FNA/Core Biopsy of Soft Tissue: Let the Category Be Your Guide

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Objectives

- Employ cytomorphology to better differentiate soft tissue lesions into diagnostic categories
- Implement selected immunohistochemical stains on FNA/core biopsy specimens to work within differential diagnoses of soft tissue lesions
- Utilize flourescence in situ hybridization (FISH) testing when appropriate on soft tissue lesions

Introduction

- Soft tissue FNA/core biopsy evaluation is a team effort (need to incorporate clinical history, radiology)
- Cytomorphology can overlap between entities so often IHC and FISH testing are needed
- Sometimes it's fine not to be definitive; broad categorization and low grade versus high grade distinction can help guide initial patient management
- Preoperative radiation typically used for high grade tumors (while it is not for low grade tumors)
- Some tumors are particular chemosensitive: synovial sarcoma, Ewing sarcoma, rhabdomyosarcoma, among others

Cast a Wide Net

- Sometimes in order to place a lesion into the mesenchymal (soft tissue) category carcinoma, melanoma and lymphoma should be excluded by ancillary studies
- In general similar IHC/FISH panels can be used for lesions within the same morphologic category (spindle cell lesions for example)
- Anatomic site can also help direct an ancillary panel (paraspinal good site for nerve sheath tumor for instance)

Benign Hints

- Superficial location
- Smaller size (<5 cm)
- Mobile (not fixed)
- Fluid on aspiration (abscess, hematoma, seroma, cyst)

Malignant Hints

- Deep seated mass (retroperitoneum)
- Larger size (>5 cm)
- Fixed to surrounding tissue
- Aggressive/infiltrative radiologic features

FNA/Core Needle Biopsy Performs with Good Accuracy

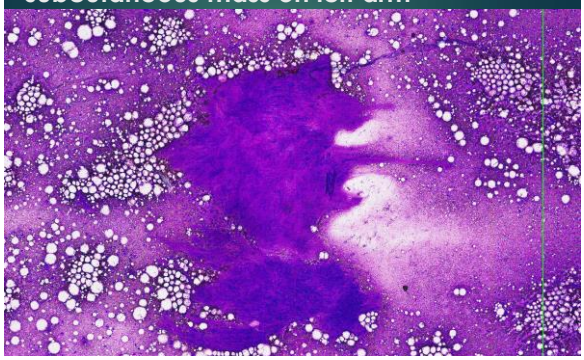
- Over a 7 year period cytologic diagnosis was concordant with histopathologic diagnosis in 96.9% (156/161)
- Most common malignant diagnoses:
  - Liposarcoma (30%)
  - Pleomorphic sarcoma (22%)
  - Leiomyosarcoma (8%)
  - Chondrosarcoma (7%)
  - GIST (26%)
- Most common benign neoplastic diagnoses:
  - Fibromatosis (22%)
  - Myxoma (17%)
  - Schwannoma (13%)

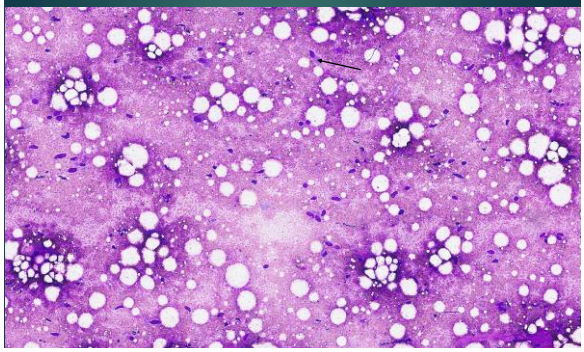
Colletti et al. Diagnostic Cytopathology. 2016;44(4): 291-298

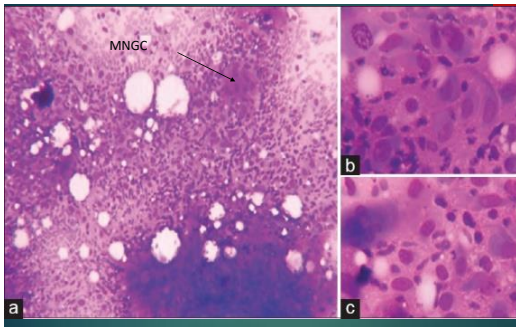
Stromal Predominant Lesions

- Intramuscular myxoma
- Nodular fasciitis
- Low grade fibromyxoid sarcoma (MUC4+, *FUS-CREB3L2* by FISH)
- Myxofibrosarcoma (low and high grade)
- Chordoma (S100+, cytokeratin+, and brachyury+)
- Chondrosarcoma (S100+)
- Myxoid liposarcoma [t(12;16) *CHOP-FUS* or *CHOP-EWS* FISH]

17-Year old male with enlarging subcutaneous mass on left arm



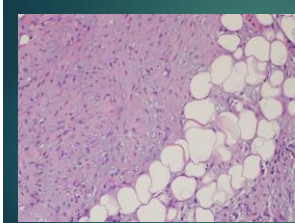




More cellular example of the same entity

Image adapted from Rani D and Gupta. J Cytol;36(4):196-199.

