Fine needle aspiration cytology of small round blue cell tumors: The use of EM

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Introduction
Small round blue cell tumor (SRBCT)= tumors characterized by small, round, relatively undifferentiated cells
• Cytology is an excellent tool for the diagnosis of these tumors.
• Cells of these tumors slightly larger or 1.5-2 times size of a lymphocyte.
• Advantage cytologic diagnosis of poorly differentiated neoplasms to which these belong is that FNA is usually rapid interpretation of the material-- allowing to triage the tissue as necessary.
• Obtain material for further studies : EM, flow cytometry and molecular studies.

ACCME/Disclosure
Dr. Turbat-Herrera has nothing to disclose
**Introduction**

- Small round blue cell tumors:
  - Ewing's sarcoma (EWS)/peripheral neuroectodermal tumor
  - neuroblastoma
  - rhabdomyosarcoma
  - synovial sarcoma
  - nephroblastoma (Wilms tumor).
  - desmoplastic small round cell tumor
  - small cell carcinomas
  - non-Hodgkin's lymphoma
  - retinoblastoma
  - hepatoblastoma
  - small cell osteogenic sarcoma

**Differential diagnosis**

- FNA of small round blue cell tumors may mimic a variety of epithelial and even lymphoid neoplasms
- Neither cytology nor surgical pathology evaluation may be sufficient to make a specific diagnosis without the help of other diagnostic techniques.
- Diagnosis must be approached by using an intelligent selection of ancillary diagnostic techniques
- The most reasonable route to address the differential diagnosis and to make a final diagnosis should be taken

**SRBCT**

- FNA has made a difference in the way Medicine is practiced today.
- Most tumors are sampled by needle.
- FNA expedites the diagnostic process by making a diagnosis or at least narrowing the diff dx.
- When the FNA practice is coupled with the powerful tool of EM, molecular and genetic studies are added, then FNA becomes a great diagnostic modality.

**ANCILLARY DIAGNOSTIC TECHNIQUES**

- Immunohistochemistry
- Electron microscopy
- Cytogenetics
- Flow cytometry
- Molecular diagnostics
SRBCT Ultrastructure

- Ultrastructure for cytology
  EM is well suited for cytology
- the samples are small
- obtained at the time of the rapid interpretation the sample can be fixed immediately.

Understanding that EM for cytology has its inherent drawbacks is paramount.
The cytologic sample can have many artifacts due to the passage of the cells through the needle.

Cytology Specimens suitable for ultrastructural examination

- FNA with 22-25(27) gauge needles are suitable for EM.
- Rinses of the needle or additional pass/passes.
- Fluids: pleural, peritoneal, pericardial, CSF, BAL

- Fluids with high cellularity are excellent for EM and stay viable after several days of refrigeration.
- If cellularity is limited the specimen can still be adequate if diagnostic cells are present.

EM for cytology

- FNA sample is normally small but may be meager in cellularity.
- A small sample may still be very helpful for diagnosis if the malignant cells are present, especially if they are present in small groups permitting the ultrastructural observation of cell interactions.
- While all the ultrastructural criteria may not be present, if the main EM features of the tumor are present, by combining these with the clinical features, cytologic impression and IHC, a firm dx can be made.

EM for FNA: Technique

- Obtaining EM samples by FNA
- A great deal of frustration can be the result of sample disruption due to cytoplasmic membrane rupture under the electron beam when cytologic samples have been damaged as they pass through the needle due in part through pressure and the shearing force the cells are subjected to.
Technical considerations

- after sample is obtained
- aspirate gluteraldehyde with the syringe
- allow a few seconds for fixation to occur, then detach needle
- express sample into specimen bottle of gluteraldehyde

Cytology of Small round blue cell tumors

- Needle aspiration has been around for decades.
- In 1847 Kun published "New instrument for the diagnosis of tumors", the needle aspiration. This appears to be the first report of needle aspiration where a microscopic diagnosis was made.
- In 1853, Sir James Paget in his "Lectures on Tumours", stated that he favored aspiration biopsy, examined as cell spread. His description of cancer cells is still applicable today. "Many of the cells of cancer...may be somewhat like gland cells or like epithelial cells, yet a practiced eye can distinguish them, even singly..."

FNA of SRBCT

- 1933 Dr. Fred W Stuart published the experience of Memorial Hospital, 2500 breast carcinomas
- In that publication he stressed the following points:
  - 1-Emphasis on technique
  - 2-Correlation of clinical information with interpretation
  - 3-Comparison of cytology with histology
  - 4-Points out the usefulness of the technique and its limitations
- **Need for ancillary techniques**

Ancillary techniques

- Even in the best hands, some cytology cases as a whole require the use of ancillary techniques for a definitive diagnosis
SRBCT by FNA

• Exact categorization may not be possible at the light microscopic level in the case of poorly differentiated tumors.
• In fact, a definitive diagnosis based on clinical and cytomorphological criteria was possible in 57% of the cases in one study.


