*Dr. Venkateswaran K Iyer, MD**

Fine Needle Aspiration Cytology Diagnosis in Paediatric Uro-oncology

**Associate Professor, Department of Pathology, All India Institute of Medical Sciences, New Delhi*

Introduction

Fine needle aspiration cytology (FNAC) has a well established role in diagnosis of malignancy in all age groups of patients including in the paediatric age group.[1,2] Specifically in children, FNAC is the primary diagnostic modality for evaluation of liver masses in children since it is safe, does not have a risk of bleeding like core needle biopsy and yields a diagnosis in most liver tumors in conjunction with radiological imaging studies.[3] Most tumors of the liver are hepatoblastoma for which pre-operative chemotherapy is needed, which is given based on FNAC diagnosis.

Tumors of the paediatric kidney are much commoner, being the commonest after leukemia/lymphoma, tumors of the central nervous system and neuroblastic tumors.[4,5] FNAC or core needle biopsies are controversial in this location, needing a risk reward evaluation. The main concern in use of biopsy in kidney tumors is upstaging. In adults, renal cell carcinoma is the most common renal tumor. It is an aggressive neoplasm which responds poorly to chemotherapy. Upstaging is therefore deleterious and unless patient is already having disseminated disease, FNAC is contraindicated in renal cell carcinoma.

Wilms tumor (WT) on the other hand has effective chemotherapy available. Concerns of upstaging following core needle biopsy has abated following the publication of reviews in which the benefits of early diagnosis outweighed the concerns and it was demonstrated that core needle biopsy is safe and does not upstage the tumor.[6] FNAC is a less traumatic procedure than core needle biopsy in WT and is therefore preferable if results are equivalent to biopsy. No study comparing FNAC with core needle biopsy has been conducted as yet. However, publications on the benefits of pre-operative evaluation of paediatric renal tumors using FNAC are available.

Benefits of FNAC in Evaluation of Paediatric Renal Tumors

A number of advantages make FNAC an attractive investigational modality. The single biggest advantage in comparison to biopsy is that it does not require anaesthesia, an important benefit in paediatric patients. FNAC is safe, does not harm the patient, gives quick results and importantly, does not upstage a tumor. In a majority of instances, the treating physician gets the answer he is looking for from FNAC, thereby eliminating the need for biopsy. However, it is important to be aware of the limitations of FNAC as well. In general, FNAC is usually indicative and not the final diagnostic modality which is histopathology. Cytology is a very subjective science and only an experienced cytopathologist will be able to adequately assist the paediatric surgeon. Cytology cannot be effectively practiced by general histopathologists and the physician must be aware of this limitation.

In the specific setting of paediatric uro-oncology, FNAC serves certain important purposes.[7] The main aim is to identify rare variants or types of renal tumor which are treated differently. Secondary usefulness is in assessment of treatment response. WT, also known as nephroblastoma, is the most common renal tumor in children, which is capable of divergent differentiation along primitive blastemal, tubular as well as mesenchymal tissue.[4] It has a good outcome with surgery and chemotherapy, with tumors in Stage 1, II and III of favourable histology having over 95% survival.[8] With such an excellent outcome, the main focus of improving treatment in paediatric renal tumors has turned to early identification and specific targeted chemotherapy for rare variants which have poor outcome. These rare variants include anaplastic WT, clear cell sarcoma of the kidney (CCSK), Rhabdoid tumor of the kidney (RTK) and a newly described anaplastic sarcoma of the kidney which is exceptionally rare.[9] Many of these variants can be identified on FNAC, making it a useful procedure.

The other main indication for FNAC comes in Stage III paediatric renal tumor which is inoperable. In these it is necessary to give pre-operative chemotherapy to shrink the tumor, for which tissue diagnosis is preferable.[10,11,12] In developing countries like India, most tumors are in Stage III, many of which are inoperable. In a study of 52 consecutive cases seen on our department, 40 (76.9%) were in Stage III or higher stage and according to protocol were slated for chemotherapy before surgery. In this situation, FNAC is desirable before start of chemotherapy, since separate protocols exist for WT, anaplastic WT, CCSK and RTK.

The FNAC Procedure

FNAC is performed using a 22 to 26 gauge needle of adequate length to reach the mass. For superficial swellings a one inch or one and a half inch needle is used, fitted on a 10 or 20 ml syringe. Using a specially constructed syringe holder permits one handed operation, freeing up the other hand for localizing and stabilizing the swelling. The needle is introduced into the swelling without suction, then 5 to 10 ml of suction pressure is applied. Three to five passes of the needle, traversing through the substance of the swelling, are made. Then suction is completely released after which the needle is withdrawn. All material collected within the needle is expelled onto a small area of a slide. A second slide is used as a spreader to spread the material on to one or more slides. Spreading should evenly spread the material without causing crushing. Smears should ideally be subjected to both alcohol and air dried fixation. The smear should be immersed immediately in alcohol before the material dries in the air. Such smears are suitable for Papanicolaou staining (or Haematoxylin and Eosin in some centers). Airdried smears are suitable for Giemsa (May Grunwald Giemsa) staining. Papanicolaou stained smears are ideal for evaluating nuclear features and making a diagnosis of malignancy as well as the architectural features for subtyping the tumor. Giemsa stained smears provide additional details regarding cytoplasmic features which may help in subtyping in some cases.