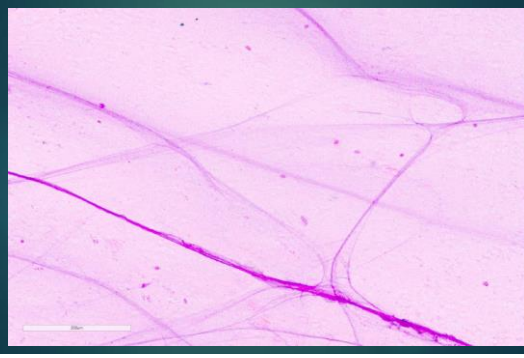
Nodular Fasciitis



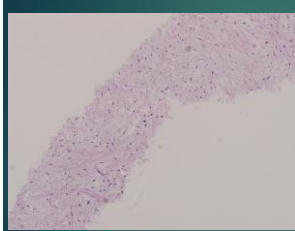
- Majority have spindled morphology with tapered cytoplasmic tails
- Open chromatin
- Can have myxoid background
- Can be hypercellular
- +/- inflammatory background
- Clinical context(superficial), rapidly growing recent timeline
- SMA and CD68 with negative desmin supportive
- Inflammatory myofibroblastic tumor is a cytologic mimic
- Diagnosis can be aided by *USP6-MYH9* gene fusion

Can recommend clinical surveillance to ensure resolution of lesion within weeks to months

42 year old female with a well demarcated intramuscular mass of the thigh

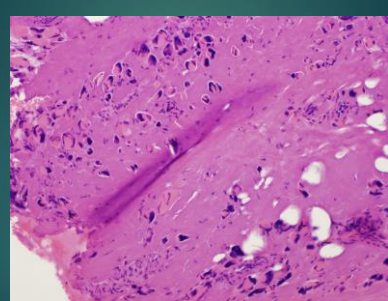


Intramuscular myxoma



- Deep seated, usually good correlation by CT imaging
- Low cellularity
- Bland uniform cells with cytoplasmic processes (stellate cells)
- Usually minimal vascularity (but can tolerate some)
- Multinucleated atrophic muscle fibers can play havoc

Degenerating muscle fibers (H+E)



Problematic differential for Myxoma

- **Low grade fibromyxoid sarcoma (usually subfascial)**
- Often has overlapping cytologic features with myxomas. Usually **less myxoid matrix**, **more cytologic atypia (hyperchromasia) and more cellular** as compared to myxomas.
- Curvilinear vessels
- Immunostaining for **MUC4** is a sensitive and specific marker for this entity
- CD34 + in the majority of myxomas/cellular myxomas and is expected to be negative in LGFMS
- FISH testing for ***FUS-CREB3L2*** is diagnostic in 95% of case
- Risk of recurrence and late metastases

Low Grade Fibromyxoid Sarcoma

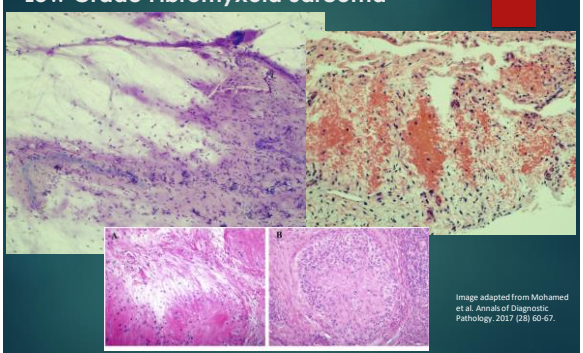
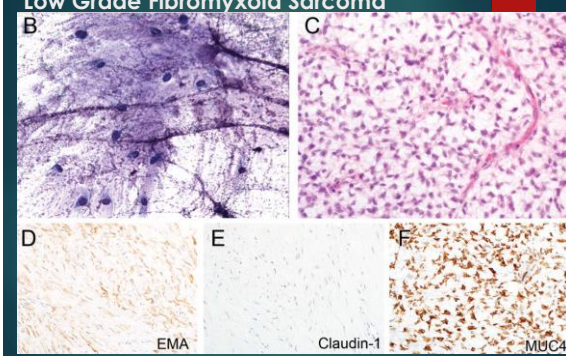


Image adapted from Mohamed et al. Annals of Diagnostic Pathology. 2017 (28) 60-67.

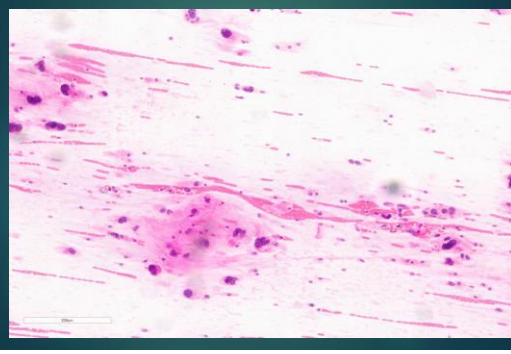
Giant hyalinizing rosettes

Low Grade Fibromyxoid Sarcoma



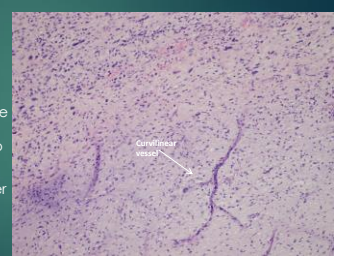
Adapted from Yang EJ, Hornick JL, Qian X. Cancer cytopathology 2016;124(9): 651-58.

62 year old male with deeply seated soft tissue mass of lower leg

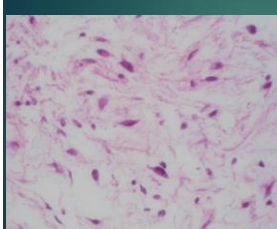


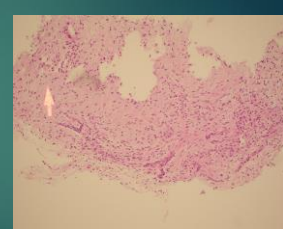
Myxofibrosarcoma

- Variable amounts of myxoid matrix
- Often curved, thick vessels ('curvilinear')
- Spindle cells show range of atypia and hyperchromasia (low to high grade)
- Low grade almost never metastasize
- No great ancillary test



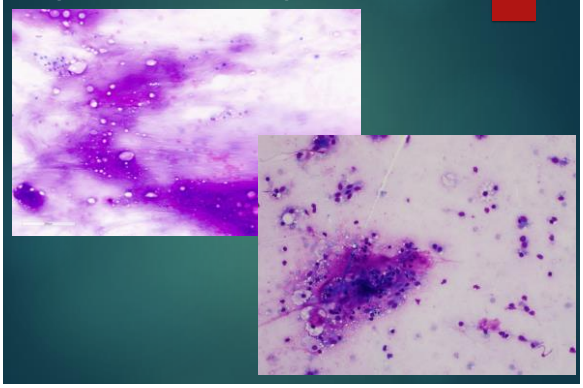
Low Grade Myxofibrosarcoma (not fair)