SRBCT by FNA

• IHC is the first approach
• IHC results may at times be equivocal.
• Many of these tumors share IHC results with others in this category
• EM can help clarify some of these issues.

SRBCT by FNA

• Cytologically a fairly uniform population of round to oval cells about 2 times the size of a mature lymphocyte with scanty, basophilic cytoplasm.
• Nucleoli can be inconspicuous, small, or quite prominent.
• Cytoplasm is generally scanty, resulting in a very high nuclear to cytoplasmic ratio.
• These tumors are referred as blue cell tumors B/C cells are basophilic with hyperchromatic nuclei and a thin rim of cytoplasm which makes them for the most part indistinguishable from other tumors in this category.

SRBCT Cytology

Cytology “look alikes” because for the most part they look like several other tumors in this category.
• Cytopathologist when confronted with such an issue is to know what to collect and in what medium to perform the necessary tests.
• There are some cytopathologists that are happy to make a dx of “malignant neoplasm”, in that case, and if that is the way one chooses to practice, one may not need anything else…
SRBCT : Cytology

- Ancillary techniques to make a definitive diagnosis or narrow differential DX
- IHC stains vary from tumor to tumor but in small blue cell tumors some markers offer no discrimination
- EM is excellent for this purpose since only a few cells are necessary in order to:
  1. make an ultrastructural diagnostic evaluation with minimal criteria,
  2. narrowing the diff dx
  3. making the diagnosis
  4. confirming the IHC results.

Ewing's Sarcoma / Primitive Neuroectodermal Tumor

- 1919 James Ewing described a tumor in bone, distinct from osteogenic sarcoma which he called “diffuse endothelioma of bone” in his seminal textbook “Neoplastic Diseases”
- In 1918 Arthur Purdy Stout described a primitive, rosette-forming tumor arising from the ulnar nerve of a young adult which later became known as PNET.
- These two entities now belong to the family of tumors known as ES/PNET. They share the cytogenic abnormality of t(11;22)(q24;q12).

EWINGS SARCOMA

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**FNA of Ewings Sarcoma / PNET**

- In FNA of Ewings, the tumor cells tend to be uniform and can be seen: dispersed in poorly differentiated cases.
  - but can be arranged in relatively small, tight, syncytial clusters.
- Nuclei may be round or irregular and indented and lack nucleoli but may have inconspicuous nucleoli. These small cells tend to have a high nuclear to cytoplasmic ratio (N/C).
- Two population of cells have been described: large chief cells and smaller dark cells. The cytoplasm is pale blue and contains variable numbers of small vacuoles which correspond to glycogen deposits. Cellular smears with dispersed monomorphic cells in a vacuolated tigroid background can be seen.

**FNA of EWS / PNET**

- In the majority of cases, abundant cytoplasmic glycogen can be demonstrated by PAS staining/diastase resistant. Presence of large amounts of intracellular glycogen is not specific.
- 35% of all Ewing's sarcoma cases do not contain detectable glycogen and other tumors contain detectable glycogen as well.
- Variable numbers of pseudorosettes may be seen in better diff cases; fibrillary matrix and Homer Wright rosettes are seen at times and mitotic figures are rarely detected.

**Ewing’s sarcoma**

- Ewings sarcoma is a very primitive neoplasm and for the most part lacks NE differentiation although Suh CH, Ordonez N, Hicks J, Mackay B demonstrated rare granules in dendritic cell processes and in perinuclear locations in 2001.

**EWS/PNET**

**IHC**

Most useful today CD99 which is a protein encoded by a gene(p30/32 MIC 2;013) located in the short arm of the X and Y chromosomes. Not pathognomonic, also found in embryonal rhabdoid, other soft tissue sarcomas and lymphoblastic lymphoma.

**Molecular genetic features of ES/PNET:** reciprocal translocations of 11:22(q24,q12) which results in fusion of EWS gene with FLI or ERG genes. The most common fusion, EWS exon 7 with FLI exon 6 demonstrated by RT-PCR or FISH.

Deletion of INK4A is present in 1/3 of cases.
EWS

- 18 year old African American male with a history of rhabdomyosarcoma S/P chemotherapy presents with a 2-3 cm subcutaneous nodule on his back.
- FNA showed single, discohesive, monotonous small cells with high N/C ratio and tiny cytoplasmic vacuoles, inconspicuous nucleoli were present in some cells.
- Material was obtained for EM.
Ewings sarcoma ultrastructural features

- Primitive small cells that have abundant glycogen
- Rare granules have been described, cytoplasmic and in processes.
- Small round ovoid cells joined by variable numbers of rudimentary cell junctions and desmosomes
- Nuclei have smooth contours with mostly inconspicuous nucleoli and margined chromatin.
- Cytoplasm with variable sized clusters of glycogen particles and a few organelles, mostly polyribosomes. Glycogen may sometimes be lost during processing and appear as holes.
- Occasionally clusters of intermediate filaments, mostly vimentin, rarely (<5%) whorls are present.