In WT aspiration, the non-aspiration technique is preferred. In this, needle passes are made without application of suction and without attachment of a syringe. This is less traumatic, since respiratory movements are accommodated with corresponding external movement of the needle hub, the entry point into the skin acting as the fulcrum. Yields are somewhat less cellular than with suction but the aspirates are also less bloody, which is better for immunocytochemistry. The risk of intra-abdominal bleeding as well as tumor spillage are likely to be reduced by using this technique, although no study has specifically examined this issue. In contrast, using a syringe attached to a syringe holder makes the aspiration apparatus rigid. As the child breathes, the respiratory movements cannot be compensated by motion in the external aspiration apparatus. However, performing the aspiration procedure quickly within seconds, overcomes the limitations of using suction, especially as the suction process yields highly cellular material adequate for diagnosis and for making extra smears. Both aspiration procedures are therefore equally useful. Deep seated swellings which cannot be reached with an ordinary one and a half inch needle require ultrasound guidance and a 22 or 23 gauge LP needle. The majority of childhood intra-abdominal masses in India are large and can be aspirated without guidance using ordinary needles in an outpatient setting.

Complications of FNAC

Complications of FNAC are extremely rare. The only real complication is haemorrage, which can be problematic in abdominal masses, since it requires emergency nephrectomy, which is clearly undesirable. The incidence of this complication is around 1 %, making it uncommon. Ideally overnight observation of the patient should be done for sudden increase in abdominal girth. If the patient is going home, the parents should be explained the circumstances for which they should return immediately to the hospital and under no circumstance should they leave the city until the next day. Prothrombin time is a good screening test which should be available before intra-abdominal aspiration is performed without admission. Needle track tumor seedling has been over-emphasized in the past as a complication. Initial fears of tumor dissemination along needle track have not been substantiated in paediatric renal tumors, and it is now accepted that FNAC does not upstage a tumor.

FNAC in Paediatric Tumors

A study of fine needle aspiration cytology done in the AIIMS between 1987-2003 found 152 aspirations had been performed in the study period. The break up of cases is given in Table-1. The procedure had a 9.2% unsatisfactory rate, in which only blood or necrosis or cyst fluid was aspirated. There were no complications during this period. However, in 57 FNACs performed subsequent to this period, there were two instances in which patient developed intra-abdominal bleeding, characterized by sudden increase in abdominal girth. In both cases the patient had to be taken up for emergency nephrectomy.

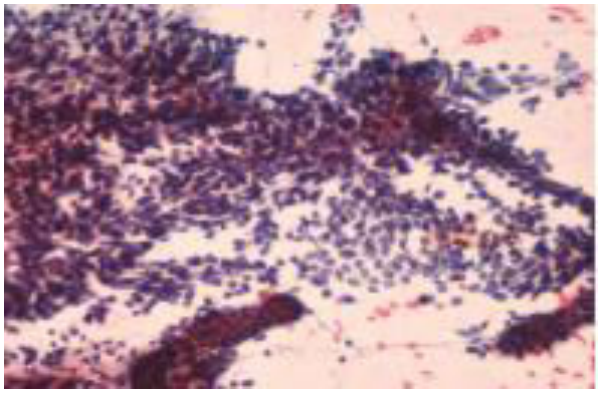
Table 1. FNAC in paediatric renal tumors seen at AIIMS from 1987 to 2003

Diagnosis	Number of cases	Percentage
Wilms tumor, favourable histology	108	71.05
Anaplastic Wilms (unfavourable histology)	2	1.32
Clear cell sarcoma	8	5.26
Rhabdoid tumor	1	0.66
Malignant round cell tumor, non-specific	19	12.5
Unsatisfactory aspiration procedure1	4	9.21
Total	152	100

FNAC of Wilms Tumor

FNAC features of WT are well described.[13,14,15,16,17,18] Aspirates from WT show varying proportions of blastema, tubules and mesenchyme. Triphasic or biphasic aspirates (having blastema and tubules) can be called WT on FNAC based on slide morphology alone. Aspirates with mesenchymal tissue almost always have tubules as well. A diagnosis of WT should not be made when blastema and mesenchyme alone are identified without accompanying tubules, since mesenchyme on cytology is difficult to distinguish with certainty from stromal tissue accompanying other malignant round cell tumors. Since the identification of tubules is important in diagnosis, criteria used for identification have to be stringent. Tubules are well defined tubular structures with anatomical borders. Vague rosetting is a common feature of blastemal cells and should not be called tubule. Similarly, vague aggregation of blastemal cells is common and this grouping should not be called epithelial differentiation.

Fig. 1. Aspirate from Wilms tumor showing blastema and two well defined tubules with abatomical borders. Papanicolaou, X 100.