Usually subcutis of older adults

32 year old female with pelvic mass



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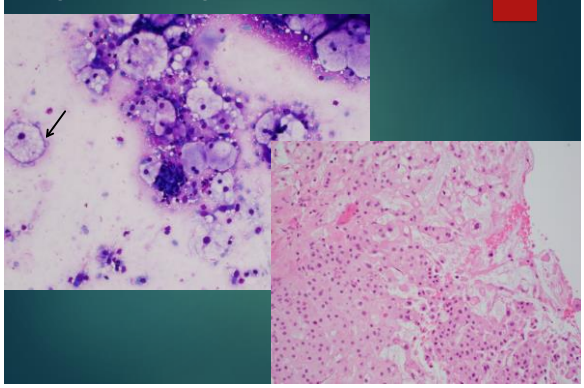
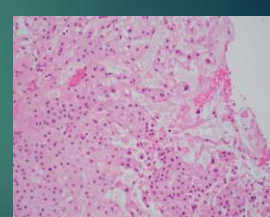
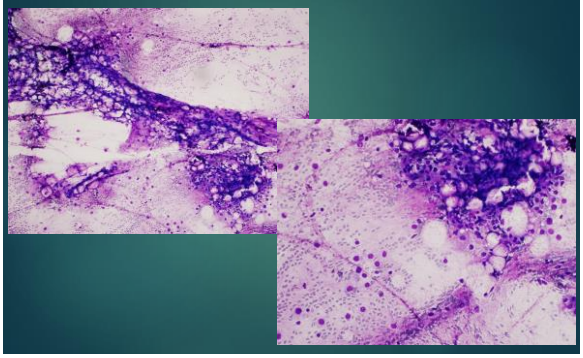


Chordoma

- Usually located in clivus, vertebral bodies or sacrococcygeal bone (small number in soft tissues)
- Physalipherous cells (big bubblers) and bland epithelioid cells embedded in abundant extracellular matrix
- Positive for S100, cytokeratins, and brachyury (highly specific)
- Up to 40% of non-cranial tumors metastasize



57 year old female with a large thigh mass



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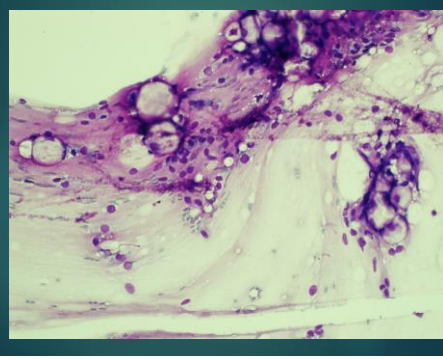
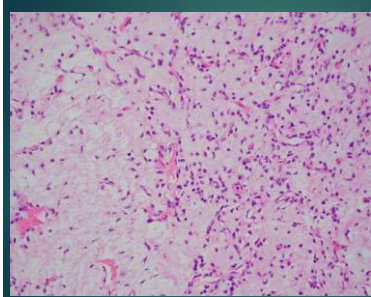


Myxoid Liposarcoma



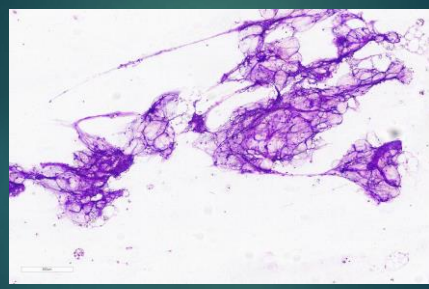
- Deep soft tissue of extremities
- 15-20% of liposarcomas
- Myxoid matrix
- Delicate vasculature
- Bland round cell proliferation
- +/- lipoblasts
- t(12;16) *CHOP-FUS* or *CHOP-EWS* FISH result is diagnostic
- High grade (>5% round cell component) is a predictor of unfavorable outcome

Adipocytic Lesions

- Lipoma
- Spindle cell lipoma/Pleomorphic lipoma
- Well differentiated liposarcoma/Atypical lipomatous tumor
- Pleomorphic liposarcoma
- Dedifferentiated liposarcoma

Bottom two entities can morphologically enter the pleomorphic or spindle cell categories

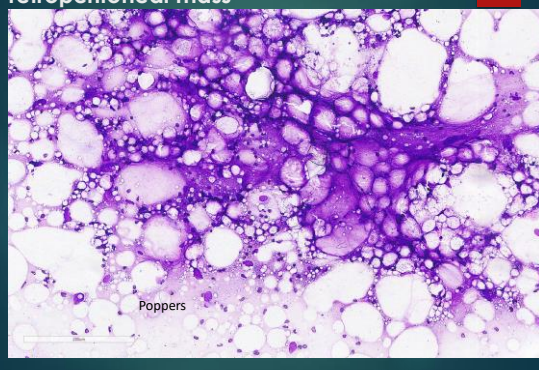
35 year old male with numerous subcutaneous masses, this from forearm



Lipoma

- Large univacuolate adipocytes with uniform size
- Peripheral small, bland nuclei (no atypia)
- If head and neck region and areas of spindled morphology or floret cells think spindle cell/pleomorphic lipoma (CD34 immunostain can clinch diagnosis)

