▶ Tumour cells express WT1 and EMA (epithelial membrane antigen).

Metanephric adenoma

- ▶ Monomorphic small tumour cells that are evenly spaced and form tight tubules.
- ▶ Bland nuclei with regular contour and indistinct nucleoli.
- ▶ Mitosis is nearly absent.
- ▶ Psammomatous calcification is noted.
- ▶ Tumour cells express WT1 and vimentin but are EMA negative.

Box 4: Features that differentiate a neuroblastoma and neuroectodermal tumour/Ewing sarcoma (PNET/ES)**Neuroblastoma**

- ▶ Tumour cells have round nuclei with coarse chromatin, nucleoli are seen only in cells with ganglionic differentiation.
- ▶ Polymorphic populations of cells, including poorly differentiated cells with scanty cytoplasm and ganglionic cells with eccentrically placed cytoplasm.
- ▶ Background neuropil and ganglion cells indicate obvious neuronal differentiation.
- ▶ Tumour cells lack MIC2 staining.

PNET/ES

- ▶ Tumour cells have slightly irregular nuclei with finely stippled chromatin; nucleoli are seen only in atypical cases.
- ▶ Monomorphic cell population with fair amount of vacuolated cytoplasm.
- ▶ Obvious neuronal differentiation is absent.
- ▶ MIC2 is positive in most cells.

neuroblastomas are described.³⁶ Overlapping cytological features include presence of malignant small cells with nuclear moulding and presence of rosettes in both tumours. Box 1 lists salient features that distinguish the two are. The most useful features are identification of neuropil entrapping the cells and the variable cytoplasm and cell sizes in cells (polymorphism) in neuroblastoma (fig 8) as against a purely blastemal Wilms' tumour which has monotonous round cells.⁵ The nuclei of neuroblastoma are also uniformly rounded with stippled chromatin in contrast to those of blastemal cells which are slightly irregular and are strongly basophilic.⁵

Non-Hodgkin lymphoma vs Wilms' tumour (compare fig 2A and fig 7)

The cell population in NHL is extremely dispersed as compared to a Wilms' tumour and the presence of lymphoglandular bodies in the background assist in recognising the lymphoid nature of cells.

Blastemal Wilms' tumour vs PNET (compare fig 2A and fig 5A–B)

Although these tumours occur in patients in different age groups, exceptions may occur. Box 2 provides a summary of

Take-home messages

- ▶ Pathologists evaluating fine needle aspiration cytology from paediatric renal tumours should be aware of crucial diagnosis/differential diagnoses that will affect management of patients; eg, non-Hodgkin lymphoma vs Wilms' tumour, or identify benign tumours in the kidney accurately.
- ▶ Classifying aspirates according to their cellular patterns as triphasic, round cell, spindle cell or epithelioid cell types assists in classifying paediatric renal tumours on cytology.
- ▶ Immunocytochemistry for WT1, cytokeratin, synaptophysin, leucocyte common antigen and MIC2 will aid in evaluating round cell tumours in the renal region, while WT1, bcl2, vimentin and desmin will be useful for spindle cell tumours in that region.
- ▶ Awareness of cytological findings in different subtypes of paediatric renal tumours helps in improving accuracy of diagnosis.

distinguishing cytological features. In some academic centres, detection of specific translocation will help in clinching the diagnosis, especially in cases of PNET with atypical nuclear features.

Wilms' tumour vs CCSK (compare fig 2A and fig 4A–B)

The cells in a CCSK often get stripped off with numerous bare nuclei resembling a round cell tumour like Wilms' tumour.¹⁸ Blastemal cells of Wilms' tumour differ from the cord cells of CCSK in having scanty cytoplasm, and a comparatively hyperchromatic nucleus which lacks nuclear grooves. The Giemsa stain will reveal the typical mucopolysaccharide ground substance in a CCSK and help in ruling out a Wilms' tumour.

Wilms' with rhabdomyoblastic differentiation vs embryonal rhabdomyosarcoma

Cytological features from a genitourinary tract embryonal rhabdomyosarcoma (ERMS) show more pleomorphic cells than a Wilms' tumour, and nuclei have much more coarser chromatin as compared to the differentiated rhabdomyoblasts in a Wilms' tumour.¹¹ On ICC, cytokeratin helps to pick out small groups of discohesive epithelial cells in Wilms' tumour.

Wilms' tumour vs metanephric adenoma

Given the wide age range in a metanephric adenoma, this is a rare but crucial differential for a Wilms' tumour. Metanephric adenoma, being benign, requires excision only, while Wilms' tumour requires chemotherapy depending on the stage. Box 3 summarises the differences in the two tumours.

PNET/ES kidney vs neuroblastoma (compare fig 5 with fig 8)

The management and chemotherapy for a PNET/ES is very different to that for a neuroblastoma. Besides the features listed in box 4, finding of the specific translocation will help in diagnosing a PNET/ES.