Ordonez NG, Hicks J, Mackay B. "Ultrastructure of the Ewings sarcoma Family of tumors, Ult Pathol 2002, 26: 67-76.

Translocation (11:22)(q24,q12): EWS/FLI-1 fusion gene

EWS

Utrastructurally

- THE EM DIAGNOSIS IS MADE NOT ONLY BY WHAT IS PRESENT ULTRASTRUCTURALLY IN THE CYTOPLASM BUT ALSO BY WHAT IS ABSENT.
- ABSENT IN THIS CASE:
  - EPITHELIAL DIFFERENTIATION
  - LYMPHOID FEATURES
  - THIN AND THICK FILAMENTS (Z LINES) (Hx of rhabdomyosarcoma)

ES/PNET may exhibit a tigroid background which is helpful but not absolutely diagnostic of EWS.

Any tumor with abundant glycogen such as germ cell tumors as seminoma, embryonal carcinoma can display a tigroid background.
Tigroid background

Ewings sarcoma

EWS

NEUROBLASTOMA

Tumor cells that have limited cytoplasmic organelles. A few mitochondria and Golgi (arrowhead). Other regions of the cytoplasm contain increased numbers of glycogen particles (arrows).
Neuroblastoma

- Neuroblastoma (NB) arises from neuroblasts, the undifferentiated precursor cells of the sympathetic nervous system.
- About 70% of neuroblastomas occur in the retroperitoneum and the majority of these involve the adrenal gland.

FNA Neuroblastoma

- FNA samples highly cellular consisting of small blue cells seen singly or arranged in small clusters.
- Cells are small and undifferentiated with a high N/C ratio. The nuclei round or irregular, hyperchromatic or with salt and pepper chromatin (Pap).
- Better differentiated tumors may form rosettes and fibrillary material (neuropil) and ganglion cell differentiation may be present.
- Early ganglion differentiation seen as larger cells with more cytoplasm and prominent nucleoli.
- Clusters of cells may be separated by pale blue to light purple fibrillar matrix (Diff Quik). These pseudorosettes may be seen in as low as 18% to as high as 72% of all cases.
- Necrosis and macrophages may be present as well as calcifications.

Neuroblastoma: IHC

- Neuroblastomas are characteristically positive for neuron-specific enolase (NSE) which is an isoenzyme of the glycolytic enzyme, enolase, which has been shown to be highly specific for neurons and neuroendocrine cells.
- Other markers evaluated in neuroblastoma include CD56, chromogranin, and synaptophysin, which are not usually helpful in distinguishing undifferentiated and poorly differentiated tumors. S100 and CD99 are negative.
- Neurofilament

- The anti-GD2 antibody, has been described that is directed against a ganglioside and may be applied on tissues. This is said to be a sensitive and reproducible marker and can be used to detect and quantify neural residual disease in neuroblastoma.
- 3F8 and Sangroblastin are monoclonal antibodies being used for treatment of NB.
Neuroblastoma ultrastructure

- EM feature of neuroblasts: processes which most of the time contain microtubules, intermediate filaments and small, 100nm, round, dense-core neurosecretory granules and occasional 40-50nm clear vesicles. Better differentiated NBs have abundant interweaving neuritic processes joined by rudimentary cell junctions.
- EM composed of neuroblast cells with smooth nuclear membranes with heterochromatin and one or two distinct nucleoli. Polyribosomes, RER cisternae and pleomorphic lysosomes in a perinuclear location. Cytoplasmic glycogen has been reported by some in neuroblastomas which may be a possible pitfall.

Cells with high N/C ratio are separated by numerous cellular processes of fairly uniform diameter. The main portion of cytoplasm contains many ribosomes, a few mitochondria and a limited number of small segments of RER. Axonal-like processes contain microtubules (arrow) and neurosecretory granules (arrowheads).
Rhabdomyosarcoma

- Stout as well as others’ criteria for rhabdomyosarcomas even in the absence of cross striations.
  
  - Horn and Enterline: certain undifferentiated round cell or spindle cell neoplasms including sarcoma botryoides and alveolar forms deserve the designation embryonal and alveolar rhabdomyosarcoma respectively.
  
  - In these poorly differentiated rhabdoses in which cross striations may not be identified in cytologic samples ancillary techniques are necessary.
  
  - IHC for muscle differentiation: desmin, myogenin
  
  - EM immature thick and thin filaments in a sarcomere, even ill defined, may be diagnostic

Currently classified into embryonal rhabdomyosarcoma (ERMS), alveolar rhabdomyosarcoma (ARMS), and pleomorphic rhabdomyosarcoma (PRMS) subtypes.