44 year old female with a 10 cm retroperitoneal mass



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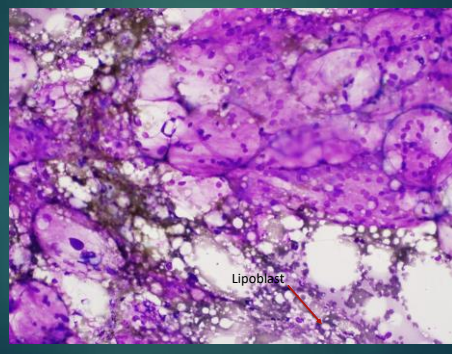
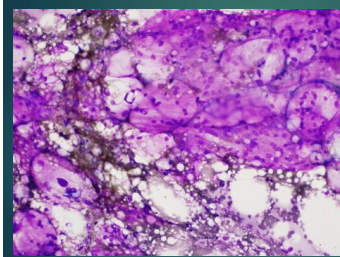
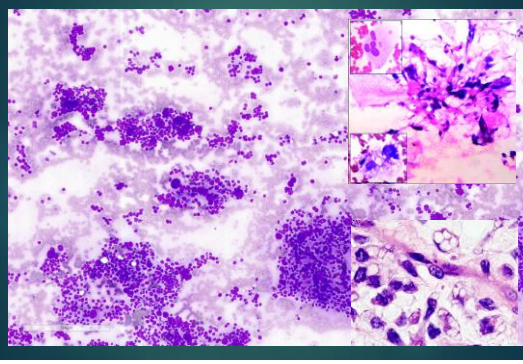


Well Differentiated Liposarcoma/Atypical lipomatous tumors



- If deep soft tissue retain term liposarcoma
- If at surgically amenable sites (limbs and trunk) then ALT
- Account for 40% of all liposarcoma
- Clusters of cells with lipid vacuoles
- Atypical stromal nuclei (hyperchromatic, large, irregular shape) are usually tipoff
- Lipoblasts often are hard to identify
- Sclerosing and inflammatory variants are recognized
- CDK4 nuclear immunopositive
- FISH testing for MDM2 amplification is diagnostic when paired with morphology and deep location

44 year old female with a 10 cm retroperitoneal mass



Pleomorphic Liposarcoma

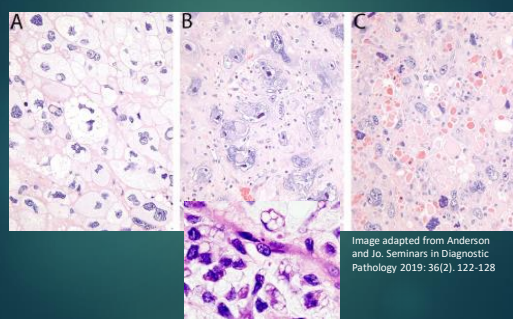


Image adapted from Anderson and Jo. Seminars in Diagnostic Pathology 2019: 36(2). 122-128

Pleomorphic Liposarcoma

- Least common subtype of liposarcoma (5%)
- Usually deep soft tissue of lower or upper extremity
- Varying proportion of lipoblasts in background of high grade, pleomorphic sarcoma
- Lipoblasts are needed in clinch the diagnosis
- Lack MDM2 amplification which separates this from dedifferentiated liposarcoma
- Most aggressive form of liposarcoma

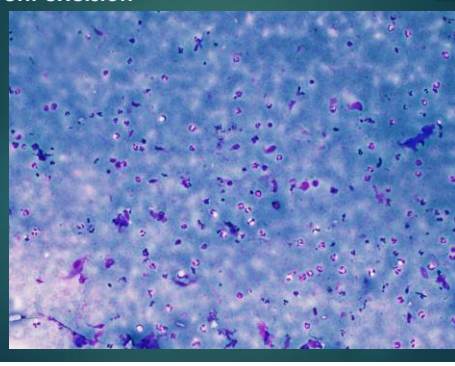
Spindle Cell Lesions

- **Reactive/reparative myofibroblastic lesions including nodular fasciitis** (SMA positive, desmin negative by IHC)
- **Fibromatosis** (B-catenin nuclear expression by IHC in 75%)
- **Schwannoma** (diffuse S100 by IHC)
- **Solitary Fibrous Tumor** (STAT6 nuclear positivity by IHC)
- **Synovial sarcoma** (TLE1+ by IHC, FISH testing for t(X;8) *SYT-SSX1/2* translocation is diagnostic)
- **Gastrointestinal stromal tumor** (DOG1 or cKit by IHC)
- **Malignant peripheral nerve sheath tumor** (focal S100, SOX10+ in 80% by IHC)
- **Leiomyosarcoma** (Desmin+ by IHC)
- **Dermatofibrosarcoma protuberans** (CD34+ by IHC)
- **Angiosarcoma** (can also be epithelioid; ERG or CD31 by IHC)
- **Don't forget about spindle cell melanoma**

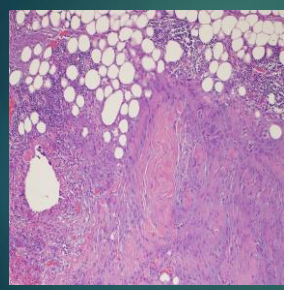
Spindle Cell Lesions

- Worrisome for malignancy features:
  - Hypercellularity
  - Hyperchromasia
  - Nuclear anisonucleosis and pleomorphism
- MPNST doesn't play fair.
  - Can be hypocellular in areas
  - Can be minimal for S100

26 year old patient with nodule near recent excision

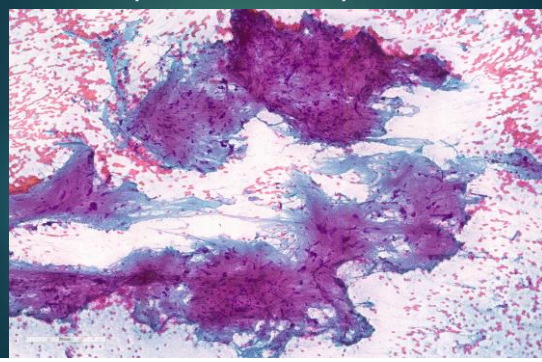


Reactive/Reparative changes

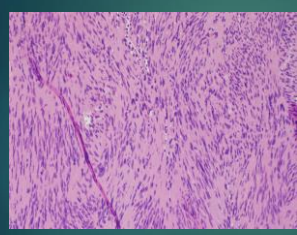


- Typically low to moderate cellularity
- Proliferating myofibroblasts with tapered cytoplasm
- Lack hyperchromasia
- Inflammatory background and multinucleated giant cells can be good clues

