



Immunocytochemistry of Small Round Cell Tumors on Fine Needle Aspiration Cytology

Authors

Ashwini Nargund, Malathi M, Akkamahadevi Patil, Siddhartha Biswas

Department of Pathology, Division of Cytology, Kidwai Cancer Institute, Bengaluru

Abstract

Background and Objectives: *The malignant round cell tumors (MRCT) comprises a group of tumors with a variable histogenesis & biological behavior. Various ancillary investigative techniques have been applied to achieve the diagnosis of MRCT. This study was carried out to confirm the diagnosis of small round cell tumors (SRCT) on FNAC smears using ICC.*

Methods: *25 cases were selected irrespective of any age and sex during the year 2005 to 2006. Fine needle aspiration cytology was performed on the lesion after informed consent from patients. Based on the preliminary diagnosis of malignant small round cell tumor on light microscopy additional specimen were obtained for ICC studies. The results were compared with the cytogenetic and histopathology studies.*

Results: *A total a 25 cases were studied. ES/PNET (14 cases) were the maximum number of cases studied. The cytomorphological diagnosis was confirmed by immunocytochemistry in 76% cases (19 of 25 cases) and revised the diagnosis 8% (2 of 25 cases) cases. The ICC results correlated well with the cytogenetic analysis results, agreement was seen in 11 of the 15 cases which had both ICC & cytogenetic analysis done (73.3%). 5 of the 9 cases which had both ICC & excision biopsy done showed correlation (55%).*

Conclusion: *Immunocytochemistry aided in the confirming of cytological findings. Invasive open biopsies can be avoided there by reducing the trauma to the patient Therefore immunocytochemistry on FNAC smears needs to be included in the list of investigation for the diagnosis of malignant small round cell tumors.*

Keywords: *FNAC, SRCT, Immunocytochemistry, Ewing Sarcoma/PNET, cytogenetics.*

Introduction

Malignant small round cell tumors (MSRCT) are a group of neoplasm characterized by small, round, relatively undifferentiated cells². They present a common diagnostic difficulty in clinical practice and may be indistinguishable by light microscopy (LM). This group of tumors generally includes Neuroblastoma (NB), Ewing Sarcoma (EW)/Primitive neuroectodermal tumor (PNET), malignant lymphoma, rhabdomyosarcoma (RMS)

and Wilms tumor (WT). Several other tumors such as retinoblastoma, hepatoblastoma, small cell osteosarcoma also belong to this category¹.

This group of tumors typically arise during childhood, plus a number of tumors occur in adults. Fine needle aspiration is a very useful technique in the diagnosis of small round cell tumors. If the cytologic features are evaluated along with ancillary study as immunocytochemistry, a correct diagnosis can be

made in most cases. Deep seated lesions have become accessible to the needle due to the advance in the imaging techniques like ultra sonography (USG) and computerized tomography (CT). If a definitive diagnosis can be offered on aspirated material, it will dramatically reduce the hospitalization costs and operative costs and at the same time reduces the time considerably before a definite treatment protocol is planned.

Aims

1. To confirm the diagnosis of Small round cell tumors on immunocytochemistry of FNA smears in our clinical set up.
2. To reduce the hospitalization costs and of biopsy costs of the lesion by performing FNA procedures.
3. To provide definitive diagnosis of small round all tumors to facilitate the initiation of definitive therapy.
4. To compare the results thus obtained with the results derived from the cytogenetic analysis and histopathological study & thus assess usefulness of immunocytochemistry in the management of patient with small round cell tumor.

Material and Methods

25 cases are retrieved during one year (Sept 2005 to Aug 2006) with clinical suspicion of small round cell tumor. The FNA smear for immunocytochemistry were done at the department of cytopathology. The age, sex date of admission, presenting symptoms, swelling, laboratory parameters, site of FNAC, previous history of surgery or any other treatment details were obtained.

Informed consent is obtained from the patient or patients kin. Proper clinical work up is done. FNA was performed by the standard technique. For superficial lesions a 22 gauge, 1.4. inch needle was attached to a disposable 10-ml syringe attached to a cameco syringe pistol. Sonographic guided FNA was done by the guidance of prob for

deep masses using 22 gauge needle. CT guidance was used for thoracic lesion.

At least two aspirations were performed. Air dried, as well as slides immediately fixed using cytofix (poly ethylene glycol & 80% Isopropanol) were used. The air dried smears were stained with May Grunwald Giemsa (MGG) to evaluate specimen adequacy & to give a preliminary diagnosis. Based on the Preliminary diagnosis of malignant round cell tumor on light microscope additional specimens were obtained for immunocytochemical studies.

In the lesions where cytomorphology was convincing limited panels of antibodies were put up for confirmation of the lesion. The antibody panels used were as given below. Number of smears available depended on the amount of the material aspirated Minimum of Six smears was made including both wet fixed & air dried smears.

The antibody panels: After the cytological evaluation & arriving at a differential diagnosis a panel of antibodies are selected, ie., leukocyte common antigen (LCA, catalogue No. NCL-LCA-RP, Novocastra) neuron-specific enolase (NSE, Catalogue No. M0873, DAKO) chromogranin (catalogue No. NCL-CHROM, Novocastra) Desmin (catalogue No. NCL-DES-DER-II, Novocastra) Epithelial membrane antigen (EMA, catalogue No. NCL-EMA, Novocastra), Cytokeratin (CK, catalogue No. NCL-PAN-CK, Novocastra) MyoD1, (Catalogue No. M3512, Dako) S100 (catalogue No. Z0311, Dako) Alpha fetoprotein (AFP, catalogue No. A0108, Dako) Synaptophysin (Syn, Catalogue No. A0010, Dako) CD20 (catalogue No. M0755, Dako), CD3 (catalogue No. NCL-CD3-PS1, Novocastra) and MIC-2 gene (catalogue No. NCL-CD99, Novocastra).

Preparation of cell block: Realizing the drawback of limited number of smears, attempt is made in all possible cases to prepare the cell blocks form the aspirated material. Aspirated materials is taken into sterile normal saline, needle is washed with saline & immediately centrifuged at 1000 rpm speed. Supernatant decanted off &

formalin is added to the cell button. The cell blocks were processed as any other surgical pathology material.