In a study of 110 aspirates from WT seen over the period of 1987-2003, morphologic diagnosis of WT was possible in 72.7% of the 110 cases. The remaining 28.3% of aspirates could not be diagnosed since they showed only blastema, which is not reliably distinguishable from other malignant round cell tumors. It is important to note that these blastema only aspirates turn out to be WT in the vast majority, over 90% of instances. So a diagnosis of WT made in a paediatric renal mass is going to be WT, regardless of cytologic features, in over 90%. The utility of cytology is therefore tested not by the correct diagnosis of WT but by analyzing what proportion of the differential diagnosis and variants are correctly picked up. The frequency of important cytological features which help in diagnosis is given below.

Cystic change in WT can be occasionally seen. They have to be differentiated from multicystic nephroma and cystic partially differentiated nephroblastoma. Although FNAC features of CPDN are reported, this distinction is better made on radiological features, since aspirates will yield mainly fluid, on which definite diagnosis is not possible.[19]

Table 2. Frequency of cytomorphologic features in 110 aspirates from WT

Cytomorphologic Feature	Percentage
Blastemal cells	100
Tubules	72.73
Mesenchymal tissue	40
Rosettes	67.27
Glomeruloid bodies	9.09
Stroma predominance	10.0
Rhabdomyoblasts	4.55
Anaplastic WT	1.82

Anaplastic WT can be picked up on FNAC, using the same criteria as in histopathology, i.e. three times increase in nuclear size, hyperchromasia and atypical mitotic figures.[20] However, on FNAC a diagnosis of anaplastic WT is possible only when the aspirate shows tubular differentiation or other immunohistochemical evidence of WT. Blastema only aspirates from anaplastic WT cannot be diagnosed on FNAC without immunocytochemical support. Anaplasia when seen on FNAC is an indication of diffuse anaplasia which responds poorly to chemotherapy and for which a more intensive chemotherapy regimen is given.[21]

Stromal predominant WT can be present in 10% of WT aspirates and correlate well with stromal predominant histology in the corresponding excision specimen. In 4.55% of aspirates, there was rhabdomyoblastic differentiation in addition to stroma predominance. These should not be confused with primary rhabdomyosarcoma of the kidney, which is very rare.[22] Stroma predominant WT have good outcome similar to triphasic WT but because of their peculiar composition, they do not shrink with pre-operative chemotherapy, although the blastemal foci are ablated.[23] This can lead to a false impression of chemoresistance and suspicion regarding the primary diagnosis of WT. In these situations, prior FNAC characterization helps in evaluation of the treatment response.

Role of Immunohistochemistry in FNAC of Wilms Tumor

Two thirds of WT aspirates can be diagnosed on cytomorphology. One third shows blastema only. In these, immunocytochemistry on the aspirate should be performed. Cytokeratin is positive in epithelial elements of WT.[24] In blastema only aspirates, we have found 20% of cases to show focal cytokeratin positivity which helps in making a positive diagnosis. Cytokeratin is a common immunostain available in most laboratories, and hence important. However 80% of blastema only aspirates will come negative.

WT-1 is a specific immunohistochemical marker which is strongly positive in the nucleus of WT cells.[25, 26] It works well in cytology slides, since it is a nuclear rather than cytoplasmic stain. Positivity for WT-1 will establish a case as WT in over 95% of FNACs. WT-1 has to be interpreted in the correct clinical and cytomorphological context, since lymphoblastic lymphoma and desmoplastic small round cell tumors are also positive for WT-1.

Another vital role for immunocytochemistry is to rule out the main differential diagnosis of WT on aspirates. Hence immunocytochemistry for Synaptophysin (for neuroblastoma) and CD99 (for primitive neuroectodermal tumors) should also be performed.[24] At least 4 unstained smears from a case suspected to be WT should be alcohol fixed. After studying the morphology, these should be used for immunohistochemistry. By means of FNAC and immunohistochemistry, over 90% of cases will be accurately classified as WT and over 90% of the variants will be picked up. The remaining 10% will mostly get diagnosed after dye excretion tests, biochemistry and serum markers and a biopsy will be required in only rare instances.

FNAC of Clear Cell Sarcoma

CCSK is the most common tumor of the paediatric kidney after WT, but is rare, forming just 5% in the NWTS series.[27,28,29] WT never metastasizes to bone, whereas CCSK does so frequently and hence is termed bone metastasizing tumor.[27] CCSK should be suspected whenever a patient with renal mass has bony metastasis. However, statistically the commonest source of bony metastasis is neuroblastoma of the adrenal. PNET of the

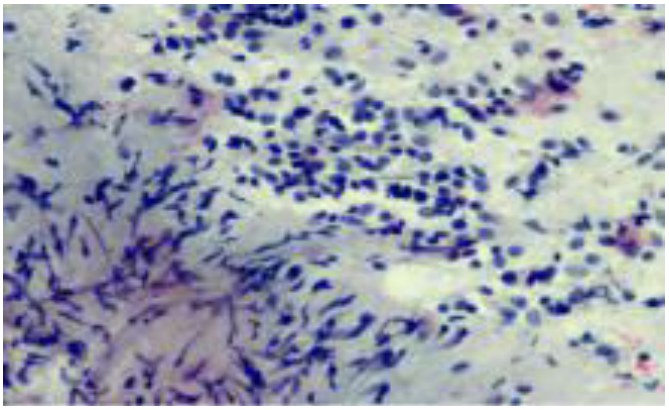
kidney and retroperitoneum are rarer than CCSK but also have a tendency to bony metastasis as does paediatric malignant pheochromocytoma and renal cell carcinoma, both of which are extremely rare in this age group.