25 year old male with a paraspinal soft tissue mass present for several years

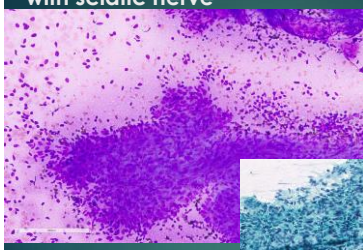


Schwannoma

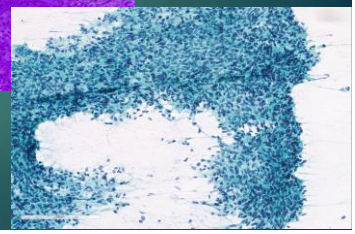


- Variably cellular depending on area sampled
- Buckled, low grade spindle cells within tissue **fragments** with a **fibrillary matrix**
- Be wary that ancient change can cause random nuclear atypia
- Diffuse S100 staining is effectively diagnostic

30 year old with NF1 and mass associated with sciatic nerve



Cellular, hyperchromatic, sense of tapered nuclei



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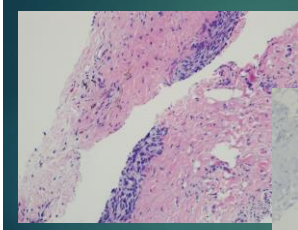
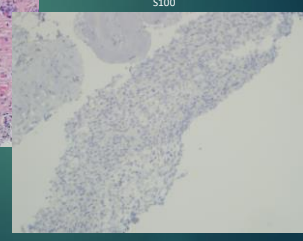


Variable cellularity with myxoid change

S100

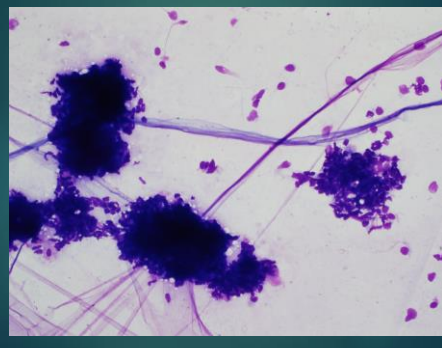


Malignant Peripheral Nerve Sheath Tumor

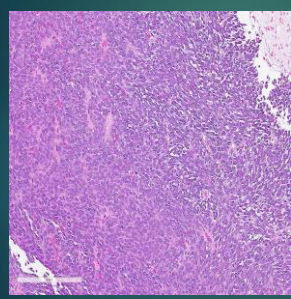
- 50% arise in patient's with Neurofibromatosis type 1
- Usually associated with a major nerve and >5cm at time of diagnosis
- Highly cellular, hyperchromatic, sense of tapered nuclei
- Typically fascicular growth pattern with alternating cellularity (variable morphology)
- Positive for S100 in <50% of cases decreases with higher grade
- SOX10 is positive in ~80%
- Extensive overlap with melanoma but rarely positive for Melan-A and MITF
- Negative for HMB-45 and BRAF (some hope)

Gaspard et al. Histopathology. 2018; 73(6): 969-982

14 year old female with a 10 cm enlarging central neck mass



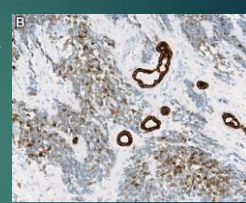
Synovial Sarcoma



- Typically deep seated in lower extremity but head and neck not infrequent
- High cellularity, dark but usually lack overt nuclear atypia
- Dense cell clusters alternating with dispersed cells
- Usually very monomorphic
- Cytokeratin/EMA usually focal in monophasic type
- Positive for CD99 and TLE1 by IHC
- FISH testing for t(X;18) *SYT-SSX1/2* translocation is diagnostic

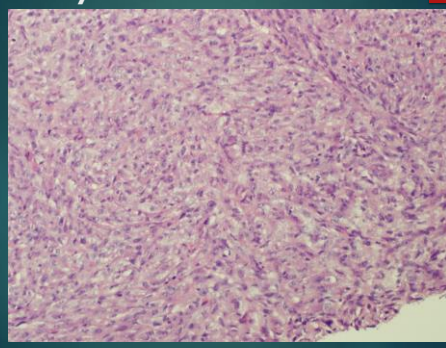
Cytokeratin Games

- 22/44 (50%) of Alveolar Rhabdomyosarcomas were positive (ranging from focal to diffuse) for Cam 5.2
- Desmin is a smart stain for small round cell differential
- Epithelioid Angiosarcoma is often pancytokeratin positive
- Epithelioid MPNST can show cytokeratin positivity
- Leiomyosarcoma can also express cytokeratin



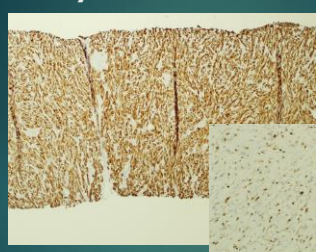
Adapted from Thompson LDR, Jo VY, Agaimy A et al. *Head and Neck Pathol.* August 2017.

