Cytology Workshop #8

Zubair W. Baloch, MD, PhD:
Consultant for Veracyte, INC

Tarik M. Elsheikh, MD:
Nothing to disclose
Controversies and Diagnostic Challenges in Head and Neck Cytopathology

Zubair W. Baloch, MD, PhD
Tarik M. Elsheikh, MD

Disclosures

• Zubair W. Baloch, MD, PhD
  – Veracyte, INC – Consultant
• Tarik M. Elsheikh, MD
  – None

Cystic Lesions of Head and Neck:

Zubair W. Baloch, MD, PhD
Objectives

- Generate a cytologic differential diagnosis for various cystic and solid head and neck lesions.
- Recognize the pitfalls in the cytologic and histologic diagnosis of primary and metastatic head and neck lesions.
- Discuss the value of special techniques in the diagnosis of head and neck and salivary gland tumors.

Case 1

- 60-year-old man with right neck mass
- ? Tail of parotid mass vs. lymph node
- US – cystic mass
  - Favor metastasis to cervical node
  - Thyroid US – no suspicious nodules
- Panendoscopy
  - No mucosal abnormalities
  - ? Mass of right tonsil

FNA of right neck mass
FNA of right neck mass

Cytology Dx: Squamous cell carcinoma

Tonsil Biopsy

Squamous cell carcinoma
Incidence of unsuspected carcinoma in cervical cystic lesions

<table>
<thead>
<tr>
<th>Study</th>
<th>Incidence (%)</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krogdahl (1979)</td>
<td>4</td>
<td>7/161</td>
</tr>
<tr>
<td>Cinberg et al. (1982)</td>
<td>22</td>
<td>4/18</td>
</tr>
<tr>
<td>Granstrom &amp; Edstrom (1989)</td>
<td>23</td>
<td>9/42</td>
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<tr>
<td>Flannagan et al. (1994)</td>
<td>16</td>
<td>4/25</td>
</tr>
<tr>
<td>Gourin &amp; Johnson (2000)</td>
<td>10</td>
<td>12/121</td>
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<tr>
<td>Sheahan et al. (2002)</td>
<td>24</td>
<td>8/33</td>
</tr>
</tbody>
</table>

Sensitivity of FNA in the diagnosis of malignancy in cystic neck lesions

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinberg et al. (1982)</td>
<td>33</td>
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<tr>
<td>Granstrom &amp; Edstrom (1989)</td>
<td>33</td>
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<tr>
<td>Flannagan et al. (1994)</td>
<td>50</td>
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<tr>
<td>Gourin &amp; Johnson (2000)</td>
<td>37.5</td>
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<td>Sheahan et al. (2002)</td>
<td>73</td>
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<tr>
<td>Moatamed et al. (2009)</td>
<td>76</td>
</tr>
<tr>
<td>Baykul et al. (2010)</td>
<td>90</td>
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Cystic Lesions of the Neck

- Congenital
- Acquired
- Majority of neck cysts in newborns and infants are developmental
- Children and adults – Inflammatory or neoplastic
### Cystic Lesions of the Neck

<table>
<thead>
<tr>
<th>Congenital / Developmental</th>
<th>Traumatic</th>
<th>Inflammatory</th>
<th>Neoplastic Benign</th>
<th>Neoplastic Malignant</th>
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</thead>
<tbody>
<tr>
<td>First Branchial Cleft Cyst</td>
<td>Serous fluid</td>
<td>Abscess</td>
<td>Cystic Schwannoma</td>
<td>Cystic Nodal Mets from SCC</td>
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<tr>
<td>Thyroglossal duct cyst</td>
<td>Laryngeal</td>
<td>Tuberculosis</td>
<td>Parathyroid cyst</td>
<td>Cystic Nodal Mets from PTU</td>
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<tr>
<td>Lymphatic malformations</td>
<td>Ludwig Angina*</td>
<td>Goiterous nodule or Cyst PTC</td>
<td></td>
<td>Cystic Mets from other organs</td>
</tr>
<tr>
<td>Epidermoid or Dermoid</td>
<td>HIV related – Lymphoepithelial cyst</td>
<td>Thymic cyst</td>
<td>Salivary gland</td>
<td></td>
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<tr>
<td>Bronchogenic &amp; Esophageal duplication cysts</td>
<td>Ranula</td>
<td>Salivary Gland</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Congenital Cysts