Paediatric spindle cell tumours in kidney

This group includes entities like CMN, mesenchymal predominant Wilms' tumour, fetal rhabdomyomatous Wilms' tumour, embryonal rhabdomyosarcoma of urinary tract, spindle cell variant CCSK, stromal tumours of kidney and synovial sarcoma. The crucial differential in this situation is to distinguish a mesenchymal predominant Wilms' tumour (a malignant tumour) from the benign congenital mesoblastic nephroma (CMN). Aspirates from mesenchymal dominant Wilms' tumour are more cellular compared to those of CMN.¹² It is highly unusual to encounter a purely stromal Wilms' tumour in untreated cases and hence a careful search for the scanty blastemal component avoids misdiagnosis.¹² Cells in CMN are also more cohesive than the loose mesenchymal cells in a Wilms' tumour.¹⁹

Features that suggest a spindle cell variant of CCSK over a mesenchymal predominant Wilms' tumour are the typical magenta ground substance, perivascular arrangement of tumour cells, and the fact that the cells of CCSK are larger and cohesive with abundant cytoplasm.¹²

Summary points to be remembered while evaluating the aspirates from a paediatric renal tumour

- ▶ Rule out other abdominal tumours, especially neuroblastoma.

- ▶ *Rule out a lymphoma or haematolymphoid malignancy* as these would be treated with non-surgical mode of chemotherapy only.
- ▶ *Decide benign vs malignant renal tumour.* Most benign tumours such as congenital mesoblastic nephroma or multicystic nephroma will be treated primarily with surgical excision however huge they are at presentation, while a large Wilms' tumour will require preoperative chemotherapy.
- ▶ *Subtype the malignant paediatric renal tumour* as Wilms' tumour vs non-Wilms' tumour (CCSK/RTK/PNET).

CONCLUSION

Paediatric renal tumours present a unique spectrum very much amenable to an accurate cytological diagnosis with a little bit of experience. A checklist of common tumours in a particular age group, relevant clinical information, awareness of distinctive and overlapping cytological features, and appropriate use of immunocytochemistry with cytogenetics all combine in ensuring an accurate cytological diagnosis.

Acknowledgements: We are grateful to Dr Brijesh Arora for his clinical inputs and Dr Ruta Goregaonkar for immunohistochemical findings in paediatric renal tumours

Competing interests: None.

Provenance and peer review: Commissioned; externally peer reviewed.