Procedure of Staining

The preserved smears are brought to room temperature. The slides are washed in two changes of Tris buffer saline (TBS) at pH 7.6 for 5 minutes each. **Smears should not get dried at any stage** of the staining. Endogenous peroxides are blocked by immersing the slides in freshly prepared 0.3% methanol-hydrogen peroxide solution in a coplin jar for 30 minutes. Wash in TBS for 2 minutes. Moist chamber is used for staining procedure. The slides are overlaid with blocking serum for 20 minutes. The blocking serum is tapped off the slide but **NOT WASHED**. The area on the slide without the smear is wiped off carefully to conserve the amount of primary antibody and to avoid dilution of the antibody with the buffer. Primary antibody is put over the smear area to be stained and incubated at 2-6 degrees Celsius overnight in refrigerator in the moist chamber. Slide is washed in two changes of TBS for 10 minutes each after decanting the antibody. Secondary antibody is applied and incubated at room temperature for 45 minutes. Antibody is decanted and washed in two changes of TBS for

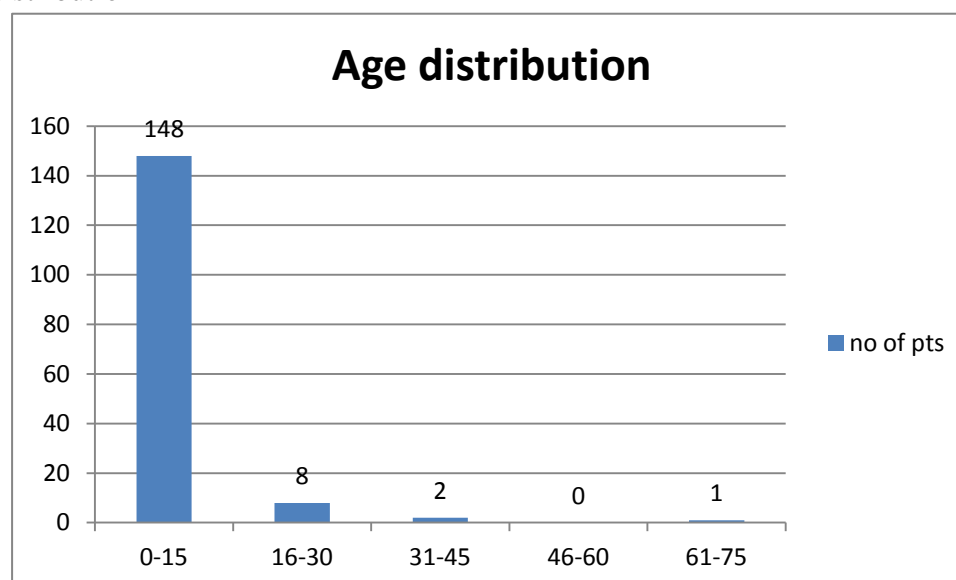
10 minutes each. The excess buffer is wiped around the smears carefully and steptavidine-peroxidase reagent is overlaid and incubated for 45 minutes in moist chamber. The reagent is tapped off and washed in two changes of TBS for 10 minutes each.

Chromogen is prepared fresh by adding 4 drops of DAB Solution to 5 ml of DAB substrate. Smear is covered with the solution and incubated for a period of 5 to 15 minutes by monitoring of development of brown colour under the microscope. The reaction is stopped by rinsing in distilled water. Counter stained with Mayer's Haematoxylin for 1 minute and washed in running water for 2 minutes. Smears are then dried and mounted in DPX. Always controls were made put up with every batch of smears. Positive reaction is indicated by brown coloured precipitate at sites of specific cellular localization.

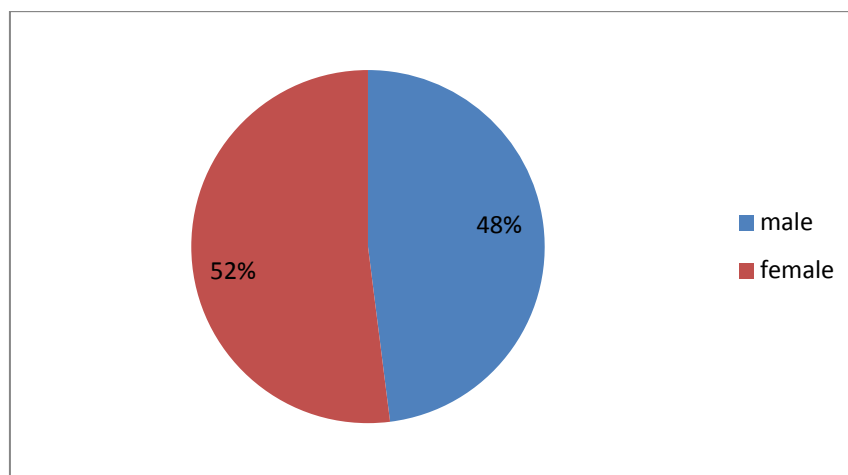
Result

A total of 25 patients of malignant small round cell tumors, diagnosed on FNAC were taken for this study. The study group had a wide age range, ranging from 6 month to 60 years with a higher incidence (n=14, 56%) in the 0-15 years age group.

Graph 1: Age distribution



Among the 25 patients, 12 (48%) were males & 13 (52%) were females.

Graph 2 : Sex Distribution

Among the 25 cases, the site of swelling were extremities 6 (24%) cases, head & neck including mouth, jaw 6 (24%) cases, Abdomen 5 (20%) cases, loin 1 (4%) case, eye 1 (4%) case, gluteal region 1 (4%) case, mediastinum 1 (4%) case, pelvic region 3 (12%) case & retroperitoneum 1 cases (4%). 10 cases (40%) among the 25 cases had associated symptom of pain. 3 cases had recurrence after 1 year of surgery, 1 case had recurrence after 3 years of surgery. All the cases had recurrence at the same primary site except for 1 case that had recurrence around knee joint with primary in gluteal region. Among the 25 cases, 10 cases (40%) had bony lesion, 8 cases (32%) had soft tissue lesion, 3 had adrenal mass (12%) & eye (4%), mediastinum (4%), liver (4%) & retroperitoneum (4%) had each of 1 case respectively. 11 patients presented with metastasis. The site of secondaries were, multiple skeletal metastasis was 6 cases (54%), Lung metastasis 1 case (9.09%), lymph node metastasis (18.18%) in 2 cases, Scalp 1 case (9.09%), encasing inferior venacava in 2 (18.18%), Infiltrating Liver (9.09%), Optic nerve 1 case (9.09%) each respectively. 3 cases had bone marrow involvement from the primary disease.

Morphological features

Relevant clinical & radiological data were available during the interpretation of FNAC smear. The light microscopic appearance of FNAC smears from various MRCT (25 cases) was characterized as follows.

ES/PNET (14 cases): The cytomorphology of ES and PNET tumors was indistinguishable and consisted of numerous malignant cells with high nuclear cytoplasmic ratio & focal nuclear molding. (Fig. 1 & 2) The nuclei had evenly dispersed chromatin pattern with small indistinct to no nucleoli seen. Cytoplasm was scanty with fine vacuolations. Mitotic activity was variable, 2 cases showed rosette formation.