- First Branchial Cleft Cyst
  - Residual embryonic tract
  - Extends from external auditory canal (EAC) through the parotid gland to the submandibular region
  - Type 1 – Periauricular
  - Type 2 – Periparotid (extending from the EAC to the angle of mandible)
- II, III, IV branchial cleft cysts

### Branchial Cleft Cyst-FNA

- Turbid white yellow fluid
  - Variable number of squamous cells and aellualr squames
  - Cellular debris
  - Inflammatory cells
  - Squamous cell with atypia?
  - Branchial cleft cyst in older patients?
Benign Squamous Cystic Lesion vs. Cystic Mets of Squamous Carcinoma

Not so easy

45-year old man with left neck cystic mass

Atypical squamous cells in the background of marked acute inflammation
45-year old man with left neck cystic mass

Inflamed branchial cleft cyst

Benign Squamous Cystic Lesion vs. Cystic Mets of Squamous Carcinoma

<table>
<thead>
<tr>
<th>Cytologic Features</th>
<th>Benign Mean/Std.Err</th>
<th>Malignant Mean/Std.Err</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-Groups</td>
<td>1.3 / 0.86</td>
<td>3.3 / 2.28</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Single Cells</td>
<td>3.3 / 0.53</td>
<td>2.2 / 0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Anucleate Cells</td>
<td>2.0 / 0.41</td>
<td>1.9 / 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Nuclear Atypia</td>
<td>13.7 / 8.26</td>
<td>1.8 / 1.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Basal/Inflammaions</td>
<td>1.0 / 1.32</td>
<td>2.7 / 4.27</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Basal-Necrosis</td>
<td>1.1 / 1.29</td>
<td>1.6 / 2.15</td>
<td>NS</td>
</tr>
<tr>
<td>P53 staining</td>
<td>1.2 / 0.08</td>
<td>1.8 / 0.34</td>
<td>NS</td>
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</table>
Benign Squamous Cystic Lesion vs. Cystic Mets of Squamous Carcinoma

- Clues
  - Clinical history – could be occult primary
  - Inflammation – common in benign
    - Acute inflammation with keratinizing squamous lesions
  - N/C ratio
    - Maintained in benign lesions
  - Nuclear atypia
  - Excisional biopsy
  - P53, p16

HPV in Squamous Cell Carcinoma
Head & Neck Squamous Cell Carcinomas (HNSCCs)

- HNSCCs - 6.5% of annual cancer cases worldwide
  - Estimated 38/100,000 new cases/yr (U.S.)
  - Median age = 60 yrs
  - 2/3 Males: 1/3 Females
  - Incidence in Western Europe and U.S increasing over last few decades

Oncogenesis of HPV

- Multistep/Multifactor process
  - Oncogenes
  - Modification of cellular genes
  - Possible genetic susceptibility of host
  - Impaired cell-mediated immunity
- Genome of dsDNA incorporation
  - “Early” Region encodes for “Early Proteins” E1-E7 (important in pathogenesis and transformation)
  - E6, E7 classified as oncogenes
    - E6 binds to p53 and degrades it
    - E7 binds to pRB and causes dysfunction
      - Results in inhibition of the cell cycle control and facilitation of tumor development

HPV in Oropharyngeal Cancer

- Significantly higher HPV prevalence found in oropharyngeal SCCs that oral or laryngeal SCCs (Kreimer et al., 2005)
- Proposed that HPV-positive oropharyngeal SCC is a distinct entity, less dependent on smoking and alcohol use (Klussmann et al, 2003)
HPV in Other Head & Neck Tumors