The diagnosis of CCSK has become important. Recent advances in chemotherapy have improved survival, so that stage for stage, the results of therapy in CCSK are getting closer and closer to WT.[30,31] However, the chemotherapy regimen used is different from WT, incorporating doxorubicin. Early identification of a tumor as CCSK before bony or other distant metastasis takes place is the single most important intervention which can save a life. Presumptive treatment of CCSK as WT in Stage 3 with the wrong chemotherapy gives time for tumor progression and metastasis. The use of FNAC is important in this regard.[32]

Aspirates from CCSK are frequently misdiagnosed as WT. In our series of 8 cases, over half had been misdiagnosed on FNAC as WT or as anaplastic WT in some instances.[32] This is because CCSK shows presence of stroma like tissue which is mistaken for mesenchyme. Entrapped normal tubules are frequent on histology and can be seen in 25% of aspirates as well, leading to a mistaken triphasic appearance. These tubules have a benign appearance and can be easily categorized by an experienced cytopathologist.

CCSK on FNAC is well described.[32,33,34,35,36] Aspirates from CCSK are cellular and are composed of two cell types. The first is cord cells which have moderate cytoplasm, eccentric placement of nucleus and a vesicular grooved pleomorphic nucleus. Cord cells are always seen. The second cell type is septal cells which come in stromal fragments of spindle shaped cells arranged around vessels and are not always seen. Myxoid material is seen in these spindled fragments and in the background in a majority of cases. Careful attention to detail is needed to separate out CCSK from WT but distinction is almost always possible.

Fig. 2. **Aspirate from clear cell sarcoma showing the cord cells on the right and septal cells on the left embedded in myxoid matrix. Papanicolaou, X100**



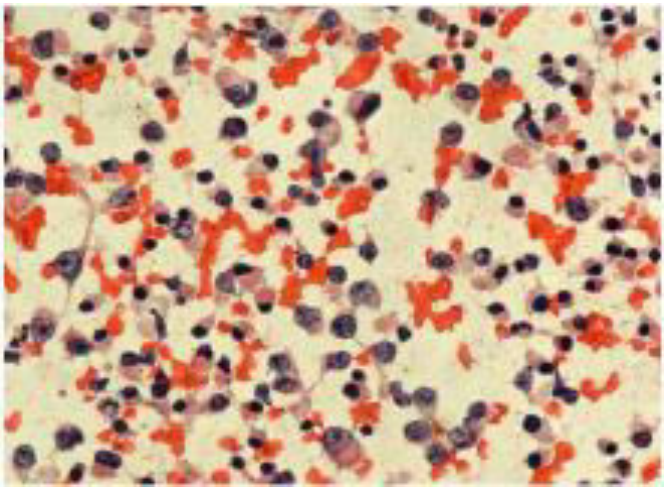
There is no positive stain or molecular marker for CCSK and its histogenesis remains uncertain. Negative immunocytochemistry for differentials (synaptophysin for neuroblastoma, cytokeratin and WT-1 for WT, CD99 for PNET) should be done before labeling a case as CCSK.[37,38,39] Since aspirates from CCSK have pleomorphism, a mistaken diagnosis of anaplastic WT can also be suspected on FNAC and needs to be ruled out with cytokeratin and WT-1 immunohistochemistry.

FNAC of Rhabdoid Tumor

This tumor is much rarer than CCSK and has a poor outcome. The single most important prognostic factor is early diagnosis before tumor progression and metastasis.[40,41] Although treatment for RTK is not as effective as for CCSK, many patients do respond and show improved survival. Hence early diagnosis based on FNAC is useful.

RTK, like CCSK, is also frequently mistaken for WT on FNAC. Aspirates from RTK are characterized by cells with cytoplasmic eosiniphilic inclusions and large nuclei with prominent nucleoli.[42] There is no tubular or mesenchymal differentiation. Morphology is characteristic but can show variability in extent which may not always be visible on FNAC. Immunocytochemistry for INI-1 is positive in all normal tissues but is negative in RTK, unlike all other tumors like CCSK and WT. This reflects deletion/translocation of the INI-1 gene in RTK, which can also be detected using molecular biological tests.