Leiomyosarcoma

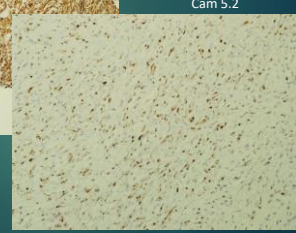


Leiomyosarcoma

Desmin



Cam 5.2

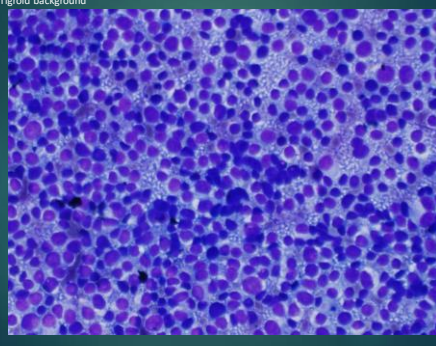


Small round cell malignancies

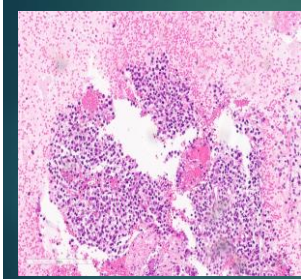
- **Lymphoma** (flow cytometry)
- **Ewing sarcoma** [diffuse CD99+, t(11;22)*EWSR/FLI-1 by FISH]*
- **Desmoplastic small round cell tumor** [Positive for keratins, desmin and WT1 (carboxy-terminus) by IHC; *EWSR1/WT1* translocation by FISH]
- **Embryonal/Alveolar Rhabdomyosarcoma** (positive for desmin and skeletal markers myoD1 and myogenin by IHC)
- **Undifferentiated round cell sarcoma or 'Ewing-like sarcomas'** (*CIC-DUX4* and *BCOR-CCNB3* gene fusions are two examples)

6 year old girl with a mass at the angle of the jaw

Tigroid background



Ewing sarcoma



- Hypercellular smears
- Predominanlty dispersed small round cells with fine chromatin
- Tigroid background
- Vacuolated cytoplasm (best visualized on cell block)
- Expect diffuse CD99 +
- FISH testing for t(11;22)*EWSR/FLI-1* is usually diagnostic (85%)

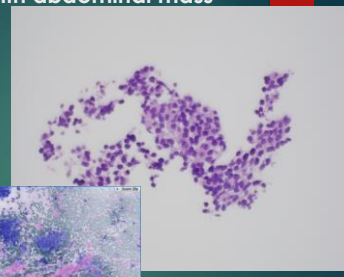
Tumors with EWSR gene rearrangements

- Ewing sarcoma
- Angiomatoid (malignant) fibrous histiocytoma
- Myoepithelioma/Myoepithelial carcinoma of soft tissue
- Clear cell sarcoma
- Desmoplastic small round cell tumor
- Extraskeletal myxoid chondrosarcoma
- Myxoid Liposarcoma

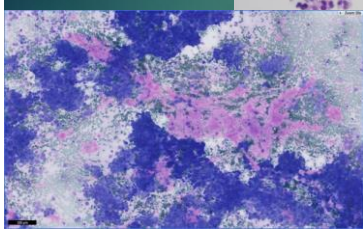
Hill DA, Pfeifer JD, Marley EF et al. Am J Clin Pathol. 2000;114(3):345-53.

Boland J and Folpe A. Advances in Anatomic Pathology. 2013;20(2): 75-85.

35 year old male with abdominal mass

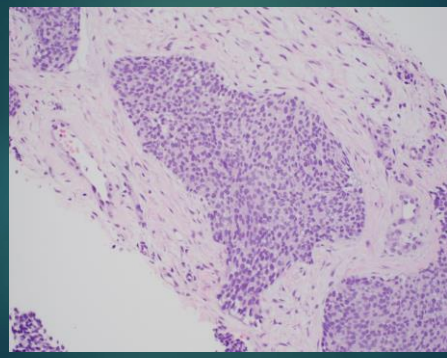


Dense stroma, small round cells

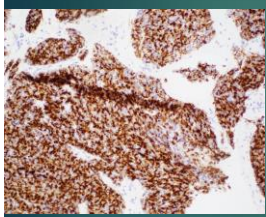


Touch preparation

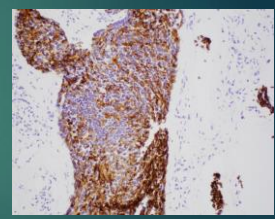
35 year old male with abdominal mass



Immunoprofile

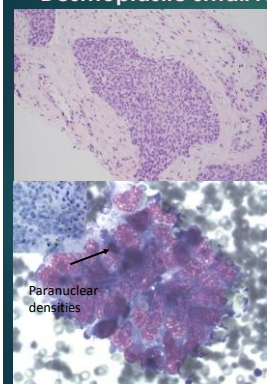


Desmin (dot-like positivity)



Keratin

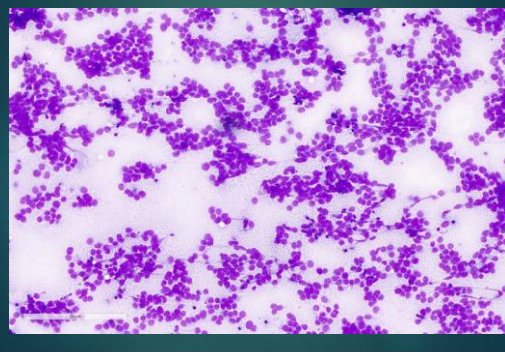
Desmoplastic small round cell tumor



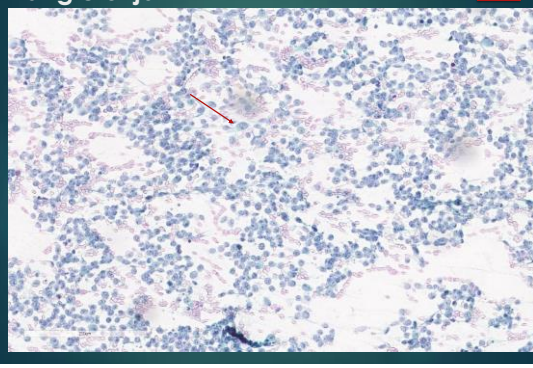
- Predominantly young males in abdominal cavity
- Smear are hypercellular from 3D clusters to single cells
- Monomorphic small to medium sized cells without marked pleomorphism
- May pick up dense stroma on smear
- Crush artifact and paranuclear densities described
- On core tumor cells embedded in fibrous stroma
- Positive for keratins, desmin and WT1 (carboxy-terminus)
- Negative for myogenin and MyoD1
- *EWSR1-WT1* translocation