Neuroblastoma (3 cases): The aspirates were hyper cellular with numerous individually scattered small cells and some cohesive groups. A few larger cells with moderate amounts of cytoplasm were also identified. The nuclei were oval to slightly irregular and had an evenly dispersed 'Salt and pepper' chromatin pattern with small to inconspicuous nucleoli. Some aspirates contained fibrillary tangles of neurotic process (neuropil) (Fig.6a&6b). Rosette formation was noted.

Ganglioneuroblastoma (2 cases): Ganglioneuroblastoma is the related lesion of neuroblastoma. This demonstrates characteristic ganglion cells with neuronal fragments. Ganglion cells have larger nuclei with prominent nuclei and moderate amounts of coarsely granular cytoplasm (Fig. 7a & 7b).

Rhabdomyosarcoma (2 cases) :FNAC smears showed pleomorphic cells in sheets, discretely and occasionally in small clusters, with scanty to moderate amounts of cytoplasm containing occasional small cytoplasm vacuoles. One case

showed binucleation. A myxoid background was also noted (Fig. 3a & 3b)

Retinoblastoma (1 case): The cells were similar to those of neuroblastoma. The cells were small, uniform and generally closely packed into clusters of variable sizes.

Hepatoblastoma (1 cases): The smears showed uniform fetal type hepatocytes appearing as somewhat cohesive groups in acinar arrangements. The cytoplasm was abundant with oval. Central nuclei and nucleoli, slight degree of pleomorphism was noted (Fig. 4a,4b).

Immunocytochemistry

Table 8: Details of Immunocytochemistry of Small round cell tumors

Antibodies	ES/PNET (14)	NB (3)	GNB (2)	RMS (2)	RB (1)	HB (1)	BL (1)	LL (1)
CD 99	14	Neg	Neg	1	Neg	Neg	Neg	1
Vimentin	14	-	Neg	1	-	Neg	-	-
Desmin	Neg	Neg	-	2	-	-	-	-
MyoD1	-	-	-	2	-	-	-	-
NSE	6	3	2	-	1	-	-	-
Chromogranin	Neg	1	2	-	Neg	-	-	Neg
Synptophysin	1	-	-	-	1	-	-	Neg
S 100	-	2	2	-	-	-	-	-
CK	Neg	-	-	Neg	-	1	-	Neg
AFP	-	-	-	-	-	1	-	-
LCA	Neg	-	-	-	-	-	1	1
CD 20	-	-	-	-	-	-	1	Neg
CD 3	-	-	-	-	-	-	Neg	1
EMA	-	-	-	-	-	1	-	-

Out of 14 cases Ewings sarcoma/PNET, all 14 were positive for C 99 (Fig.2a,2b) & vimentin (Fig.2d), while 6 of 14 were positive for NSE (Fig.2c) & 1 of 14 were positive for Synaptophysin. All cases of ES/PNET were negative for CK, LCA and Desmin.

Out of 3 cases of neuroblastoma, all were positive for NSE, 2 of 3 were positive for S100 & 1 of 3 were chromogranin positive. All three were negative for CD 99 and Desmin.

Both the cases of Ganglioneuroblastoma were positive for NSE, chromogranin and S100 both were negative for CD 99 & VIMENTIN.

Both the cases of Rhabdomyosarcoma was positive for MyoD₁ (Fig.3c, 3d, 3e), & Desmin , 1 of 2 cases was positive for CD 99.1 of 2 case was positive for vimentin. Both were negative for CK.

Burkitt Lymphoma (1 case) : The smears were hyper cellular composed of uniform lymphocytes with round nuclei, conspicuous nucleoli, and small but variable cytoplasmic vacuoles. (Fig.5a ,5b) there wre tangible body macrophages seen throughout the smear.

Lymphoblastic Lymphoma (1 case) : The smear contained monotonous Lymphoblast's having round or convoluted nuclei, finely granular chromatin inconspicuous nucleoli & scant amount of cytoplasm . Tingible body macrophages were variably present.

One case of Lymphoblastic Lymphoma was positive for CD99, LCA , CD 3 & negative for CD 20, CK, chromogranin synaptophysin.

Histopathology

Out of 25 cases of malignant small round cell tumors, 9 cases were subjected to the biopsy 5 of 9 were concluded as case of ES/PNET supported by IHC & 2 of 9 was diagnosed as Non Hodgkins lymphoma & was advised for IHC for final diagnosis 2 of 9 was diagnosed as malignant round cell tumor & was advised for IHC for further categorization.

Cytogenetics

Out of 25 cases of malignant small round cell tumors, 15 cases were available for cytogenetic analysis. 11 of 15 cases had satisfactory sample and showed characteristic cytogenetic abnormalities as shown blow in the table 9. 4 of 15 samples were unsatisfactory for opinion.

Table 9 Characteristic Cytogenetic Abnormalities

	Diagnosis	Characteristic cytogenetic abnormalities	Number of cases
1	ES/PNET	t(11,22) (q24,q120)	7
2	ES/PNET	t(7,22) (q22, q12)	1
3	Neuroblastoma	del 1 (p32,p36)	2
4	Burkitt lymphoma	t(8,14) (q24,q32)	1

Treatment details

Table 10 : Table below shows the details of treatment received.

Case	Chemotherapy	Radiation therapy	Excision	Supportive and symptomatic	Lost of follow up
ES/PNET (14)	8	5	5	-	2
NB (3)	1	-	-	1	1
GNB(2)	1	-	-	1	-
RMS (2)	1	-	-	-	1
RB (1)	-	-	-	-	1
HB (1)	-	-	-	1	-
BL (1)	1	-	1	-	-
LL (1)	1	1	-	-	-

Metastatic status at onset

Table 11: Metastatic status at onset

Case	Bone	Lung	Lymph node	Liver	Inferior venacava
ES/PNET (7 cases)	6	1	3	-	-
GNB (2 cases)	-	-	-	-	2
NB (2 case)	-	-	-	1	1

Table 12 below shows the clinical, pathological and radiological details of the 25 cases taken for this study.