- Embryonal RMS typically occurs in young children, whereas alveolar RMS usually occurs in older children and young adults, and PRMS occurs in older adults.

- Embryonal and alveolar rhabdo are considered small blue cell tumors unlike pleomorphic rhabdo and therefore the discussion of this tumor I will be referring to those two variants.
Rhabdomyosarcoma

- The cytology of embryonal rhabdomyosarcoma is characterized by the triad: small blue cells, spindle (strap cells - rhabdomyoblasts) and a myxoid matrix.
- The smears are usually highly cellular; single cells predominate but small tissue fragments may be present. The cells are small and round (rare nuclear irregularities seen at times) with hyperchromatic nuclei and scant cytoplasm with rare vacuoles containing glycogen.
- Spindle strap cells may predominate over the small blue cells. Cross striations usually not visible but cytoplasmic condensations may be seen.
- Myxoid stromal fragments are characteristic and a tigroid background (stripped appearance due to the presence of glycogen). The stromal fragments appear magenta or metachromatic in Diff Quik stain (for rapid interpretation).

Alveolar rhabdo

- FNA produces very cellular aspirates with similar appearance as in those described for embryonal with predominance of small round blue cells.
- More cytologic variability is usually present and the cells can be slightly larger, with coarser chromatin and one or more nucleoli.
- Binucleation and multinucleation may be present.
- Floret cells (giant tumor cells) may be common and are characteristic of alveolar rhabdo unlike in embryonal.

Embryonal Rhabdomyosarcoma

- FNA of embryonal rhabdo
- Rare giant cells may be seen like in pleomorphic rhabdo but these appear in the myxoid stroma.
Embryonal rhabdomyosarcoma

- Aveolar RMS shows predominantly dissociated cells or chance formations.
- Embryonal type shows large tissue fragments with abundant eosinophilic material and various numbers of dissociated cells.
- The relative proportion of poorly to better and well-differentiated rhabdomyoblasts varies in both types and in all patterns.
- In a study, the embryonal type was found to be composed of mainly early rhabdomyoblasts.
- Tadpole or ribbon-shaped tumor cells were also observed in embryonal RMS.
- In contrast, most tumor cells were small and lymphocyte-like in alveolar RMS, with finely granular chromatin.

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Alveolar rhabdomyosarcoma, Cytology

Rhabdo: IHC

- The use of desmin on cytological material has been reported as being useful in differentiating between different small round cell tumors of childhood, and is a specific marker for RMS.
- Desmin positivity is seen not only in large rhabdomyoblasts, but also in more numerous, smaller, less well differentiated tumor cells since it is expressed during the early differentiation of skeletal and smooth muscle cells.
- Antibodies recognizing the muscle-specific intermediate filament-desmin, muscle-specific actin, and myoglobin, are valuable in differentiating them but may lack sensitivity or specificity in some cases.
- Myogenin Ab: also positive
Rhabdomyosarcoma

- Myf-4/myogenin belonging to the family of MyoD genes, regulates the differentiation of pluripotent, primitive mesodermal cells in the skeletal muscle. A very useful monoclonal antibody against MyoD1 detects myogenic regulatory proteins and was introduced for use in pathology in 1990. In a study conducted by Wang et al., on paraffin sections, myogenin and MyoD1 nuclear expression was noted in 91% of RMS cases, whereas neither was detected in any of the neuroblastomas or Ewing's sarcomas/ PNETs.
- In a large series of 956 RMS cases reported by Morotti et al., the two markers, MyoD1 and myogenin, were positive in 97% of the cases but in none of the 96 non-RMS tumors tested in the series. Both ARMS and ERMS stain positively for myogenin, although the number of cells staining in the latter subtype is clearly fewer. Thus, MyoD1 and myogenin are highly sensitive and specific markers for RMS as compared to desmin, and can differentiate alveolar RMS from the embryonal type.

Rhabdomyosarcoma, ultrastructure

- Electron microscopy plays an important role in the characterization and diagnosis of rhabdomyosarcoma and is a valuable tool in the differential diagnosis, skeletal muscle differentiation.

Minimal criteria for EM diagnosis are the recognition of alternating thick and thin filaments (sarcomeric constituents), Z-disc (band) material.