Fig. 3. **Aspirate from Rhabdoid tumor showing eosinophilic cytoplasmic inclusions and focal prominent nucleoli. Papanicolaou, X200.**

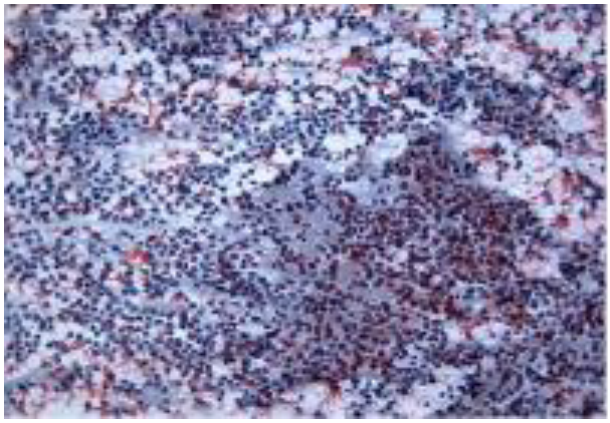


Differential Diagnosis

Radiology plays a vital role in diagnosis of tumors which occur in relation to the kidney and adrenal in the paediatric age group. In cytology practice, the main radiological concern is the confidence of the radiologist in placing a tumor as renal or supra-renal. In our experience, very large tumors replacing the whole kidney are difficult to place as primary renal tumor or a suprarenal tumor secondarily invading and destroying the kidney. Secondly, tumors arising in the upper pole of kidney need to be viewed with suspicion, since frequently they turn out to be suprarenal.

The main differential diagnosis of WT is with neurblastoma. FNAC of neuroblastoma is characteristic in a majority of cases, since it shows presence of a fibrillary neuropil background. Tumor cells are embedded in this fibrillary matrix and show prominent rosette formation.[43] A minority of cases look like malignant round cell tumors. Immunocytochemical markers like synaptophysin, chromogrannin, NB84 and GD-2 are positive in neuroblastoma and can help make a diagnosis in difficult cases. FNAC is an important diagnostic modality which can help in performing molecular biological tests as well.[44,45]

Fig. 4. **Aspirate from neoroblastoma showing neuropil material around which the tumor cells are arranged in rosettes.**

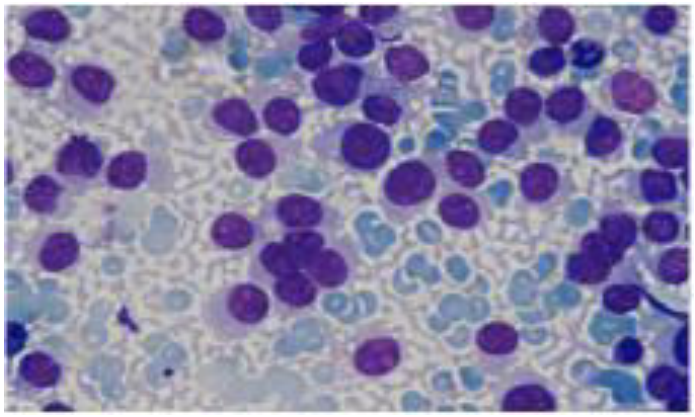


Papanicolaou, X100.

Pheochromocytoma and adrenocortical carcinoma are rare in the paediatric age group. These have characteristic biochemical markers in the form of norepinephrine, urinary VMA and cortisol levels which should be estimated. Anaplastic WT can be a differential diagnosis in the above tumors which can show considerable pleomorphism

Renal and retroperitoneal PNET are very rare in this age group. PNET can show presence of moderate cytoplasm and also have bony metastasis.[46] A mistaken diagnosis of CCSK should be avaoided in these. CCSK usually replaces most of kidney. If a good amount of kidney tissue is seen separate from the mass, confident FNAC diagnosis of CCSK should not be made without immunocytochemistry. PNET is positive for CD99 (Mic-2) which helps in diagnosis.

Fig. 5. Aspirate from PNET showing moderate cytoplasm. Giemsa, X 400



Rhabdomyosarcoma of the retroperitoneum is very rare.[47,48] Most aspirates have a round cell tumor appearance and only occasionally shows well differentiated rhabdomyoblasts and strap cells. Immunocytochemistry for myogenic markers are needed in difficult cases. Blastemal cells of WT are desmin positive in many instances, making desmin a poor marker when trying to differentiate WT and rhabdomyosarcoma. Myogenin is the best marker for myogenic differentiation in this situation, which being a nuclear stain is also easier to interpret in cytology material. Rhabdomyoblasts can be seen in WT aspirates which would also be myogenin positive. However, they usually come in triphasic aspirates in which a lot of stromal tissue is present, and hence do not come in the differential diagnosis.