Table 12: Clinico Pathological details of Small round cell tumors

Case No.	Age (yrs)	Sex	Site	Fever	Pain	Past History	Biochemistry/Blood
1	8	F	Thigh	-	-	Operated 3 yrs back recurrence-same site	Normal
2	30	F	Shoulder	-	-	-	↑ Total count
3	16	M	Temporla region	-	+	-	AST, ALT, ALP
4	8	F	Thigh	+	+	-	↑ AST, ALT ALP TC
5	61	M	Scalp, Temple	-	-	-	AST, ALT, ALP
6	6	F	Mouth (Palate)	-	-	-	ALP TC
7	13	F	Back	+	+	-	ALP, LDH TC
8	8	F	Jaw (lower)	+	+	-	ALP, LDH TC
9	19	M	Cheek	+	-	-	ALP, LDH TC
10	11	F	Leg	-	+	Operated 1 yr back	Total count
11	2	F	Abdomen	+	-	-	LDH, AST
12	19	F	Loin	-	-	Operated 1 yr back	Normal
13	19	F	Mandible	-	-	-	Normal
14	32	F	Thigh	-	-	-	Normal
15	6 months	M	Abdomen	+	-	-	TC, AST AFA
16	3	F	Eye	-	-	-	TC
17	4	M	Abdomen	+	+	-	Normal
18	31/2	M	Gul teal	+	-	-	Normal
19	32	M	Pelvic region	-	+	-	ALP
20	25	M	Iliac mass	-	+	-	Normal
21	3	M	Abdomen scalp, Preauricular	+	-	-	Normal
22	22	M	Mediastinum	-	-	-	Normal
23	2 ½	F	Abdomen	+	-	-	LDH
24	20	M	Knee Joint	-	+	Operated an (Rt) Gul teal region	TC AST
25	4	F	Abdomen	+	+	-	TC ALP ST AL

Case No.	FNAC	IHC	CG	Histopathology	X-ray site	USG Abdomen	Metastatic work up	BMA	Bone/Soft tissue
1	SRCT	ES/PNET	ES/PNET	ES/PNET	Soft tissue mass	Normal	-ve	Normal	Soft tissue
2	SRCT (Small Cell Carcinoma/ES/PNET)	ES/PNET	ES/PNET	Round Cell tumor, Advice IHC	Metastatic deposit on bone	Not done (ND)	Bone, Lymph node	ND	Bone
3	SRCT	ES/PNET	ES/PNET	Not done (ND)	Ct-Scan Bone :ES/PNET	Not done (ND)	-ve	Normal	Bone
4	ES/PNET	ES/PNET	ES/PNET	Not done (ND)	Bone lesion : ES	Normal	-VE	Normal	Bone
5	ES/PNET	ES/PNET	ES/PNET	Not done (ND)	Bone	Not done (ND)	Lymph node, Bone	Normal	Bone
6	SRCT	ES/PNET	ES/PNET VARIANT TRANSLOCATION	Not done (ND)	Bone lesion CT Scan (Maxila)	Not done (ND)	-ve	ND	Bone
7	SRCT	ES/PNET	ES/PNET	Not done (ND)	Bone disseminated ES	Normal	Bone	Involved by SRCT	Bone
8	SRCT	BL	BL	NHL Advice IHC	ND	Normal	Not done (ND)	ALL-L3	Bone
9	SRCT	BL	BL	NHL Advice IHC	ND	Normal	Not done (ND)	ALL-L3	Bone
10	ES/PNET	ES/PNET	ES/PNET	ES/PNET	Bone -ES	Not done (ND)	-VE	Normal	Bone
11	NB	Not conclusive	NB	Not done (ND)	Adrenal mass, Infiltration of @ lobe of liver	Not done (ND)	Not done (ND)	Normal	Adrenal
12	SRCT	ES/PNET	Not done (ND)	Extraskeletal PS/PNET	Not done (ND)	Not done (ND)	-VE	ND	Soft tissue
13	SRCT	ES/PNET	Not done (ND)	ES/PNET (Parotid region)	Not done (ND)	Not done (ND)	Not done (ND)	Normal	Soft tissue
14	SRCT	ES/PNET	Not done (ND)	Not done (ND)	Soft tissue mass	Normal	Bone Mets +ve	ND	Soft tissue
15	Hepatoblastoma	HB	Not done (ND)	Not done (ND)	CT abdomen: liver lesion	Normal	Not done (ND)	ND	Liver
16	SRCT	Not conclusive Not done (ND)	Not done (ND)	Not done (ND)	CT : S/o. RB infloptic nerve a muscles	Normal	Not done (ND)	Normal	Eye
17	GNB	Not conclusive Not done (ND)	Not done (ND)	Not done (ND)	CT Adrenal with advanced lesion (IVC)	Normal	Not done (ND)	Normal	Adrenal
18	SRCT	RMS	Not done (ND)	Not done (ND)	Not done (ND)	Normal	Not done (ND)	Normal	Soft tissue
19	ES/PNET	ES/PNET	Not done (ND)	Not done (ND)	Hip bones B/L- secondaries	Not done (ND)	Bone	Involved by SRCT	Bone
20	SRCT	ES/PNET	Not done (ND)	ES/PNET	Bone lesion	Not done (ND)	Lung, Bone	Normal	Bone
21	GNB	Not conclusive	Not done (ND)	Not done (ND)	CT-Adrenal-advanced lesion (IVC)	Normal	Inferior Vena Cava (IVC)	Normal	Adrenal
22	SRCT (PNET/lymphoma)	Lymphoblastic lymphoma	Not done (ND)	NHL (advise IHC)	Not done (ND)	Normal	Not done (ND)	Normal	Soft tissue
23	RMS	RMS	Not done (ND)	Not done (ND)	CT abdomen ; Large retroperitoneal mass	Normal	Not done (ND)	Normal	Soft tissue
24	ES/PNET	ES/PNET	Not done (ND)	SRCT	Knee joint	Normal	Paratracheal Lymphadenopathy	Normal	Soft tissue
25	NB	Not conclusive	NB	Not done (ND)	CT abdomen S/o. NB. Encasing IVC, Renal	Normal	Encasing inferior venacava	Normal	Retroperitoneum

