Nutn’ but a Small Biopsy
How to approach small biopsy samples of the sinonasal tract

Lester D. R. Thompson
www.lester-thompson.com

Learning Objectives
Following the presentation, participants should be able to:
1. Interpret and incorporating radiology findings into determining the best diagnosis for small biopsies.
2. Create a hierarchy of immunohistochemistry evaluation to achieve the best diagnosis with limited material.
3. Learn which entities can be combined versus separated into groups for management purposes.

Case
- 28 year old woman
- Presented with progressive sinus pain with recent onset of diplopia and headaches
- Left proptosis and exophthalmos were noted
- By computed tomography, there is a 1.8 x 1.1 cm left frontoethmoid sinus mass that involved the medial superior bony orbital wall, and extends to involve the soft tissues of the orbit and maxillary sinus
- During physical exam, there was a palpable mass through the left upper eyelid nasal side
- At biopsy there was a soft, somewhat necrotic to degenerated appearance to the tissue removed
Best diagnosis based on the H&E findings:

1. Melanoma
2. Mesenchymal chondrosarcoma
3. Rhabdomyosarcoma
4. Sinonasal undifferentiated carcinoma
5. Poorly differentiated squamous cell carcinoma
6. Lymphoma
7. Olfactory neuroblastoma
8. Ewing sarcoma
9. Pituitary adenoma

Differential Diagnosis: Mesenchymal Chondrosarcoma

- Malignant mesenchymal tumor with cartilaginous differentiation
- Rare, but 2nd - 3rd decades
- Cartilage is frequently limited, requiring many sections or levels
- Biphasic microscopic pattern:
  - Abrupt islands of cellular hyaline cartilage
  - Small, undifferentiated round to spindled cells
  - Cell arranged in a solid pattern with staghorn-shaped vessels
- **Positive**: CD99, Sox9; **Negative**: S100 protein
- **HEY1-NCOA2** fusion detected by FISH in ~80%
Differential Diagnosis:
Ewing/PNET

High-grade primitive small round cell sarcoma
with (variable) neuroectodermal differentiation
defined by presence of translocation (EWSR1)

- Small round blue cell tumor, sheets, tumor necrosis, finely distributed chromatin, mitoses
- **Positive:** CD99, FLI1, Erg, SNF5, p63 (~20%)
- **Variable:** NSE, S100 protein, synaptophysin, chromogranin, NFP or GFAP
- **Negative:** myogenic; hematolymphoid, keratin (focal up to 30%)
- **EWSR1/FLI1** translocation most often
Differential Diagnosis: NK/T-cell lymphoma, Sinonasal Type

- Extranodal, NK/T cell lymphoma, sinonasal type
  - Cytologic atypia, perivascular distribution, geographic necrosis
  - Often shows pseudoepitheliomatous hyperplasia
  - **Positive:** CD3, CD56, CD138, granzyme, EBER
  - **Negative:** CD20, CK-pan, desmin, vascular markers
• 42 year old
• Woman
• Congestion, difficulty breathing
• Imaging showed a sphenoid sinus mass
• Biopsy performed

CSP – Saturday Case Seminar
Ectopic Sphenoid Sinus Pituitary Adenoma

**Clinical**

*Benign pituitary gland neoplasm occurring separately from and without involvement of sella turcica (a normal anterior pituitary gland)*

- Direct extension from intrasellar pituitary tumors in about 2% should be excluded
- Incidence: Rare in ectopic locations
- Age: Wide range: 16–84 years
  - Mean: 54 years
- Gender: Female > Male (1.3:1)
- Symptoms: Obstruction, sinusitis, pain, discharge, headache, visual disturbances, endocrine syndrome

**Imaging Findings**

- Intrasphenoidal mass with expansion and/or erosion
- Sella may be involved by upward extension, but usually normal
- Strong enhancement post contrast
- Define extent and location of tumor
- Imaging usually suggests chordoma, nasopharyngeal carcinoma, or metastatic tumor
Pituitary Adenoma Pathology Findings

- Polypoid and pedunculated mass within sphenoid sinus, bone erosion
- Size: Range: 0.5 to 8.0 cm (mean, 2.9 cm)
- Intact surface epithelium
- Submucosal location, unencapsulated tumor
- Invades into subepithelial stroma and bone
- Necrosis (up to 25%); pleomorphism
- No perineural or vascular invasion;
- No atypical mitoses
- Many patterns
  - Solid, organoid, glandular, insular, festoons, ribbons, single file, rosettes—pseudorosettes, papillary, cystic
- Epithelial cells
  - Polygonal, plasmacytoid, cuboidal, spindled, round or oval nuclei with "salt-and-pepper," clumped chromatin, small nucleoli, intranuclear inclusions and variable cytoplasm
  - Profound pleomorphism
Immunohistochemistry