Fig. 6. Aspirate from rhabdomyosarcoma shows a malignant round cell tumor in which the tumor cells show cytoplasm to one side. Papanicolaou, X400

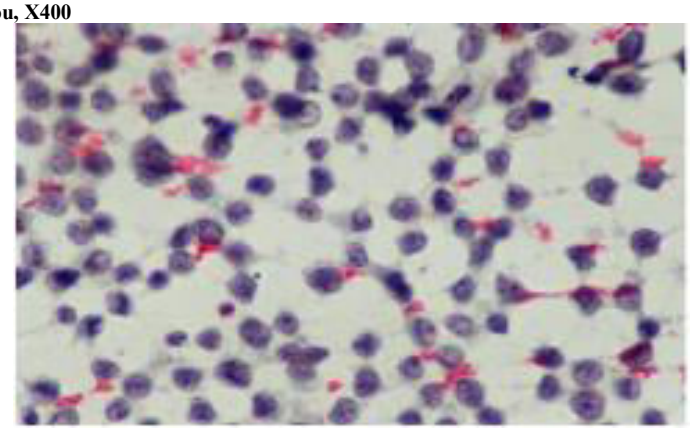
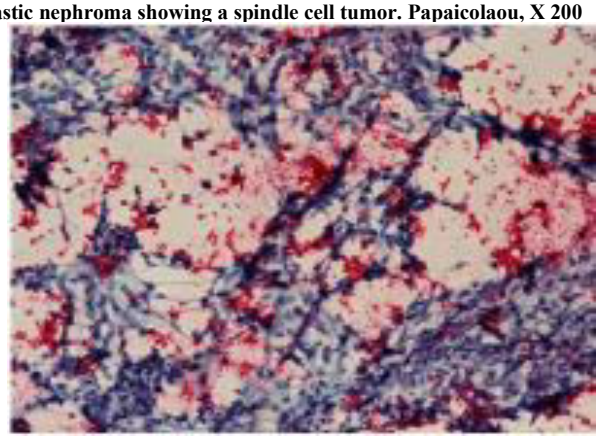


Fig. 7. Aspirate from mesoblastic nephroma showing a spindle cell tumor. Papaicolaou, X 200



Exceptionally, unusual presentations of lymphoma, leukemia or other tumors may be seen in the paediatric kidney.[49] Mesoblastic nephroma is a renal tumor seen in the neonatal period or in infancy and has a spindle cell appearance, which allows easy diagnosis on cytology.[50]

Conclusion

The main purpose of FNAC in paediatric uro-oncology is identification of tumor type so that specific targeted chemotherapy can be given. Separate chemotherapy regimens exist for WT, Anaplastic WT, CCSK, RTK, Neuroblastoma and PNET. FNAC from renal and supra-renal masses helps in selection of the correct chemotherapy regimen, especially in Stage 3 and above renal masses. In India over three fourths of cases present in Stage 3 or above, making FNAC valuable. Immmunocytochemistry plays an important role in diagnosis. There are specific markers for WT, RTK, neuroblastoma and PNET which helps in differential diagnosis.

Reference:

1. Cohen MB, Bottles K, Ablin AR, Miller TR. The use of fine needle aspiration biopsy in children. *West J Med,* 150: 665-667, 1989.
2. McGahey BE, Moriarty AT, Nelson WA, Hull MT. Fine needle aspiration biopsy of small round blue cell tumors of childhood. *Cancer,* 69: 1067-1073, 1992.
3. Iyer VK, Kapila K, Agarwala S, Verma K. Fine needle aspiration cytology of hepatoblastoma. Recognition of subtypes on cytomorphology. *Acta Cytol*, 49: 355-364, 2005.
4. Schmidt D, Beckwith JB. Histopathology of childhood renal tumors. *Hematol Oncol Clin North Am*, 9: 1179-1200, 1995.
5. Beckwith JB. Chapter 43. Renal neoplasms of childhood. In Diagnostic Surgical Pathology, Third edition, Sternberg SS (Ed). *Lippincott Williams and Wilkins, Philadelphia,* pp1825-1852, 1999.
6. Vujanic GM, Kelsey A, Mitchell C, Shannon RS, Gornall P. The role of biopsy in the diagnosis of renal tumors of childhood: Results of the UKCCSG Wilms tumor study 3. *Med Pediatr Oncol*, 40: 18-22, 2003.
7. Goregaonkar R, Shet T, Ramadwar M, Chinoy R.Critical role of fine needle aspiration cytology and immunocytochemistry in preoperative diagnosis of pediatric renal tumors. *Acta Cytol*, 51: 721-729, 2007.
8. Metzger ML, Dome JS. Current Therapy for Wilms' Tumor. *Oncologist,* 10: 815-826, 2005.
9. Vujanic GM, Kelsey A, Perlman EJ, Sandstedt B, Beckwith JB. Anaplastic sarcoma of the kidney. A clinicopathologic study of 20 cases of a new entity with polyphenotypic features. *Am J Surg Pathol*, 31: 1459-1468, 2007.
10. Beckwith JB, Palmer NF. Histopathology and prognosis of Wilms tumors: results from the First National Wilms' Tumor Study. *Cancer*, 41(5): 1937-1948, May 1978.
11. Green DM, Thomas PR, Shochat S. Treatment of Wilms tumor: Results of the National Wilms Tumor Studies. *Hematol Oncol Clin North Am*, 9: 1267-1274, 1995.
12. Ritchey ML, Pringle KC, Breslow NE, Takashima J, Moksness J, Zuppan CW, Beckwith JB, Thomas PR, Kelalis PP. Management and outcome of inoperable Wilms tumor: A report of National WIlms Tumor Study-3. *Ann Surg*, 220: 683-690, 1994.
13. Quijano D, Drut R. Cytologic characteristics of Wilms' tumors in fine needle aspirates. A study of ten cases. *Acta Cytol*, 33: 263-266, 1989
14. Sharifah NA. Fine needle aspiration cytology characteristics of renal tumors in children. *Pathology*, 26: 359-364, 1994.
15. Dey P, Radhika S, Rajwanshi A, Rao KLN, Khajuria A, Nijhawan R, Banarjee CK. Aspiration cytology of Wilms' tumor. *Acta Cytol*, 37: 477-482, 1993.
16. Hazarika D, Narasimhamurty KN, Rao CR, Gopinath KS. Fine needle aspiration cytology of Wilms tumor: A study of 17 cases. *Acta Cytol*, 38: 355-360, 1994