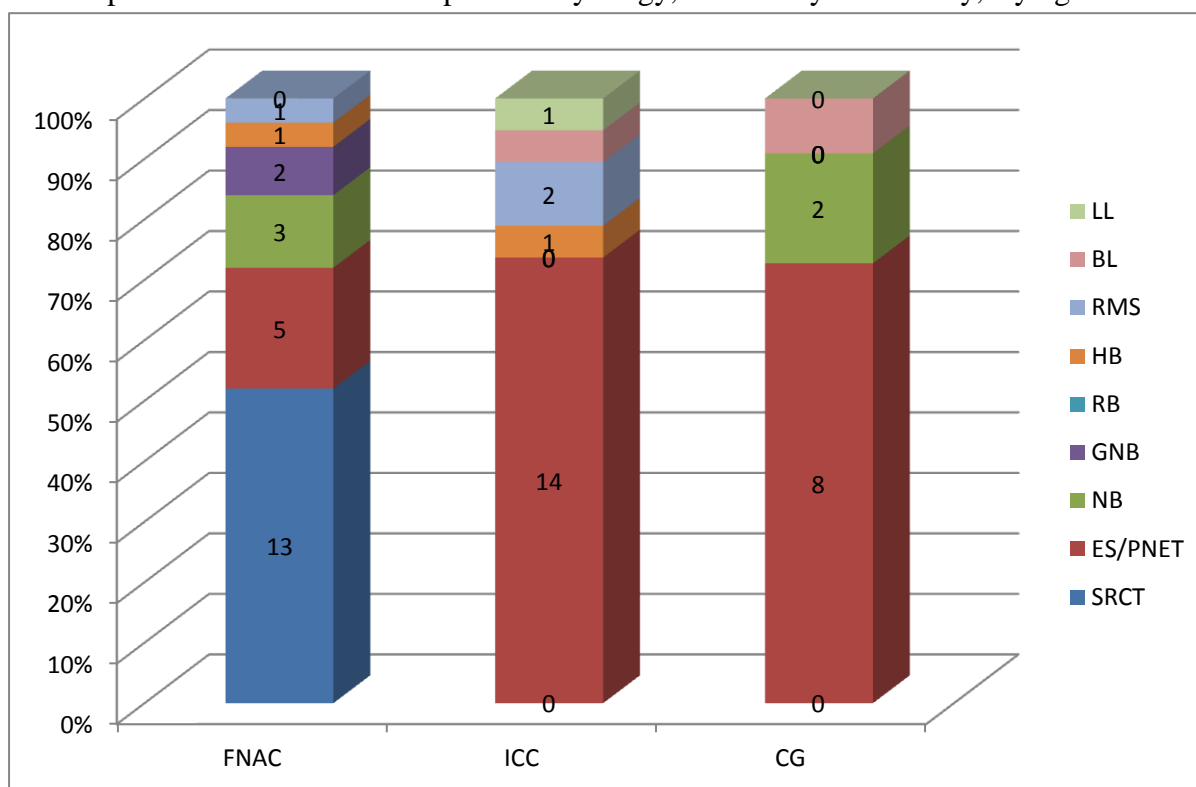
Statistical analysis: The data obtained by our study is analysed graphically. Table No.13 shows details of Small round cell tumors

diagnosed by fine needle aspiration cytology, immunocytochemistry and cytogenetics.

Table 13: Details of Small round cell tumors diagnosed by fine needle aspiration cytology, Immunocytochemistry and Cytogenetics

Diagnosis	FNAC	ICC	CG
SRCT	13	0	0
EST/PNET	5	14	8
NB	3	0	2
GNB	2	0	0
RB	0	0	0
HB	1	1	0
RMS	1	2	0
BL	0	1	1
LL	0	1	0
Total	25	19	11

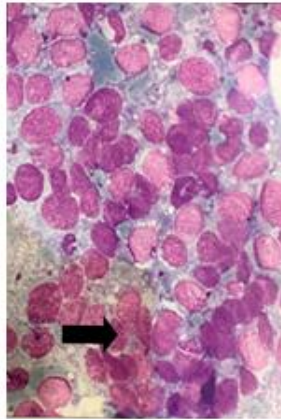
Graph 5: Comparison of Fine Needle Aspiration Cytology, Immunocytochemistry, Cytogenetics



Graph No.5 depicts the comparison of fine needle aspiration cytology, immunocytochemistry and cytogenetic result. On FNAC only 12 cases (48%) were sub-categorized. But when ICC was combined with FNAC 19 out of 25 cases (76%) of SRCT were sub-categorized. ICC was helpful in diagnosing all the 14 cases of ES/PNET. Cytogenetics was helpful in comparing our

results. 11 patients showed characteristic chromosomal abnormality. They had agreement with ICC study. ES/PNET (8 cases showed characteristic cytogenetic abnormality). Immunocytochemistry when combined with fine needle aspiration cytology will definitely increase the diagnostic accuracy.

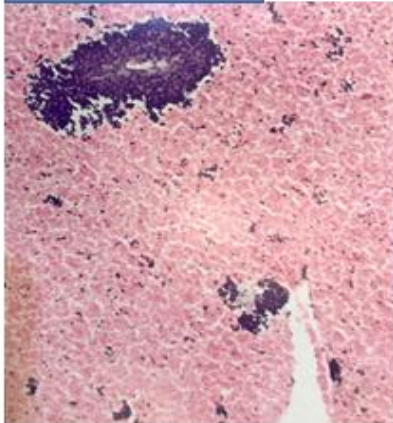
Fig 1



ES/PNET
Exhibit nuclear molding

Fig 2 & 2a

ES/PNET CELL BLOCK
SHOWING Rosette



CD 99

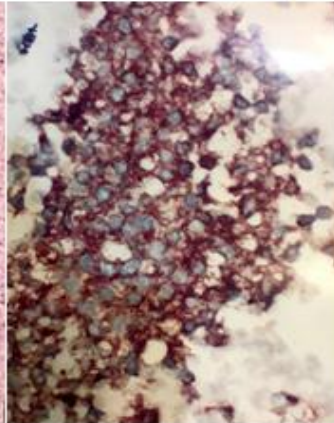
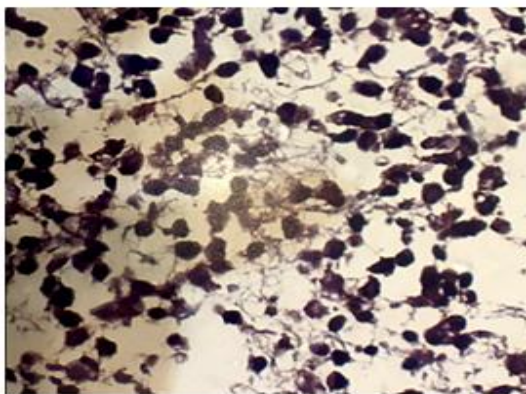