Rhabdo EM

- The less differentiated rhabdomyoblasts are conspicuous in alveolar and blue cell embryonal rhabdo.
- Erlandson divides Rhabdomyoblasts into
  1- presumptive - no z lines , only sparse organelles, thin filaments, some glycogen
  2- undifferentiated- cells with rigid 15nm myosin filaments and numerous ribosomes
  3- poorly diff-z dics with a periodic structure w/wo myofilaments. Dense masses of tangled myofilaments and ribosomes
  4- Mod Diff- variable #s of haphazardly arranged rudimentary sarcomeres or more mature sarcomeres where recognizable bands are seen
  5- Well diff where numerous well defined sarcomeres.
Embryonal rhabdomyosarcoma

Minimal criteria for rhabdo

EM Rhabdomyosarcoma
Wilms Tumor

- Wilms' tumor (WT) or nephroblastoma—morphologically resembles embryonal renal tissue.
- > 90% children under the age of ten years.
- Believed to arise from the nephrogenic blastema.
- Wilms' tumor—variety of patterns including blastematous or epithelial-predominant, mixed blastematous, and epithelial and sarcomatous types.
- The first cytologic description of Wilms tumor was described by Dey et al. who described 15 cases of renal, extrarenal, and metastatic Wilms' tumor; this was followed by several other reports.

Wilms Tumor

- 1814 earliest record of Wilms being mentioned
- 1872-histologic description
- 1879 William Osler published paper with 4 cases from lit and 2 of his own
- 1899 Wilms published 7 cases
- Most cases sporadic some related to the WT-1 gene (chromosome11p13)

Wilms Tumor

- FNA: blastemal cells with evidence of epithelial and mesenchymal differentiation—found in 90% of the cases, have scanty, deep blue cytoplasm with ill-defined borders and round to oval nuclei having fine, regular, evenly distributed chromatin.
- Epithelial component shows cells forming tubules or solid strands or cords, and the cells exhibit a paler blue cytoplasm.
- Stromal elements are usually composed of spindle-shaped cells. Occasional, large, pleomorphic cells with abundant cytoplasm may be seen.
- Other variants include mesenchyme-predominant Wilms' tumor, which shows clusters of spindle cells associated with the matrix material, with or without evidence of rhabdomyoblastic differentiation.
Wilms Tumor

- Blastema-predominant Wilms’ tumor should be distinguished mainly from neuroblastoma with which it shares clinical and cytological overlaps.
- The WT1 gene, identified as a tumor suppressor gene located at 11p13, is involved in the development of Wilms’ tumor.
- Unique features: stroma, epithelial (tubules or glomeruloid structures) and blue cells (blastema cells).
- Rosettes with metachromatic basement membrane material = Wilms.
- With fibrillary material (neuropil) = NB.
- If in adults Diff Dx is with met small cell carcinoma.

Wilms Tumor

- FNA highly cellularity
- Blastema, epithelium and stroma
- IHC
- Brahmi et al. reported positivity for cytokeratin, NSE, EMA, and vimentin

Blastema
- WT1, CD56 and vim +
- CK, LCA, NSE, S100 and FLI -

Wilms Tumor

EM
- Cells- small round held by rudimentary cell junctions to ovoid and high N/C ratio
- Tight junctions hold the structures with primitive kidney collecting duct appearance. The lumens of these lined by occasional, rare immature microvilli, a cilium and ciliary bodies
- Immature cilia seen in 30-100% of cases.
Wilms EM

- Mierau G found EM useful to study possible cases of Wilms, blastemal, adult Wilms and extrarenal primaries and mets
- 3/19 cases studied by Erlandson showed embryonal rhabdo diff
- According to Erlandson, the finding of an array of tight junctions surrounding rudimentary often closed lumen to be very useful diagnostic marker of Wilms

Desmoplastic small round cell tumor (DSRCT)

- Desmoplastic small round cell tumor (DSRCT) rare neoplasm first described by Gerald and Rosai in 1989.
- Highly malignant mesenchymal neoplasm growing along serosal surfaces, mostly involving the abdominal cavity, and is associated with poor prognosis. May involve pleura, scrotum, and ovary.
- Gerald et al. followed up on 17 DSRCT patients: 15 patients died of tumor progression with an average survival of about 22 months.

DSRCT

- Tumors with following features: predominant intrabdominal primary location with common peritoneal seeding
- Round to oval small cells in desmoplastic stroma (histologically)
- EM and IHC with multidirectional phenotypic expression (divergent diff); children to young adults
DSRCT EM

- Swanson et al. reported myogenic differentiation in two of their 12 cases.
- Swanson, Dehner and Wick MR. Polyphenotypic small cell tumors of childhood. Lab Invest 1988;58:9P

(DSRCT)

- FNAC smears are characterized by high cellularity, round blue cells arranged in clusters and stromal fragments
- Nuclei are round to oval, some of them with inconspicuous nucleoli and display nuclear molding.
- Cells have high N/C ratio with granular chromatin.
- The cytoplasm is scant to moderate, pale blue, and occasionally vacuolated.
- Loose rosette formation is also seen frequently.
- Stromal fragments which stain metachromatically have also been identified in all cases. Naked nuclei may be numerous.
- Rhabdoid cells with dense paranuclear inclusions may occur in about 50% of cases.