Positive:
- CK-Pan (AE1/AE3): 79%
- Synaptophysin: 97%
- CD56: 91%
- NSE: 76%
- Chromogranin-A: 71%
- CD99: 40%
- Prolactin: 59%
- FSH: 47%
- LH: 37%
- ACTH: 33%
- TSH: 29%
- GH: 26%

Neuroendocrine +
Epithelial markers +

CSP – Saturday Case Seminar
Treatment

- Surgery is treatment of choice
- Medical/hormonal manipulation
  - Dopamine-agonists (bromocriptine), somatostatin analogs (octreotide), corticosteroids (hydrocortisone, prednisone), thyroxine
- Stereotactic radioablation
  - For larger or incompletely removed tumors
- Excellent prognosis
  - 96% have no evidence of disease (mean follow-up: 10.5 years)
  - 4% died with disease (0.8 years)
  - 14% persistence/recurrence (mean, 2.1 years)
    - Managed with surgery or radiation

---

33 year old female
Presented with seizure
The computed tomography scan and the MRI demonstrate a 4.3 x 3.7 x 2.5 cm mass pushing the left frontal brain lobe, extends through the left ethmoid sinus
Calcifications noted

---

Small Round Blue Cell Tumors

Small Blue Round Cell: MRS LEEP
- Melanoma
- Rhabdomyosarcoma
- Sinonasal undifferentiated carcinoma (SNUC)
- Lymphoma
- Esthesioneuroblastoma (Olfactory neuroblastoma)
- Ewing sarcoma
- Pituitary adenoma
Olfactory Neuroblastoma
Clinical and Imaging

- Arises from the specialized sensory neuroepithelial (neuroectodermal) olfactory cells
- Normally in upper part of the nasal cavity
  - Superior nasal concha, upper part of septum, roof of nose, cribriform plate of ethmoid sinus
- Age: Bimodal age presentation (young or old)
- Sex: Equal gender
- Symptoms: Non-specific; Anosmia <5%
- Imaging: A “dumbbell-shaped” mass
  - Extends across the cribriform plate of ethmoid sinus
  - Bone erosion of the lamina papyracea
  - CT may show speckled calcifications
  - MR T1-weighted images after gadolinium show marked enhancement

Olfactory Neuroblastoma
Microscopic features

- Circumscribed lobules or nests of tumor in syncytial arrangement with neural processes
- Intact mucosa (olfactory mucosa in many cases)
- Tumor cells are “small, round, blue” cells
  - Slightly larger than mature lymphocytes
  - High nuclear to cytoplasmic ratio
  - Nuclei are small and uniform with hyperchromatic, delicate nuclear chromatin distribution
  - Nucleoli are inconspicuous
Olfactory Neuroblastoma
Microscopic features

- Two types of rosettes, although only in up to 30% of cases
  - Pseudorosettes (Homer Wright) common
    - The delicate, neurofibrillary and edematous stroma forms in the center of a cuffed or palisaded arrangement
  - True rosettes (Flexner-Wintersteiner) less common
    - “Gland-like” tight, annular arrangement
Olfactory Neuroblastoma
Grading

- Grade based on the degree of differentiated, presence of neural stroma, mitotic figures, and necrosis
- Grade I to Grade IV
- Grade correlates with prognosis
- Increased grade of the tumor is more difficult to diagnose

<table>
<thead>
<tr>
<th>Feature</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Mitoses</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Anaplasia</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Matrix</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
<td>–</td>
</tr>
<tr>
<td>Rosettes</td>
<td>HW</td>
<td>HW</td>
<td>FW</td>
<td>FW</td>
</tr>
<tr>
<td>Necrosis</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>
### Olfactory Neuroblastoma Immunohistochemistry

#### Positive
- Synaptophysin, chromogranin, neuron specific enolase, NFP, CD56, calretinin
- S100 protein or GFAP found at the **periphery** of the tumor lobules and correspond to Schwann (sustentacular) cells
- Rarely, focal reactions with keratin (especially LMW cytokeratin Cam 5.2)

#### Negative
- Desmin
- SMA
- MSA
- Myogenin
- HMB45
- Melan A
- CD45RB
- CD99

---

**Synaptophysin**

**Chromogranin**

**CD56**
Olfactory Neuroblastoma

Immunohistochemistry to Order

- **Positive**
  - Synaptophysin/CD56/Chromogranin
  - S-100 protein
- **Negative**
  - CK-pan
  - Myogenin/MYOD1/Desmin

**Olfactory Neuroblastoma Management and Outcome**

- Complete radical surgical eradication
  - Craniofacial resection including cribriform plate by trephination or by endoscopic approach
  - Combined with radiotherapy or chemotherapy
- Outcome:
  - Recurrence rate: 15–30%
  - Lymph node metastasis: 10–20%
  - Distant (lung/bone) metastasis: 10%
- Overall 5-year survival: 60–80%
  - Low grade: 80% 5-year survival
  - High grade: 40% 5-year survival