Fig 2b



CD99 POSITIVE SMEAR

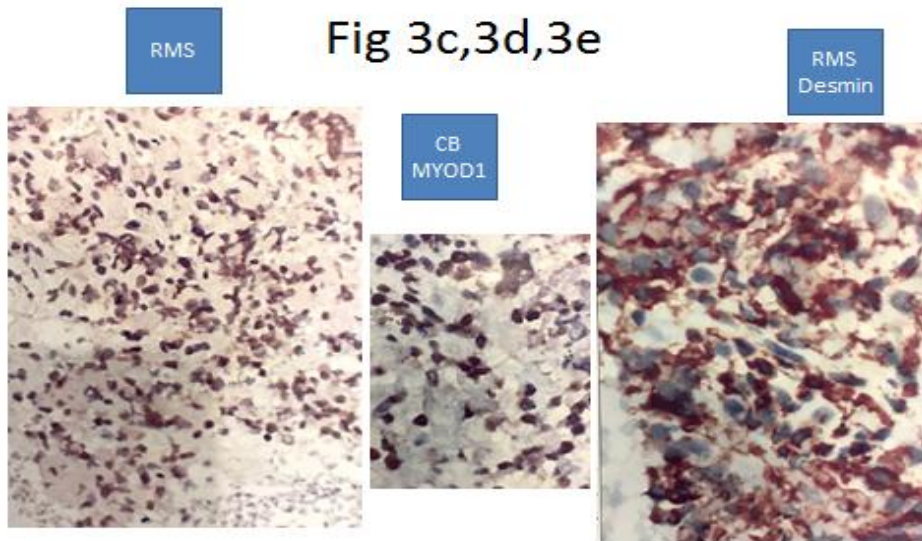
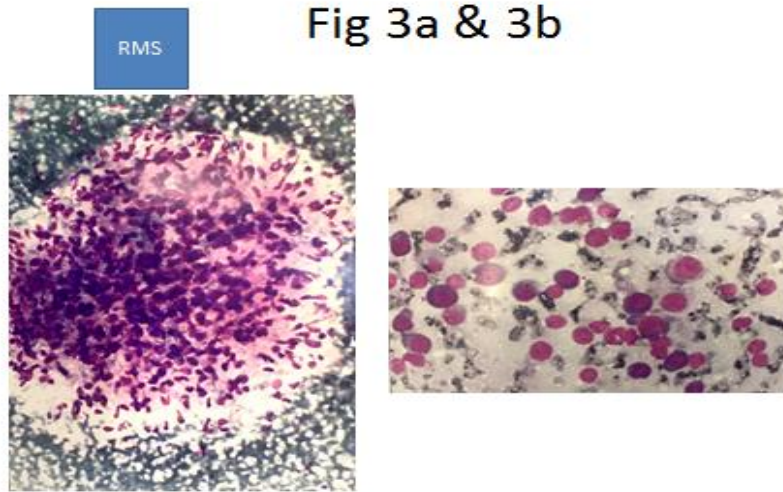
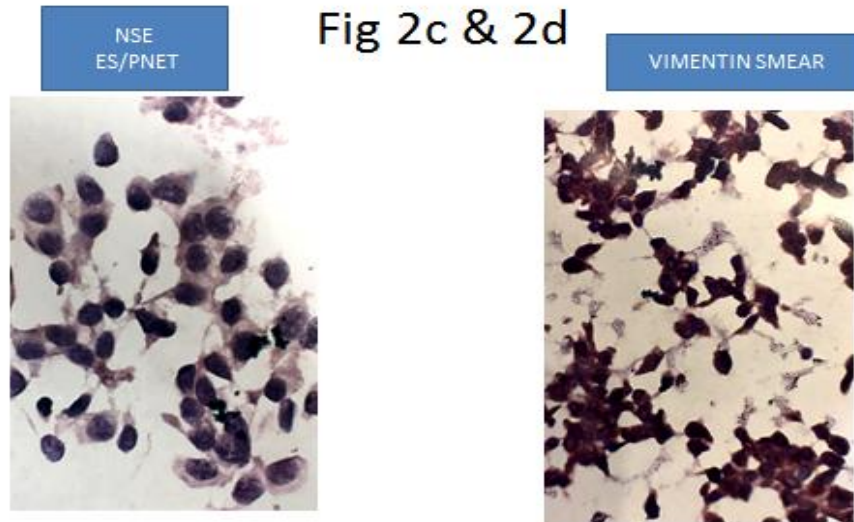