17. Iyer VK, Kapila K, Agarwal S, Dinda AK, Verma K. Role of fine needle aspiration and ploidy by image analysis in the prognostication of Wilms tumour. *Analyt Quant Cytol Histol*, 21: 505-511, 1999.
18. Radhika S, Bakshi A, Rajwanshi A, Nijhawan R, Das A, Kakkar N, Joshi K, Marwaha RK, Rao KLN. Cytopathology of uncommon renal neoplasms in the pediatric age group. *Diagn Cytopathol*, 32: 281-286, 2005.
19. Nayak A, Iyer VK, Agarwala S, Verma K. Fine needle aspiration cytology of cystic partially differentiated nephroblastoma of the kidney. *Cytopathology*, 17(3): 145-148, June 2006.
20. Drut R, Pollono D. Anaplastic Wilms' tumor. Initial diagnosis by fine needle aspiration. *Acta Cytol*, 31: 774-776, 1987.
21. Dome JS, Cotton CA, Perlman EJ, Breslow NE, Kalapurakal JA, Ritchey ML, Grundy PE, Malogolowkin M, Beckwith JB, Shamberger RC, Haase GM, Coppes MJ, Coccia P, Kletzel M, Weetman RM, Donaldson M, Macklis RM, Green DM. Treatment of anaplastic histology Wilms tumor: results from the fifth National Wilms Tumor Study. *J Clin Oncol*, 24: 2352-2358, 2006.
22. Iyer VK, AlSalti W, Morland B, Parkes S. Embryonal rhabdomyosarcoma of the kidney in childhood. *Ann Diagn Paediatr Pathol*, 4: 31-34, 2000.
23. Saba LMB, Camargo C, Gabriel-Aran M. Experience with six children with Fetal rhabdomyomatous nephroblastoma: review of the clinical, biologic, and pathologic features. *Med Pediatr Oncol*, 30: 152-155, 1998.
24. Brahmi U, Rajwanshi A, Joshi K, Ganguly NK, Vohra H, Gupta SK, Dey P. Role of immunocytochemistry and DNA flow cytometry in the fine-needle aspiration diagnosis of malignant small round-cell tumors. *Diagn Cytopathol*, 24: 233-239, 2001.
25. Ramani P, Cowell JK. The expression pattern of Wilms' tumour gene (WT1) product in normal tissues and paediatric renal tumours. *J Pathol*, 179: 162-168, 1996.
26. Satoh F, Tsutsumi Y, Yokoyama S, Osamura RY. Comparative immunohistochemical analysis of developing kidneys, nephroblastomas and related tumors: considerations on their histogenesis. *Pathol Int*, 50: 458-471, 2000.
27. Argani P, Perlman EJ, Breslow NE, Browning NG, Green DM, D'Angio GJ, Beckwith JB. Clear cell sarcoma of the kidney. A review of 351 cases from the National Wilms' Tumor Study Group pathology center. *Am J Surg Pathol*, 24: 4-18, 2000.
28. Sotelo-Avila C, Gonzalez-Crussi F, Sadowinski S, Gooch WM 3rd, Pena R. Clear cell sarcoma of the kidney: a clinicopathologic study of 21 patients with long-term follow-up evaluation. *Hum Pathol*, 16(12): 1219-1230, December 1985.
29. Cheah PL, Looi LM, Lin HP. Clear cell sarcoma of kidney: a clinicopathological study of eight cases from Malaysia. *Histopathology*, 21: 365-369, 1992.
30. Green DM, Breslow NE, Beckwith JB, Moksness J, Finklestein JZ, D'Angio GJ. Treatment of children with clear-cell sarcoma of the kidney: a report from the National Wilms' Tumor Study Group. *J Clin Oncol*, 12: 2132-2137, 1994.
31. Seibel NL, Li S, Breslow NE, Beckwith JB, Green DM, Haas GM, Ritchey ML, Thomas PR, Grundy PE, Finklestein JZ, Kim T, Shochat SJ, Kelalis PP, D'Angio GJ. Effect of duration of treatment on treatment outcome for patients with clear-cell sarcoma of the kidney: a report from the National Wilms' Tumor Study Group. *J Clin Oncol*, 22: 468-743, 2004.
32. Iyer VK, Agarwala S, Verma K. Fine needle aspiration cytology of clear cell sarcoma of the kidney: Study of eight cases. *Diagn Cytopathol*, 33: 83-89, 2005.
33. Iyer VK, Kapila K, Verma K. Fine needle aspiration cytology of clear cell sarcoma of the kidney with spindle cell pattern. *Cytopathology,* 14: 160-164, 2003.
34. Akhtar M, Ali MA, Sackey K, Burgess A. Fine needle aspiration biopsy of clear-cell sarcoma of the kidney: Light and electron microscopic features. *Diagn Cytopathol*, 5: 181-187, 1988.
35. Drut R, Pomar M. Cytological characteristics of clear- cell sarcoma of the kidney (CCSK) in fine needle aspiration biopsy (FNAB): A report of 4 cases. *Diagn Cytopathol*, 7: 611-614, 1991.
36. Srinivasan R, Nijhavan R, Dey P, Samujh R. Fine needle aspiration cytology of clear cell sarcoma of the kidney and its distinction from Wilms' tumor (letter). *Acta Cytol,* 41: 950-951, 1997.