**Differential Diagnosis: Mucosal Melanoma**

- Dyscohesive epithelioid to spindled tumor cells, junctional proliferation, pigmented, intranuclear cytoplasmic inclusions, eccentric nuclei, mitoses, peritheliomatous growth
- **Positive**: S100 protein, HMB-45, Melan-A
- **Negative**: Keratin, neuroendocrine markers, pituitary hormones and transcription factors
Rhabdomyosarcoma

- Looks like a polyp
- Can be embryonal, alveolar, or undifferentiated types
- Small round cells to ribbon or strap shaped cells to large cells
- Desmin, myogenin, myoglobin, MYOD1, SMA, MSA reactive
  - Also positive with CK-pan (8%) and CD56
- 5-year survival dependent upon stage
CSP – Saturday Case Seminar
Immunohistochemistry Evaluation

Initial panel for poorly differentiated tumor:
- CK-pan
- Desmin
- CD56
- S100 protein
- CD45RB

- 69 year old
- Man
- Congestion, difficulty breathing
- Epistaxis
- Imaging showed a nasal cavity centered mass
- Biopsy performed

CK: HWM & LMW

CSP – Saturday Case Seminar
Sinonasal Undifferentiated Carcinoma

High grade carcinoma without squamous or glandular differentiation
- Older patients
- Men > Women
- Rapidly growing clinically
- Midline destructive with bone destruction, necrosis and lymph-vascular invasion
- High frequency of metastatic disease
- Poor outcome

CSP – Saturday Case Seminar
Sinonasal Undifferentiated Carcinoma

- Undifferentiated histologic appearance
- May have rosettes
- High mitotic index

**Positive:** CK-pan, EMA, CK7 (~50%), NSE, Ki-67, p16, CD117

**Sometimes:** Synaptophysin, chromogranin, CD56, p63

**Negative:** CK5/6, desmin, CD34, HPV, S100 protein, HMB45, EBER

---

Neuroendocrine carcinoma

- Tends to have more salt-and-pepper nuclear chromatin
- Slightly different immunohistochemistry

**Positive:** CK-pan/CAM5.2: Dot/punctate; neuroendocrine markers CD56, synaptophysin, chromogranin, NSE

**Negative:** p16, HPV, NUT, CD34

---

CSP – Saturday Case Seminar
Synaptophysin
CD56

CK5/6, S100 protein, Prolactin, Desmin, CD34
S100 protein

CK5/6
p63

CSP – Saturday Case Seminar
Best classification after studies:

1. Nasopharyngeal carcinoma
2. Poorly differentiated squamous cell carcinoma, pending IHC
3. Poorly differentiated squamous cell carcinoma
4. Sinonasal undifferentiated carcinoma

Additional Considerations

- There is abrupt squamous differentiation or keratinization
- Otherwise poorly differentiated
- Negative sarcoma and neuroendocrine markers
- Additional evaluation can be done
- **NUT midline carcinoma**
**NUT Midline Carcinoma**

- NUT midline carcinoma (NMC) is an aggressive subset of squamous cell carcinoma, genetically defined by rearrangement of the NUTM1 gene
  - Nuclear protein in testis (NUT, aka NUTM1) gene
- Most common (70%) rearrangements are BRD4-NUTM1 fusion, although NUT-variant fusions are well recognized
- ~35% affect the head and neck area
  - Most in sinonasal tract > orbit
  - Majority in mediastinum
- Generally younger patients
- Usually midline but can be anywhere

**NUT1 Midline Carcinoma**

- Prognosis is usually poor (median 6.7 months)
- Diagnosis is only confirmed by nuclear immunoreactivity to NUT protein
  - Specific fusion oncogenes for clinical trials
- Conventional chemotherapeutic regimens are ineffective
- Molecular targeted therapies (bromodomain inhibitors [BETi] and histone deacetylase inhibitors [HDACi]) may yield growth arrest
- Targeted therapies increase survival to >18 mo

**Conclusion**

- Think very broadly when confronted with Sinonasal Tract “Small Round Blue Cell” tumors (MR. SLEEP)
- H&E features are often characteristic
- Let H&E guide ancillary/pertinent studies
- There will be immunohistochemistry overlap
- Targeted molecular studies as needed

**Take Home Points**

- Always use radiology and/or clinical findings
- Consider what will be done with + or – findings when ordering special studies
- Preferentially and sequentially order studies
- Wide differential diagnoses are common, but they can be narrowed significantly with clinical, imaging and laboratory findings

CSP – Saturday Case Seminar