The cytological diagnosis of paediatric renal tumours

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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 lesions5; 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 aspira-

ions 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).
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 nuclei indicate a rhabdoid tumour of kidney (RTK) and lack of nuclei favour a blastemal Wilms’ tumour. If a smear shows variable presence of nuclei 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.

### Table 1 Age distribution of renal tumours in children

<table>
<thead>
<tr>
<th>Age group range</th>
<th>Renal tumours to be expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 year</td>
<td>Congenital mesoblastic nephroma, Wilms’ tumour, Rhabdoid tumour</td>
</tr>
<tr>
<td>1-4 years</td>
<td>Wilms’ tumour, Rhabdoid tumour of kidney, Clear cell sarcoma</td>
</tr>
<tr>
<td>&gt;4-5 years</td>
<td>Wilms’ tumour</td>
</tr>
<tr>
<td>&gt;5-10 years</td>
<td>Clear cell sarcoma, Renal cell carcinoma</td>
</tr>
<tr>
<td>&gt;10-19 years</td>
<td>Renal cell carcinoma, Primitive neuroectodermal tumour/Ewing sarcoma</td>
</tr>
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### 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 experience and that of others 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. 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...
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. 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,” all of which reflect
anaplasia in a Wilms’ tumour. Patients with anaplastic Wilms’

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 ×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
×400). (C) Aspirate from a triphasic Wilms’ tumour shows stromal fragments with entrapped glomeruli, tubules and blastema (Papanicolaou, original
magnification ×100). (D) Wilms’ tumour with anaplasia on cytology shows a very large nucleus (lower half) with brisk mitosis and apoptosis
(Papanicolaou, original magnification ×400).

Figure 3  (A) Rhabdoid tumour of
kidney, showing cells with stripped
cytoplasm; the prominent nucleolus is
seen in most cells (Papanicolaou, original
magnification ×400). (B) Aspirate from a
rhabdoid tumour of kidney with the
typical eccentrically placed cytoplasmic
inclusion (thin arrows) (Papanicolaou,
original magnification ×400).
tumour show resistance to chemotherapy and a reduced recurrence free survival rate, even after intensive chemotherapy. 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 anaplasia 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. The fetal rhabdomyomatous variant of nephroblastoma shows long rhabdomyoblasts with cross striations similar to an embryonal rhabdomyosarcoma. 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 similar to an embryonal rhabdomyosarcoma.

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Clear cell sarcoma of the kidney
On cytology CCSK shows varying proportions of cord cells, septal cells, arborising vasculature and mucopolysaccharide substance. Aspirates typically reveal polygonal cord cells with eccentrically placed grooved nuclei of cords cells (thin arrow) and septal cells around blood vessels (Papanicolaou, original magnification ×400).

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. 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. 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).
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, our experience (unpublished observations) and reports by some authors indicate that translocation associated RCC have unique cytomorphology. 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 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 a 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. Conversely a cellular CMN shows cellular smears with groups of and isolated spindle 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.

Non-Hodgkin lymphoma

Renal involvement in non-Hodgkin lymphoma (NHL) is usually secondary, but some cases of primary renal NHL have been described. 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
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)/cytology 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,31 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 cytoplasmic negativity. The vimentin staining in CCSK is at best moderate and a strong cytoplasmic staining with vimentin actually points away from this diagnosis.32
- **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.33 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.34 Bcl-2 is positive in all stromal Wilms’ tumours, all synovial sarcomas and some CCSK, but negative in CMN.35

**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.31 While cell processes,
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. 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. 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. FNAC material is generally of suitable quality to perform traditional cytogenetic chromosome analysis, as well as PCR based molecular techniques. 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.

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.

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. To add to the problem, rare intrarenal...
neuroblastomas are described.\textsuperscript{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. 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. The cells in a CCSK often get stripped off with numerous bare nuclei resembling a round cell tumour like Wilms’ tumour.\textsuperscript{36} 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.\textsuperscript{11} On ICC, cytokeratin helps to pick out small groups of discohesive epithelial cells in Wilms’ tumour.

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.\textsuperscript{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.\textsuperscript{12} Cells in CMN are also more cohesive than the loose mesenchymal cells in a Wilms’ tumour.\textsuperscript{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.\textsuperscript{12}

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

\begin{itemize}
  \item Rule out other abdominal tumours, especially neuroblastoma.
\end{itemize}
CONCLUSION
Paediatric renal tumours present a unique spectrum very much amenable to an accurate cytolological 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 cytogenticuses all combine in ensuring an accurate cytolological diagnosis.

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