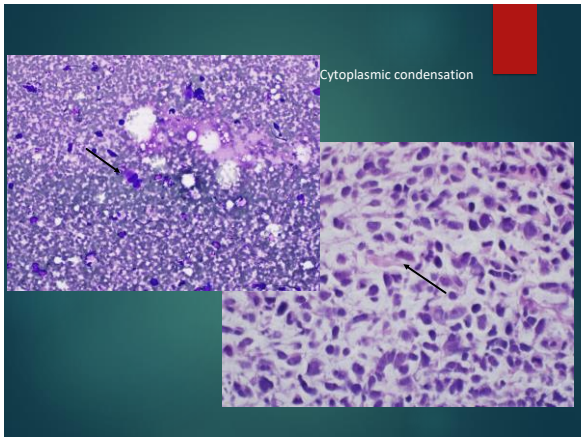
Klijanienko et al. Cancer Cytopathol 2014;122:386-93

6 year old girl with mass near angle of jaw

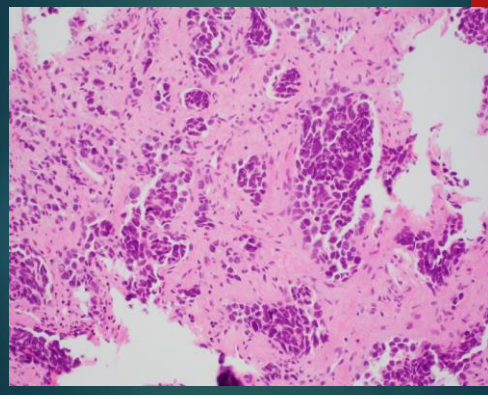


6 year old girl with mass near angle of jaw





Alveolar Rhabdomyosarcoma



Alveolar RMS

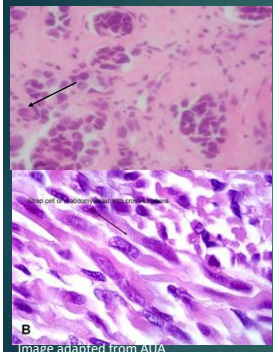


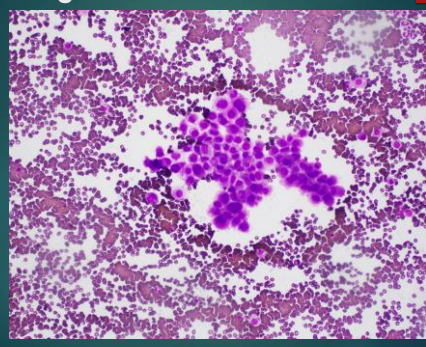
Image adapted from AUA Update Series. Volume 39.2020

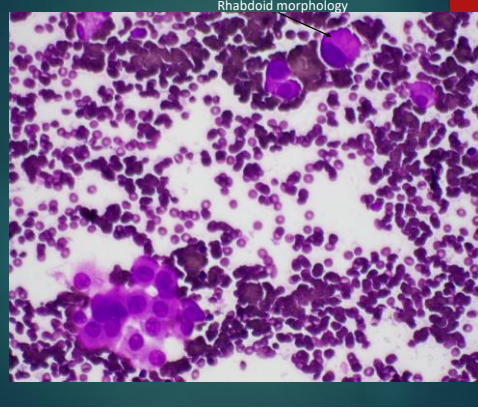
- Typically head and neck or limbs of children and adolescents
- Alveolar and Embryonal RMS have similar appearance
- Hyperchromatic round cells, loosely dispersed
- Cells with rhabdomyoblastic appearance are variable
- Alveolar RMS has fibrovascular septae dividing tumor cells into discrete nests
- Express desmin and skeletal markers myoD1 and myogenin
- Myogenin typically diffuse in Alveolar RMS, focal in Embryonal RMS
- Molecular detection of *PAX3-FOXO1* or *PAX7-FOXO1* is critical for management as translocation positive tumors are more aggressive

Epithelioid Tumors

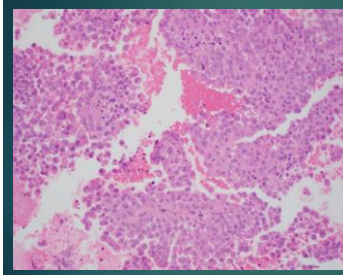
- **Epithelioid sarcoma** (INI-1 deleted by IHC, positive for vascular markers and cytokeratins)
- **Clear cell sarcoma of soft parts** (EWSR1 translocation, positive for melanocytic markers by IHC)
- **Alveolar soft part sarcoma** (TFE3 nuclear positivity by IHC, TFE3 gene fusion by FISH)
- **Epithelioid angiosarcoma** (ERG positive, often CK+ by IHC)
- **Gastrointestinal Stromal Tumor** (DOG-1 positive by IHC)
- **Myoepithelial carcinoma** (p63, calponin, S-100, pancytokeratin; don't give up can lose several markers)