- Prevalence varies in the literature
  - Possibly due to methods of analysis
    - 14-35% by PCR
    - 25% by Southern Blot
    - 18% by FISH
  - Most common HPV locations (other than oropharynx) - [Dahlstrand & Dalianis, 2005]
    - Tongue Cancer (19-100%)
    - Laryngeal Cancer (10-50%)

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Termine et al. 2008

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Number of Studies</th>
<th>Mean prevalence (%)</th>
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<tbody>
<tr>
<td>HNSCC n.s.</td>
<td>15</td>
<td>24.1</td>
</tr>
<tr>
<td>OSCC</td>
<td>47</td>
<td>28.1</td>
</tr>
<tr>
<td>ISH based</td>
<td>13</td>
<td>32.9</td>
</tr>
<tr>
<td>PCR based</td>
<td>52</td>
<td>34.8</td>
</tr>
<tr>
<td>HNSCC n.s. ISH based</td>
<td>2</td>
<td>n.c.</td>
</tr>
<tr>
<td>HNSCC n.s. PCR based</td>
<td>13</td>
<td>20.8</td>
</tr>
<tr>
<td>OSCC PCR based</td>
<td>36</td>
<td>28.8</td>
</tr>
<tr>
<td>OSCC ISH based</td>
<td>11</td>
<td>29.8</td>
</tr>
<tr>
<td>Overall</td>
<td>62</td>
<td>29.5</td>
</tr>
</tbody>
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HPV Status and Prognosis
HPV and Prognosis in Oropharyngeal Cancer

• HPV may be a favorable prognostic factor (Gillison et al., 2000; Mellin et al., 2000)
  – Mellin et al., 2000
  • 60 pts with tonsillar cancer
    – 52% of pts with HPV +ve tumors were disease free after 3 years
    – 21% of pts with HPV –ve tumors were disease free after 3 years
  • Pts with HPV +ve tumors had significantly increased 5-year survival rates compared to HPV –ve tumors (53% vs. 31%, p=0.047)
  • HPV +ve tumors favorable independent of tumor stage, gender, age or differentiation

HPV and Prognosis in Oropharyngeal Cancer

Gillison et al., 2000

• 253 head and neck cancer patients
  – 60 oropharyngeal cancers (mostly tonsil)
• Results:
  – Disease-specific survival significantly higher for HPV +ve tumors
  – No change in disease-specific survival for other head and neck cancers
• Multiples studies have shown no change in survival for HPV +ve tumors (except oropharynx) (reviewed by Dahlstrand & Dalia, 2005)

How Adequate are Head and Neck Fine-needle Aspiration Specimens for HPV Molecular Analysis?
How Adequate are Head and Neck Fine-needle Aspiration Specimens for HPV Molecular Analysis?

- 42 specimens in 40 patients
  - 37 LN's & 5 others ites
  - On-site evaluation in 41 (98%)
  - Final diagnosis – SCC in all
  - 9 cases >80% tumor necrosis
  - Adequate DNA for molecular analysis -28 (67%)
    * 7 (25%) necrotic specimens had adequate for HPV analysis

Thyroglossal Duct Cyst

- Most common congenital neck mass
- Located in mid-line or paramedian (left side)
- Closely related to hyoid bone
  - 20% Suprahoid, 65% infrahyoid & 15% at the level of hyoid bone (Grossman & Yousem 1994)
- Characteristic appearance on US, CT and MRI
  - Hypoechoic thin walled cyst
  - Debris – Hemorrhage or infection
  - Solid mass – Carcinoma (95% PTC & 5% SCC)

Thyroglossal Duct Cyst

- Differential diagnosis
  - Dermoid cyst
  - Necrotic lymphadenopathy
  - Cystic goitrous nodule arising from thyroid isthmus
  - Thymic cyst
  - Branchial cleft cyst – paramedian location
  - Cystic hygroma – paramedian location
Thyroglossal Duct Cyst - FNA

Thyroglossal Duct Cyst

Lined by respiratory or squamous epithelium, or both
Mucus glands – seen in 60% of cases
Infection - Granulation tissue or scar
Thyroid tissue – Routine section 5%; serial sections 40%