REFERENCES

1. **Ellison DA**, Silverman JF, Strausbauch PH, *et al.* Role of immunocytochemistry, electron microscopy, and DNA analysis in fine-needle aspiration biopsy diagnosis of Wilms' tumour. *Diagn Cytopathol* 1996;**14**:101–7.
2. **Gommersall LM**, Arya M, Mushtaq I, *et al.* Current challenges in Wilms' tumour management. *Nat Clin Pract Oncol* 2005;**2**:298–304.
3. **Vujanic GM**, Sandstedt B, Harms D, *et al.*, on behalf of the SIOP Nephroblastoma Scientific Committee. Revised International Society of Paediatric Oncology (SIOP) working classification of renal tumours of childhood. *Med Pediatr Oncol* 2002;**38**:79–82.
4. **Vujanic GM**, Kelsey A, Mitchell C, *et al.* The role of biopsy in the diagnosis of renal tumors of childhood: results of the UKCCSG Wilms tumor study 3. *Med Pediatr Oncol* 2003;**40**:18–22.
5. **Goregaonkar R**, Shet T, Ramadwar M, *et al.* Critical role of fine needle aspiration cytology and immunocytochemistry in preoperative diagnosis of paediatric renal tumours. *Acta Cytol* 2007;**51**:721–9.
6. **Hazarika D**, Narasimhamurthy KN, Rao CR, *et al.* Fine needle aspiration cytology of Wilms' tumour. A study of 17 cases. *Acta Cytol* 1994;**38**:355–60.
7. **Dey P**, Radhika S, Rajwanshi A, *et al.* Aspiration cytology of Wilms' tumor. *Acta Cytol* 1993;**37**:477–82.
8. **Quijano G**, Drut R. Cytologic characteristics of Wilms' tumours in fine needle aspirates. A study of ten cases. *Acta Cytol* 1989;**33**:263–6.
9. **Iyer VK**, Kapila K, Agarwala S, *et al.* Wilms' tumour. Role of fine needle aspiration and DNA ploidy by image analysis in prognostication. *Anal Quant Cytol Histol* 1999;**21**:505–11.
10. **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**:909–20.
11. **Drut R**, Pollono D. Fetal rhabdomyomatous nephroblastoma: diagnosis by fine-needle aspiration cytology—a case report. *Diagn Cytopathol* 2000;**22**:235–7.
12. **Radhika S**, Bakshi A, Rajwanshi A, *et al.* Cytopathology of uncommon malignant renal neoplasms in the pediatric age group. *Diagn Cytopathol* 2005;**32**:281–6.
13. **Drut R**. Cystic nephroma: cytologic findings in fine-needle aspiration cytology. *Diagn Cytopathol* 1992;**8**:593–5.
14. **Hughes JH**, Niemann TH, Thomas PA. Multicystic nephroma: report of a case with fine-needle aspiration findings. *Diagn Cytopathol* 1996;**14**:60–3.
15. **Gupta R**, Dhingra K, Singh S, *et al.* Multicystic nephroma: a case report. *Acta Cytol* 2007;**51**:651–3.
16. **Dey P**, Das A, Radhika S. Fine needle aspiration cytology of cystic partially differentiated nephroblastoma. A case report. *Acta Cytol* 1996;**40**:770–2.
17. **Barroca HM**, Costa MJ, Carvalho JL. Cytologic profile of rhabdoid tumor of the kidney. A report of 3 cases. *Acta Cytol* 2003;**47**:1055–8.
18. **Iyer VK**, Agarwala S, Verma K. Fine-needle aspiration cytology of clear-cell sarcoma of the kidney: study of eight cases. *Diagn Cytopathol* 2005;**33**:83–9.
19. **Portugal R**, Barroca H. Clear cell sarcoma, cellular mesoblastic nephroma and metanephric adenoma: cytological features and differential diagnosis with Wilms tumour. *Cytopathology* 2008;**19**:80–5.
20. **Premalata CS**, GayathriDevi M, Biswas S, *et al.* Primitive neuroectodermal tumor of the kidney: a report of two cases diagnosed by fine needle aspiration cytology. *Acta Cytol* 2003;**47**:475–9.
21. **Maly B**, Maly A, Reinhartz T, *et al.* Primitive neuroectodermal tumor of the kidney. Report of a case initially diagnosed by fine needle aspiration cytology. *Acta Cytol* 2004;**48**:264–8.
22. **Barroca H**, Correia C, Castedo S. Cytologic and cytogenetic diagnosis of pediatric renal cell carcinoma associated with t(X;17). *Acta Cytol* 2008;**52**:384–6.
23. **Gupta R**, Mathur SR, Singh P, *et al.* Cellular mesoblastic nephroma in an infant: report of the cytologic diagnosis of a rare pediatric renal tumor. *Diagn Cytopathol* 2009;**37**:377–80.
24. **Kaw YT**. Cytologic findings in congenital mesoblastic nephroma. A case report. *Acta Cytol* 1994;**38**:235–40.
25. **Truong LD**, Caraway N, Ngo T, *et al.* Renal lymphoma. The diagnostic and therapeutic roles of fine-needle aspiration. *Am J Clin Pathol* 2001;**115**:18–31.
26. **Khayyata S**, Grignon DJ, Aulicino MR, *et al.* Metanephric adenoma vs. Wilms' tumor: a report of 2 cases with diagnosis by fine needle aspiration and cytologic comparisons. *Acta Cytol* 2007;**51**:464–7.
27. **Carpentieri DF**, Nichols K, Chou PM, *et al.* The expression of WT1 in the differentiation of rhabdomyosarcoma from other pediatric small round blue cell tumors. *Mod Pathol* 2002;**15**:1080–6.
28. **Parham DM**, Roloson GJ, Feely M, *et al.* Primary malignant neuroepithelial tumors of the kidney: a clinicopathologic analysis of 146 adult and pediatric cases from the National Wilms' Tumor Study Group Pathology Center. *Am J Surg Pathol* 2001;**25**:133–46.
29. **Hoot AC**, Russo P, Judkins AR, *et al.* 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**:1485–91.
30. **Abosoudah I**, Ngan BY, Grant R, *et al.* WT1 expression and hemihypertrophy in congenital mesoblastic nephroma. *J Pediatr Hematol Oncol* 2008;**30**:768–71.
31. **Shao L**, Hill DA, Perlman EJ. Expression of WT-1, Bcl-2, and CD34 by primary renal spindle cell tumors in children. *Pediatr Dev Pathol* 2004;**7**:577–82.
32. **Barroca H**. Fine needle biopsy and genetics, two allied weapons in the diagnosis, prognosis, and target therapeutics of solid pediatric tumors. *Diagn Cytopathol* 2008;**36**:678–84.
33. **Adem C**, Gisselsson D, Dal Cin P, *et al.* ETV6 rearrangements in patients with infantile fibrosarcomas and congenital mesoblastic nephromas by fluorescence in situ hybridization. *Mod Pathol* 2001;**14**:1246–51.
34. **Biegel JA**, Tan L, Zhang F, *et al.* Alterations of the hSNF5/INI1 gene in central nervous system atypical teratoid/rhabdoid tumors and renal and extra renal rhabdoid tumors. *Clin Cancer Res* 2002;**8**:3461–7.
35. **Kilpatrick SE**, Bergman S, Pettenati MJ, *et al.* The usefulness of cytogenetic analysis in fine needle aspirates for the histologic subtyping of sarcomas. *Mod Pathol* 2006;**19**:815–9.
36. **Serrano R**, Rodríguez-Peralta JL, De Orbe GG, *et al.* Intrarenal neuroblastoma diagnosed by fine-needle aspiration: a report of two cases. *Diagn Cytopathol* 2002;**27**:294–7.