DSRCT: IHC

- Positivity for cytokeratin, desmin, (paranuclear, dot-like), Myo D, EMA, and WT1 in rare cases.
- Cytology coupled with IHC may not be considered as diagnostic but can serve as a mode of preoperative diagnosis in cases where a biopsy may be difficult to obtain.
(DSRCT)

• In 1992, Sawyer et al. identified a novel reciprocal translocation of chromosomes 11 and 22, t(11;22)(p13;q12).
• Alava et al. detected EWS-WT1 chimeric transcripts in 11/12 DSRCTs by RT-PCR by using primers for the EWS gene exon 7 (EWS 22.3) and the WT1 gene exon 10 (WT1 10.1).

EM of Desmoplastic Small Round Blue Cell Tumor

• Neoplastic cells were seen in nests surrounded by a thin basal lamina.
• Cell junctions can be few and poorly developed.
• Small, well-formed desmosomes with tonofilaments can be seen.
• Dendritic-like processes containing microtubules and dense-core granules were seen in four of the cases.
• Sparse neurosecretory-like granules were the only indication of neuroendocrine differentiation in two other cases.

Multidirectional phenotypic expression along lines of epithelial, neural and muscle phenotype.

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DSRCT

• (A) Tightly packed cluster of neoplastic cells surrounded by basal lamina. A cytoplasmic process contacting the basal lamina is seen at the periphery of the nests (arrow) (* 2,100). (B) Higher magnification showing the thin basal lamina and another cytoplasmic process containing neurosecretory-type granules (* 12,000).
DSRCT

- (A): Small lumen into which are protruding microvilli (× 9,000). (B): Group of tumor cells, one of which is showing an intracytoplasmic lumen containing amorphous material (× 5,200).

SMALL CELL NE CARCINOMA

- The neuroendocrine nature of these tumors was suspected before the light microscopic studies of Azzopardi in 1959 and the demonstration of neuroendocrine granules in 1968 by Bensch et al.
- Dense core granules called neurosecretory granules.
- FNA is usually the first diagnostic approach.
- Small cell carcinomas are more common in the lung.
- FNA cytology of the lung is a daily dilemma.
- These tumors can be seen in other organs: Larynx, salivary glands, esophagus, colon, pancreas, uterine cervix, and prostate.
Small cell carcinoma

- By FNA, small cell carcinomas can be diagnosed
- May be difficult to distinguish them from other poorly diff carcinomas such as adenocarcinomas or squamous cell carcinomas.

Small Cell carcinoma

- IHC
- small cell carcinoma illustrates the limited sensitivity of immunohistochemistry when the degree of differentiation of neuroendocrine markers is itself limited.
- Most small cell carcinomas are negative by chromogranin and positive for synaptophysin and CD56
Small Cell Carcinoma

- EM
- Higher magnification shows a few neurosecretory granules are present in the cytoplasm.
- The size and characteristics of the secretory granules along with the proper cytologic features are sufficient for the diagnosis of small cell neuroendocrine carcinoma.
- Of course IHC for Chrom, Synapto and CD56 are also correlated for good measure.

Cohesive medium size cells with small processes which interdigitate

NE small cell tumors

- Other small cell neuroendocrine tumors can be found in the prostate, liver, cervix and so on.
- Small neoplastic cells in these other locations, one must r/o metastatic disease.
Lymphoma Look alikes

- NEC
- Melanoma
- Poorly differentiated plasmacytomas
- PD carcinoma
- Some sarcomas, epithelioid/round cell sarcomas

Lymphoma Look alikes

- These cases when metastatic can be confused with lymphomas.
Small Cell Carcinoma

Lymphoma

- Lymphoma, (small cell types) is in the differential diagnosis of these tumors
- FNA of Lymphoma:
  - cellular and discohesive
  - crush artifact
  - molding

Flow Cytometry

- FC has been used extensively for the immunophenotypic evaluation of bone marrow specimens in acute and chronic leukemia and lymphoma.
- FC in conjunction with FNAC is a valuable method for diagnosing by establishing clonality and subclassifying NHL; can eliminate the need for a more invasive surgical biopsy in many cases.
Flow Cytometry

- FC done on FNA can be tricky for the same reason that we already discussed about EM of FNA
- The passage of cells through the needle, especially when blood clots and the sample must be expelled by a thrust of the plunger, the pressure damages the cells

- Another important point: The RPMI must become turbid or cloudy in a non-bloody sample for the FC to be adequate.

EM for Lymphoma

- A battery of IHC and flow cytometry are the usual and best approach for Dx of lymphoma
- EM is usually not the way we normally approach lymphoma.
- Unless other tumors is suspected
- Great mimicker of lymphoma, small cell carcinoma

Lymphoma
Conclusion

- Understanding the limitations of the ancillary technique of choice is paramount.
- Favoring a technique to the exclusion of others places pathologists at a disadvantage.
- A judicious combination of ancillary techniques often is the best approach.