Fig 4a & 4b

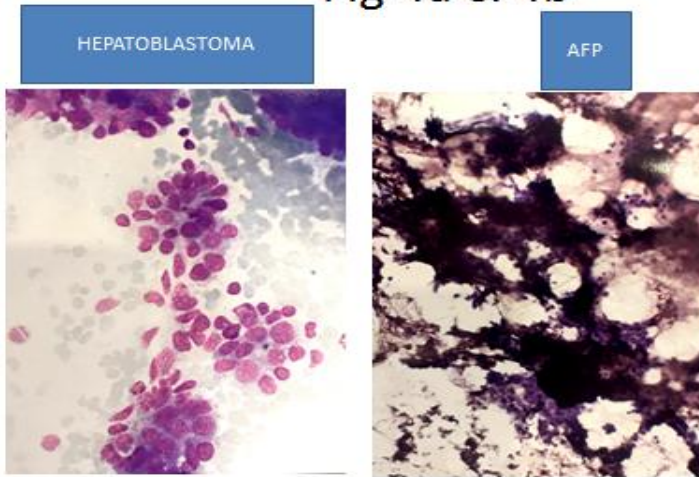


Fig 5a & 5b

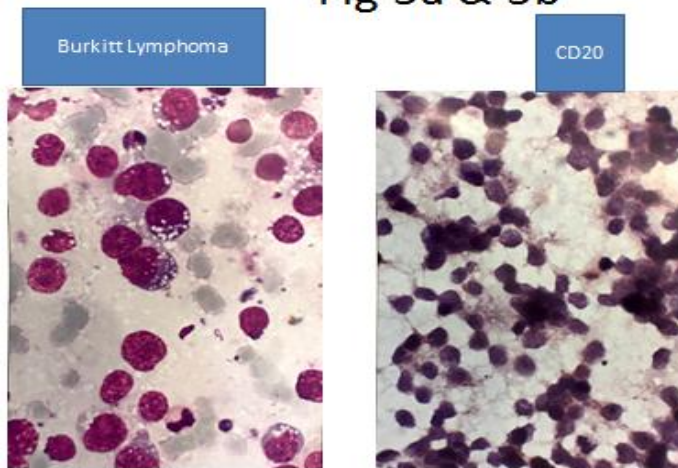


Fig 6a & 6b

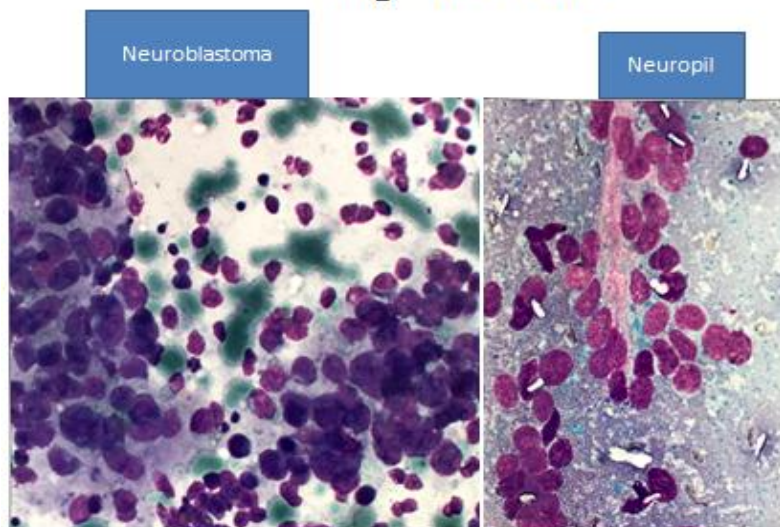
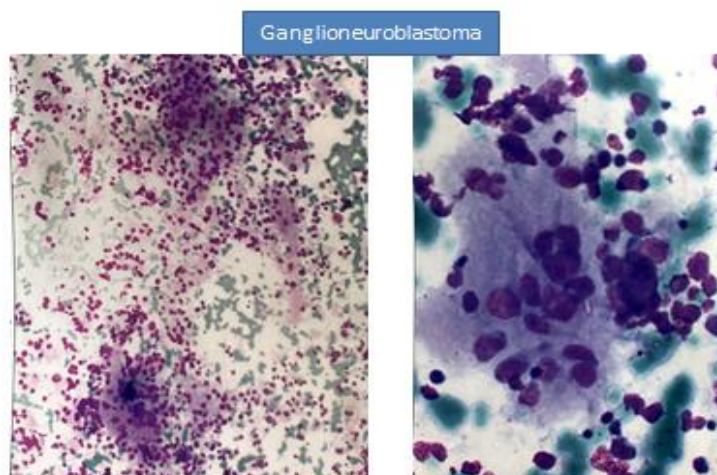


Fig 7a & 7b



Discussion

Malignant round cell tumor is a group of morphologically almost indistinguishable tumors, and the pathological diagnosis is not enough to predict tumor behavior and outcomes.¹ This study demonstrates the value of immunocytochemical staining in resolving diagnostic problems.⁸ Immunocytochemistry on FNAC smears can help to make a definitive diagnosis and thus reduce the hospitalization costs & operative costs and at the same time reduces the time considerably before a definitive treatment protocol is planned.

O. The maximum number of patients belonged to 0-15 years of age group, which is similar to those described by Khadilkar et al and Arvind et. al². The frequency of SRCT was also high in extremities (24%) cases & head & neck region (24%) second was intra abdominal (20%) cases. The characteristic features that helped us to diagnose small round cell tumors were, presence of rosettes and fibrillar background in neuroblastoma, nuclear grooves in Lymphoblastic lymphoma, cytoplasm with lipid vacuoles in Burkitt lymphoma. These features were also described by Rajendra P. Tripathi's et al.

Prerequisites for adequate ICC studies are a well spread film of diagnostic cells and adequate fixation. Adequate fixation to maintain good morphological detail is also essential not only for ICC but also for cytomorphological study. For

cytological specimen, Flens MJ et al advocate acetone fixation at room temperature while Dabbs DJ comments that the fixative at room preferred varied with the cytopathologist.²⁶ We preferred air-drying of smears and cold acetone fixation and wet-fixation of smears using cytofix (Polyethylene glycol 80% Isopropanole), In this study both the air-dried smears fixed in cold acetone and wet fixed smears using cytofix gave equal good results. All the slides used were silane coated for immunocytochemistry.

Inclusion of controls is a mandatory requirement in immunocytochemistry. With a good team work, immunocytochemistry on FNAC smears of small round cell tumors in this study, has highlighted a few points. This study demonstrates the value of immunocytochemical staining in resolving diagnostic problems & refining the morphological diagnosis of 25 cases of SRCT. It allowed more a definitive and accurate diagnosis. One case of small cell carcinoma on cytology was revised & diagnosed as ES/PNET after Immunocytochemistry. One case of lymphoma/ES/PNET was concluded as lymphoblastic lymphoma after ICC study.

In the present study antibodies to LCA were very helpful in differentiating lymphoma from other SRCT. MIC-2 was found to be a highly sensitive and specific marker for the ES/PNET group of tumors. It was positive in all cases ES/PNET

(14/4). But it could not distinguish PNET from Ewing tumors. Probably this is due to an intimate histogenic relationship between ES & PNET, MIC-2 was helpful in distinguished the ES/PNET group of neoplasm from other SRCT. Most NB were positive for NSE, but NSE has significant limitation, as it was seen to be positive in Ewing family also. Chromogranin was not found to be especially sensitive for NB.

The diagnosis of RMS can be established by the cytomorphological and immunocytochemical demonstration of MyoD1, desmin & Vimentin, MyoD1, was found to be a highly sensitive & specific marker for the RMS & showed nuclear positivity.

Out of 25 cases of small round cell tumors, only 12 cases were subcategorized on FNAC. But when ICC was combined with FNAC 19 out of 25 cases SRCT (76%) were sub-categorized. The ICC results correlated well with the cytogenetic analysis. Agreement was seen in 11 of the 15 cases which had both ICC and cytogenetic study.

Conclusion

Immunocytochemical analysis on FNAC smears confirmed with cytogenetics in clinically suspicious cases of small round all tumors is economical and reliable is proper hands of an experienced cytopathologist. Hence inclusive of immunocytochemistry of FNAC smears into the list of investigation in the diagnosis of FNAC smears into the list of investigation in the diagnosis of malignant round cell tumors is crucial and mandatory.

References

1. Kim Devoe, Noel weidner: Immunohistochemistry of Small Round-cell tumors. Semin Diagn Pathol 2000; 17:216-224
2. Urmil Brahmi, Radhika Srinivasan, Harbhajan SINGH Komal, Kusum Joshi, Subhash Kumari Gupta, Arind Rajwanshi: Comparative Analysis of Electron Microscopy and Immunocytochemistry in the cytologic Diagnosis of Malignant Small round cell tumors. Acta cytol 2003; 47(3):443-449.
3. Chang, Chin-Yang Li, Smithson, W.A: Immunocytochemical study of Small round cell tumors in Routinely Processed Specimens Arch Pathol Lab Med 1989; 113:1343-1348.
4. Halliday.B.E., Slagel D.D., Elsheikh T.E, : Diagnostic utility of MIC -2 Immunocytochemical staining in the differential diagnosis of Small Blue cell Tumors. Diagn cytopathol 1998 ; 19 (6) : 410-416.
5. Joshi V., Balarezo F., John Hicks, Tsongalis G J; Review Approach to Small round cell tumors of Childhood. Pathology Case reviews 2000; 5 (1) : 26-49.
6. McGahey B.E., Moriarty A.T Nelson W.A., Fine-Needle Aspiration Biopsy of Small Round Blue Cell Tumors of Childhood. Cancer 1992: 69 (40):1067-1073.
7. Saho K. Pai RR, Khadilkar, UIN : Fine Needle Aspiration Cytology of the Ewing Sarcoma Family of Tumors. Actal Cytol 2000; 44 (3): 332-336.
8. Brahmi U, Rajwanshi .A, Joshik, Ganguly NK, Vohra. H, Gupta, SK; Role of immunocytochemistry and DNA Flow cytometry in the Fine Needle Aspiration Diagnosis of malignant Small round cell tumors. Diagn cytopathol 2000;24 (4):233-238.
9. Das DK, Bhambhani .S, Chachra K.L., Murthy NS: Small round cell tumors of the Abdomen and Thorax Role of fine needle aspiration cytologic features in the diagnosis and differential diagnosis, Acta Cytol 1996;41(4) : 035 – 1047.
10. Cohn SL: Diagnosis and Classification of the Small Round-Cell Tumors of Childhood. Pathology 1999; 155:11-15
11. Silverman JF, Dabbas DJ, Ganick DJ, Geisinger K R: Fine Needle Aspiration