37. Looi LM, Cheah PL. An immunohistochemical study comparing clear cell sarcoma of the kidney and Wilms' tumor. *Pathology*, 25: 106-109, 1993.

38. Hsueh C, Wang H, Gonzalez-Crussi F, Lin JN, Hung IJ, Yang CP, Jiang TH. Infrequent p53 gene mutations and lack of p53 protein expression in clear cell sarcoma of the kidney: immunohistochemical study and mutation analysis of p53 in renal tumors of unfavorable prognosis. *Mod Pathol*, 15: 606-610, 2002.

39. Cheah PL, Looi LM. Implications of p53 protein expression in clear cell sarcoma of the kidney. *Pathology*, 28: 229-231, 1996.

40. Tomlinson GE, Breslow NE, Dome J, Guthrie KA, Norkool P, Li S, Thomas PR, Perlman E, Beckwith JB, D'Angio GJ, Green DM. Rhabdoid tumor of the kidney in the National Wilms Tumor Study: age at diagnosis as a prognostic factor. *J Clin Oncol*, 23: 7641-7645, 2005.

41. Reinhard H, Reinert J, Beier R, Furtwängler R, Alkasser M, Rutkowski S, Frühwald M, Koscielniak E, Leuschner I, Kaatsch P, Graf N. Rhabdoid tumors in children: prognostic factors in 70 patients diagnosed in Germany. *Oncol Rep*, 19: 819-823, 2008.

42. Barroca HM, Costa MJ, Carvalho JL. Cytologic profile of rhabdoid tumor of the kidney. A report of 3 cases. *Acta Cytol*, 47(6): 1055-1058, November-December, 2003.

43. Akhtar M, Ali MA, Sabbah R, Bakry M, Sackey J, Nash NJ. Aspiration cytology of neuroblastoma. *Light and electron microscopic correlations*.

44. Moorthy G, Agarwala S, Sharma A, Iyer VK, Srinivas M, Bajpai M, Bhatnagar V, Gupta DK. Cytogenetic evaluation of neuroblastoma using fine needle aspiration cultures. *Pediatric Surgery International 2009*.

45. Luttikhuis MO, Iyer VK, Dyer S, Ramani P, McConnville CM. Detection of MYCN amplification in neuroblastoma using competitive PCR quantitation. *Laboratory Investigation,* 80: 271-273, 2000

46. Silverman JF, Berns LA, Holbrook CT, Neill JSA, Joshi VV. Fine needle aspiration cytology of primitive neuroectodermal tumors. A report of three cases. *Acta Cytol,* 36: 541-550, 1992.

47. Seidal T, Walaas L, Kindblom LG, Angervall L. Cytology of embryonal rhabdomyosarcoma. A cytologic, light microscopic, electron microscopic and immunohistochemical study of seven cases. *Diagn Cytopathol,* 4: 292-300, 1988.

48. Iyer VK, Verma K. Convoluted and cleaved nuclei in rhabdomyosarcoma: An unusual morphologic feature causing diagnostic difficulty. *Acta Cytol*, 49: 465-467, 2005

49. Jindal B, Agarwala S, Bakhshi S, Jain V, Gupta AK, Kumar R, Bal CS, Iyer VK, Gupta SD. Bilateral primary renal lymphoma with orbital metastasis in a child. *Pediatr Blood Cancer,* 52: 539-541, 2009.

50. Gupta R, Mathur SR, Singh P, Agarwala S, Gupta SD. Cellular mesoblastic nephroma in an infant: report of the cytologic diagnosis of a rare paediatric renal tumor. *Diagn Cytopathol*, 37: 377-380, 2009.