"The problem can often be solved by using one technique or an intelligent combination of a small number of various methods, but rarely by excluding one technique in favor of another..."

Dr. P. U. Heitz
Gracias

SYNOVIAL SARCOMA

SS cytology
SS cytology

Monophasic SS

(p11.2;q11.2) (X;18)
Translocation X and 18
Merkel Cell Carcinoma

- Monomorphic cellular tumors that occur in the dermis have been called trabecular carcinomas or Merkel cell tumors.
- Merkel cells are at a higher concentration in the skin of the digits, lips and oral cavity, in the outer root sheath of hair follicles and in the tactile hair disks.

Merkel cell tumors

- Cytology: Dispersed chromatin with invaginated nuclear membranes, inconspicuous nucleoli in round nuclei, scant cytoplasm with a parallel array of CK filaments in the paranuclear zone, and many mitoses. Trabeculae and pseudorosettes may be identified.
- IHC: NI and neoplastic Merkel cells may express NSE and a number of NE markers.
- IHC: The cells react with keratin in a paranuclear fashion.
- Electron microscopy is required for definitive diagnosis. Like normal Merkel cells, tumor cells contain electron-dense granules (80-200 nm), Filaments and desmosomes. Filament-rich cytoplasmic spikes were found in four tumors. These resemble corresponding protrusions of normal Merkel cells and have not been described in other APUDomas. Characteristic membrane bound dense core granules that may be associated with unmyelinated neurites.

Merkel cell tumor

- 56 year old man with a mass in the scalp. FNA cytology was composed of small blue cells with the appropriate IHC of focal CK 20 dot –like positivity.
- EM showed small cells with primitive cell junctions and rare cytoplasmic membrane bound granules.
Merkel cell tumor

Clusters of perinuclear cytokeratin filaments CK20 +

Hepatoblastoma

- Hepatoblastoma (HB), is a rare embryonal neoplasm that occurs predominantly in infants and children younger than 3 yr of age. Hepatoblastoma, although rare, is the most common primary malignant neoplasm of the liver in children.
- A 3 year old AA male presented with a large hepatic mass and metastatic nodules in both lungs. Intraoperative biopsy revealed a hepatoblastoma.
- Aspiration biopsy yielded a highly cellular aspirate with cords of pleomorphic cells embedded in a mucoid matrix. Histologic sections showed a diffusely infiltrative neoplasm composed of sheets and cords of highly pleomorphic cells.
- The neoplastic cells stained strongly positive for cytokeratin CAM 5.2 and AE1 and focally positive for alpha-fetoprotein, ferritin, carcinoembryonic antigen and vimentin.
- Ultrastructurally, the neoplastic cells had abundant intercellular junctions and intracytoplasmic aggregates of intermediate filaments.
Hepatoblastoma, ultrastructure

Advantages of a cytologic biopsy over a surgical biopsy
- Less invasive
- Less expensive
- Less morbidity
- Practically no risk
- Less time to perform
- Can be performed as an outpatient
Advantages of EM over IHC for the practice of Cytology

- Diagnosis more precise - subcellular elements identified
- Adequacy of small samples
- Effective: 1) makes the diagnosis 2) narrows the diff dx 3) confirms IHC findings 4)
- EM immunogold: more precise than IHC since Ag-Ab reaction can be observed in its subcellular location.

CONCLUSION

- Selection of the appropriate ancillary technique to solve the diagnostic problem.
- IHC is the technique of choice when a few Abs can solve the diagnostic problem.
- EM is the technique of choice when a large battery of Abs is necessary to solve a diagnostic problem or when IHC is inconclusive.

Cases in which EM is confirmatory

Electron Microscopy

- Is the technique of choice in many cases where making a precise diagnosis is imperative for patients to obtain the precise therapy or be placed in a particular protocol.
Other techniques useful in the diagnosis of sarcomas and look-alike in addition to IH and EM

FLOW CYTOMETRY
extremely useful when hematologic conditions are in the differential diagnosis

CYTOGENETICS
certain soft tissue neoplasms have characteristic cytogenetic abnormalities
i.e. synovial sarcomas- TRANSLOCATIONS INVOLVING CHROM X AND 18, Ewing’s sarcomas (EWS-Fli1), clear cell sarcomas (eWS-ATF1), myxoid liposarcomas (FUS-CHOP), among others

MOLECULAR DIAGNOSTICS

Synovial Sarcoma

- Described by Simon in 1865, although the first use of the term is ascribed to Fisher.
- Some variants of synovial sarcoma such as the small cell (or round cell variant) may be considered under the category of MSRCT.
- Highly malignant tumor
- Usually seen in the extremities in the vicinity of joints, most commonly the knee and lower thigh region. The peak incidence is in the 3rd decade and males are affected more often than females.
- Four recognized subtypes of synovial sarcoma: biphasic tumors, monophasic fibrous tumors, monophasic epithelial tumors, and poorly differentiated tumors.
- The three types of poorly differentiated SS are a large cell epithelioid variant. There is a small cell variant which falls in the small blue cell tumor category and a high-grade spindle cell variant.