38 year old male with soft tissue mass in groin and LAD





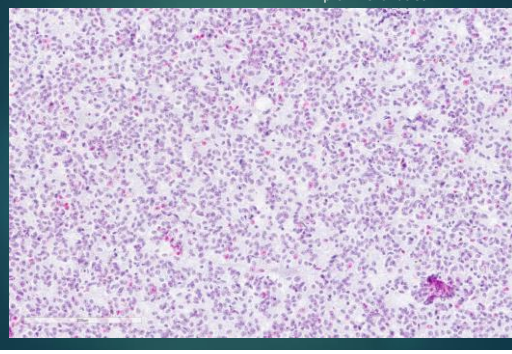
Epithelioid Sarcoma



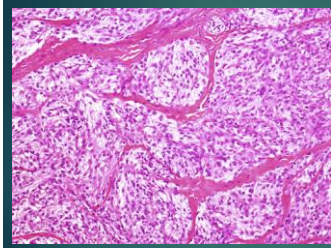
- Classic type in distal extremities
- Proximal type in pelvic regions
- Cytologically mostly large cells with marked atypia, frequent rhabdoid features
- Histologically nodular pattern around central necrosis (granulomatous pattern)
- IHC shows panCK+, EMA +, CD34+, ERG, loss of SMARCB1 (INI-1 deficient)
- The latter distinguishes from epithelioid vascular tumors

30 year old female with deep-seated tumor of ankle

Tigroid background, prominent nucleoli



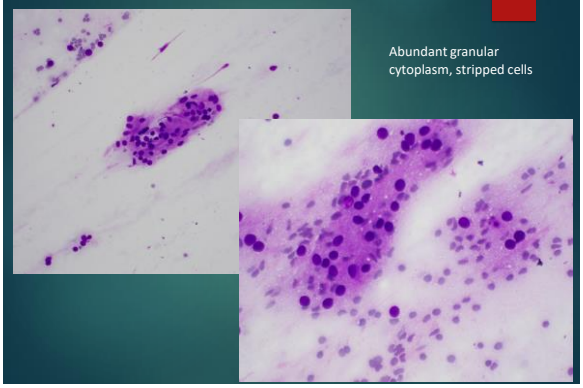
Clear Cell Sarcoma of Soft Parts



- Overwhelming majority are deep-seated tumors of extremities in young adults
- Cytologically epithelioid cells with prominent nucleoli, MNGCs, tigroid background
- Solid/nested pattern on histology
- Reactive for melanocytic markers (S100, MITF)
- *EWSR1-ATF* fusion by FISH

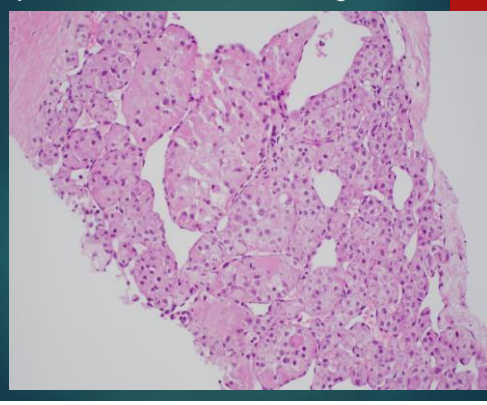
Image adapted from Thway et al. Surgical Pathology Clinics 2019. 12(1):165-190

43 year old female with anterior thigh mass

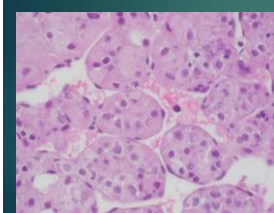


Abundant granular cytoplasm, stripped cells

43 year old female with anterior thigh mass



Alveolar soft part sarcoma

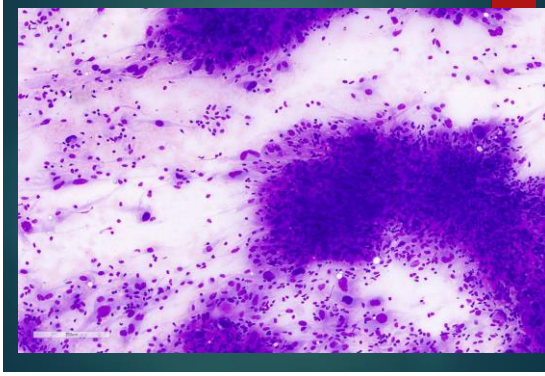


- Often deep soft tissue of thigh, can be head and neck in children (tongue/orbit)
- Uniform epithelioid cells with abundant granular cytoplasm, naked nuclei, nucleoli
- Looks like RCC
- Positive nuclear staining for TFE3

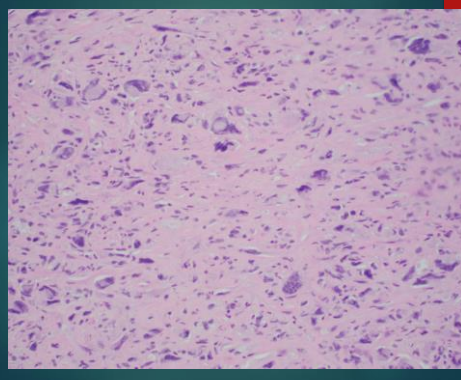
Pleomorphic Tumors

- Undifferentiated pleomorphic sarcoma (UPS, formerly MFH, diagnosis of exclusion)
- Pleomorphic liposarcoma (UPS morphology with lipoblasts, MDM2 amplification negative)
- Dedifferentiated liposarcoma (MDM2 amplified by FISH)
- Pleomorphic rhabdomyosarcoma (desmin, MyoD1 and myogenin positive by IHC)

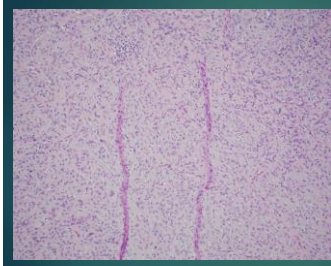
70 year old male with 7 cm deep thigh mass



70 year old male with 7 cm deep thigh mass



Undifferentiated pleomorphic sarcoma



- Account for 5-10% of sarcomas in patients 40 and older
- Hypercellular smears with loose cohesion/dispersed cells showing marked pleomorphism and giant cells
- Storiform pattern
- Essentially diagnosis of exclusion (no specific positivity for any lineage)

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