- Cytology of Neuroblastoma, Including Peripheral Neuroectodermal Tumor with immunocytochemical and ultrastructural confirmation. *Acta cytol* 1988; 32 (3) : 367-376.
12. Jelen M., Wozniak U: Cytologic Appearance of Esthesionuroblastomas in a fine needle aspirate *Acta Cytol* 1988;32 (3):377- 380.
 13. Neuhol N, Artlieb U, Wimmer M, Krisch I: Aspiration Cytology, Immunocytochemistry and electron microscopy of a malignant peripheral neuroectodermal tumor A case report *Acta cytol* 1989; 33 (1) : 74-79.
 14. Antonius S H, Jongde, Heerde PV ; Fine Needle Aspiration Biopsy diagnosis of rhabdomyosarcoma An Immunocytochemical Study. *Acta cytol* 1987; 31(5);573-577.
 15. Silverman JF, Berus A, Joshi V: Fine Needle Aspiration Cytology of Primitive Neuroectodermal Tumors A report of Three Cases. *Acta cytol* 1992;; 36(4) : 541-550.
 16. Otal-Salaverri C, Compora R.G. Vazques A H, Davidson H G: Retroperitoneal Ganglioneuroblastoma Report of a case Diagnosed by fine needle aspiration cytology and electron microscopy. *Acta cytol* 1989;33(1):80-84.
 17. Gupta RK, Alausari A G: Fine needle aspiration cytodiagnosis in a cases of hepatoblastoma. *Diagn cytopathol* 1994;5:114-117.
 18. Elison DA, Silverman JF, Straubauch PH, Joshi V: Role of Immunocytochemistry, Electron Microscopy and DNA analysis in Fine Needle Aspiration Biopsy Diagnosis of Wilms Tumor. *Diagn Cytopathol* 1996;14(2):101-107
 19. Ravindra S, Kini. U: cytomorphology and morphometry of small round cell tumors in the Region of the kidney. *Diagn Cytopathol* 2005; 32(4):211-216.
 20. Mierau GW, weeks DA, Hicks MJ; Role of Electron Microscopy and other Special Techniques in the Diagnosis of childhood Round Cell Tumors. *Human Pathol* 1998 ; 29 (12):1347-1355.
 21. Beustein M, Kovar H, Paulussen M, Juergensy H: Ewing Sarcoma family of tumors: Current Management *Pediatric oncol* 2006-22:210-230.
 22. Carpentieri DF. Qualman sj, Knaussz T: Protocol for the examination of specimens from pediatric and Adult Patients with osseous and Extra Osseous Ewing sarcoma Family & Tumors including Peripheral Primitive Neuroectodermal Tumor and Ewing Sarcome. *Arch Pathol Lab Med* 2005: 129: 866-880.
 23. Mallone M: Soft tissue tumors in childhood. *Histopathol* 1993;23:203-216
 24. Folpe AL, Rubin BP, Weiss SW: Morphologic and Immunophenotypic Diversity in Ewing family tumors a study of 66 genetically confirmed cases. *Am J Surg Pathol* 2005;29(8):1025-1033.
 25. Ushigoma S, Shimoda T, Takaki K, I shikawa E, Marlan J: *Cancer* 1989: 64:52-62.
 26. Wombart Bosch A, Contesso G : Histology Immunohistochemistry and Electron Microscopy of Small round cell tumors of Bone *Semin Diag Pathol* 1996; 13(3):149-169.
 27. Rosa Noguera: Cytogenetics and Tissue culture of Small round cell tumors of Bone and Soft Tissue, *Semin Diag Pathol* 1996;13(3): 171-183.
 28. Amore EG, Ninfo V: Soft tissue Small round cell tumors : morphological Parameters *Semin Diag Pathol* 1996; 13(3): 184-203.
 29. Meiss-Kindblom JM, Stevman G, Kindbsom LG: Differential Diagnosis of Small round cell tumors, *Semin Diag Pathol* 1996; 13 (3):213-241.

30. Terrier P, Wombart Bosch A, Contesso G: Small Round Blue Cell Tumors in Bone: Prognostic Factors correlated to Ewing sarcoma and Neuroectodermal Tumors Semin Diag Pthol 1996;13(3);250-257.
31. Flarsharan K.S, Silvermand JF: Paediatric Tumors chap 17 in : Orell SR, Steerett GF, Whitaker D, Eds, Fine Needle Aspiration Cytology, 4th Edition, Churchill Livingstone 2005:445-468.
32. Brown FM : Soft Tissue chap 16: In cytology, Diagnostic Principles and Clinical correlates, 2nd edition, Edmund.S, Cibas, Ducatman B.S. Saunders company 2003: 423-456.
33. Jambhakar NA, Bagwan IN, Ghule P, Chinory RF, Agaral S, Kadam PS: Comparative Analysis of Routine Histology, Immunohistochemistry, Reverse Trscriptase Polymerease Chain Reaction and Fluorescence In situ Hybridization in Diagnosis of Ewing Family of Tumors. Arch pathol Lab Med 2006; 130(12): 1813-1820.
34. Jambhekar NA, Kadam PS, Chinoy RF: The utility of ancillary studies in pediatric Fine Needle aspiration Cytology Diagn Cytoplathol 1992; 8: 137-147.
35. Rosai Juan : Soft tissues chap 25: In Rosai and Ackerman's Surgical pathology, 9th edition,. Rosai J. Mosby company 2005:2237-2372.
36. Rosai Juan: Bone and Joints chap 24: In Rosa and Ackerman's Surgical pathology, 9th edition, Rosai J, Mosby company 2005:2137-2236.