Synovial Sarcoma, cytology

- Cytology of conventional synovial sarcoma was described by Akemann et al.
- The cytoplogic features are: tumor cells were small to medium in size, with rounded, ovoid, or fusiform bland nuclei with inconspicuous nucleoli.
- Small glandular or acinar-like structures seen in some biphasic variant cases. Cellular pleomorphism was seen in the pleomorphic variant. Ewing et al. have elaborated on the cytological features of the monophasic variant of synovial sarcoma where tissue fragments and single cells containing scant granular cytoplasm, medium-sized nuclei, and coarse chromatin can seen. A monotonous spindle pattern with comma-shaped or oval and spindled or round nuclei can be seen.
**The cytology of the small cell variant of synovial sarcoma** shows numerous, small round cells with high N/C ratio.

- Cytology of poorly differentiated, small cell, and monophasic variants, for these, the importance of ancillary techniques such as immunohistochemistry, EM and molecular analysis is even greater.
- The molecular and cytogenetic abnormalities may be the defining factor.

**SS**

- **IHC may not very helpful with many markers positive**
  - Cytokeratin and the epithelial membrane antigen (EMA) may be negative.
  - Usually CK7 and CK19 and EMA + in the epithelial areas, in the spindle cell areas these are + in about 50%, in these areas vimentin is also positive.
  - Since CKs and EMA usually neg in peripheral nerve sheath tumors and EWS/PNET they re discriminatory
  - SS express S100 in about 30% abd CD99(MIC2) in 60% and BerEp4 in90% of the cases
  - Desmin, CD34 and WT1 are negative.

**Synovial Sarcoma**

- The translocation, t(X: 18) (p11:q11), was first identified in 1989 by Limon et al. and was further confirmed in more than 90% of synovial sarcomas,
- Cloning of the translocation, t(X;18) (p11.2;q11.2), from human synovial sarcoma revealed a fusion between the SYT gene located on chromosome 18 and the SSX gene located on the X chromosome, resulting in the formation of chimeric proteins localization.
- The Xp11 breakpoint actually involves two closely related genes, SSX1 and SSX2.

**Synovial Sarcoma**

- In equivocal cases, ultrastructural examination coupled with demonstration of the characteristic (X;18) chromosomal translocation in synovial sarcoma may be the only means for establishing a definitive diagnosis.

Small cell SS

- 23-yr-old female who had a synovial sarcoma involving the left infratemporal region, diagnosed at 7 yr of age, followed by a metastatic lesion involving the lung and chest wall 16 yr later.
- FNA of chest wall metastases consisted of numerous, small, round cells with high N/C ratio
- features similar to other small round cell tumors.
- IHC positive staining for cytokeratin, epithelial membrane antigen (EMA), and CD99.

SS Cytology

- Poorly differentiated Ss:
  - pleomorphic cell
  - Large epithelioid cell
  - Small blue cell
  - Small blue cell SS has vague biphasic patterns. Mitosis and necrosis are common

Poorly differentiated SS

- Three types of poorly differentiated synovial sarcoma can be recognized: a large cell epithelioid variant, a small cell variant, and a high-grade spindle cell variant.
- EMA reactivity was seen in 95% of cases
- cytokeratin was seen in 42%.
- S100 antigen was expressed in 63% of cases.
- Electron microscopic findings in poorly differentiated synovial sarcoma parallel those found in usual type synovial sarcoma.
- Molecular studies; 9 of 10 cases showed the presence of t(X;18) or the associated fusion gene product.
- These data indicate that poorly differentiated synovial sarcoma is a lesion that shares immunologic, ultrastructural, and molecular characteristics with the usual synovial sarcoma.

Synovial Sarcoma, ultrastructure

- Biphasic synovial sarcoma exhibits true glandular differentiation
- By EM, spaces are lined by cuboidal or columnar cells with apical microvilli which can be of the intestinal type. The glandular epithelial areas of the tumor are separated from the spindle areas by a basement membrane.
- These cells exhibit juxtaluminal junction complexes, commonly long tight junctions along with rows of lateral desmosomes.
- The cytoplasm of the epith cells have lysosomes, small clusters of glycogen particles and randomly oriented arrays of intermediate filaments.
- Rare tonofilaments can be seen especially in areas of squamous metaplasia.
Synovial Sarcoma, ultrastructure

- Monophasic SS
- Spindle cells in fascicles separated by foci of amorphous mesenchymal mucins and scattered collagen fibrils. Similar EM features as biphasic SS