Thyroglossal Duct Cyst

• Carcinoma – 95% PTC & 5% SCC
• Criteria
  – Demonstration of thyroglossal remnant
  – Normal thyroid gland (US exam?) to differentiate from PTC metastasis from thyroid
Thyroglossal Duct Cyst Carcinoma

35-year-old woman with left lateral neck cystic mass
35-year-old woman with left lateral neck cystic mass

Cytology Diagnosis: Lymphocytes and few macrophages in a background of "colloid" type material. Rule-out metastatic papillary thyroid carcinoma

Histology Diagnosis: Metastatic papillary thyroid carcinoma; TTF-1 and Thyroglobulin positive
35-year-old woman with left lateral neck cystic mass
Total Thyroidectomy

Histology Diagnosis: Papillary microcarcinoma 0.9 cm

Cystic neck mass with no history
• Lymphocytes
• Colloid like background material
• Macrophages
• No epithelial cells
• Suspicious for thyroid cancer metastasis
• What next?

Cystic neck mass suspicious for thyroid carcinoma metastasis
• On-site evaluation – suspicious
  – Ultrasound examination of thyroid
  – Aspiration of suspicious thyroid nodule
  – Thyroglobulin level assessment of the aspirate

• No on-site evaluation
  – Recommend ultrasound evaluation of thyroid
  – Repeat FNA with thyroglobulin level assessment of the aspirate
Thyroglobulin measurement in the lymph node aspirates of patients with PTC

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Dyk 100 ng/ml</th>
<th>Dyk 100 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTC</td>
<td>Other CA</td>
</tr>
<tr>
<td></td>
<td>PTC</td>
<td>Other CA</td>
</tr>
<tr>
<td>PTC</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Other CA</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>NDX</td>
<td>30</td>
<td>1</td>
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<tr>
<td>ATYP</td>
<td>90</td>
<td>0</td>
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<tr>
<td>OTHER</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NDTHN</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Dx = Diagnosis, TG = Thyroglobulin, F/U = surgical pathology follow up, PTC = Papillary thyroid carcinoma, CA = Carcinoma, NDX = No tumor seen, NDS = Non-diagnostic, ATYP = Atypical/Suspicious, *= includes cases of tall cell variant of papillary carcinoma; ** = metastatic well-differentiated follicular derived carcinoma; *** = poorly differentiated carcinoma; **** = carcinoma not otherwise specified.

32-year-old man with bilateral parotid gland masses
32-year-old man with bilateral parotid gland masses- HIV +

Lymphoepithelial Cyst

Warthin’s Tumor

**Primarily occurs within parotid gland**
Second most common salivary gland neoplasm – 5-10%
Believed to originate from salivary duct remnants entrapped within glandular lymphoid tissue

**Clinical features:**
- 50-79 year-old
- Common in men
- Bilateral

Warthin’s Tumor

**Cytology**
Mixed population of lymphocytes

Sheets of oncocytes

Background debris (grossly mobile oil consistency)
Overlapping Cytologic Features

- **Lymphocytes**
  - Chai et al. (Diag Cytopathol 1997)
  - 61 cases with prominent lymphoid component
    - Warthins 33 cases
      - Warthins-31, Benign cyst-1, SCCA-1
    - Other epithelial malignancies 6 cases
      - Oncocytoma; Pleomorphic adenoma, ACC
    - Lymphomas 12 cases
    - Benign 10

Overlapping Cytologic Features

- **Lymphocytes**
  - Intraparotid LN
  - Lymphoepithelial cyst
  - Chronic Sialadenitis
  - Warthin’s
  - Acinic cell carcinoma
  - Mucoepidermoid Carcinoma
  - Lymphoma

Approach to cystic neck lesion

Background – Mucoid

- Histiocytes & lymphocytes
  - Mucus retention cyst
- Salivary gland
  - Chronic sialadenitis
  - Sialolithiasis
  - Mucoepidermoid carcinoma
  - Myxoid and chondroid fragments – Pleomorphic adenoma – rare
- Atypical cells
  - Malignancy – Mets vs. primary
Approach to cystic neck lesion

Background – watery proteinaceous fluid

- **Lymphocytic infiltrate & few epithelial cells**
  - Lymphoepithelial cyst
  - Thyroglossal duct cyst – midline location
- **Salivary gland**
  - Lymphocytes and oncocytes
  - Warthin’s tumor
  - Atypical keratinized cells
    - Squamous cell carcinoma
    - Metaplasia in benign tumor – history of previous FNA
- **Keratinized cells**
  - Atypical – Squamous cell carcinoma
  - Branchial cleft cyst

Approach to cystic neck lesion

- Benign vs. malignant squamous cystic lesion
  - Background – inflammation?
  - Necrotic debris
  - Cellularity – increased in SCC?
  - Abnormal keratinization / Dyskeratosis
  - Nuclear atypia

Approach to cystic neck lesion

- Cystic mass suspicious for thyroid cancer
  - Recommend ultrasound of thyroid and aspiration of suspicious nodules
  - TTF-1 and thyroglobulin (should do both) if enough cells
  - Thyroglobulin assessment of FNA specimen
Salivary Gland Cytology
Diagnostic challenges and potential pitfalls

Tarik M. Elsheikh, MD

Case #1: Parotid mass, 56 yo male

What is your Diagnosis?

A. Pleomorphic adenoma
B. Basal cell adenoma
C. Adenoid cystic carcinoma
D. LG mucoepidermoid carcinoma
Case #2: Submandibular mass, 37 yo man

Diagnosis?
A. PA
B. Basal cell adenoma
C. Adenoid cystic CA
D. LG mucoepidermoid CA

Diagnostic Challenges and Problems

- Cystic lesions
- Low grade malignancies
- Cellular benign neoplasms
- Atypical inflammatory changes
- Atypical lymphoid infiltrates
- Unusual cytologic presentation of common lesions
- Rare unusual lesions

Primary Salivary Gland Neoplasms

**Benign**
- Pleomorphic adenoma
- Basal cell adenoma
- Warthin tumor

**Malignant**
- LG Mucoepidermoid CA
- Acinic cell carcinoma
- Adenoid cystic carcinoma
- HG and Undifferentiated CA
Key Cytologic Features

- Low power architectural appearance
- Size of cells and amount of cytoplasm
- Nucleoli
- Character of single cells in background
- Character of background substance

Basal Cell Adenoma

- 2% of SG tumors
- Small cells
- Tightly cohesive clusters
- Background naked nuclei
- Can not distinguish from malignant basaloid tumors

- Minimal cytoplasm
- Round to oval nuclei
- Finely-coarsely granular chromatin
- Occasional small nucleoli
Amorphous extracellular hyaline material may be seen at periphery of cell clusters.

Not specific for membranous BCA

Adenoid Cystic Carcinoma

Cytology

Cribriform (Well Differentiated)

- Clusters and branching multilayered groups of basaloid cells surrounding globules of homogenous acellular material (reduplicated basal lamina PAS+)

Solid (Poorly Differentiated)

- Loosely cohesive groups of cells with increased nuclear atypia and prominent nucleoli
- Acellular material (globules) lacking
Salivary Gland Neoplasms with Basaloid Cell Features

- Architectural features are most important
- Never make a definitive Dx of BCA
- Cribriform ACC can be accurately diagnosed, but must exclude membranous BCA
- Solid BCA and solid ACC are indistinguishable
- *Basal cell adenocarcinoma* indistinguishable cytologically from BCA and ACC

Case study

- Architectural features are most important
- Never make a definitive Dx of BCA
- Cribriform ACC can be accurately diagnosed, but must exclude membranous BCA
- Solid BCA and solid ACC are indistinguishable
- *Basal cell adenocarcinoma* indistinguishable cytologically from BCA and ACC
Sample Cytologic Diagnosis

DX: Cellular neoplasm with basaloid cell features, see comment

Comment: Differential diagnosis includes basal cell adenoma and adenoid cystic carcinoma (Ki 67). Basal cell adenoma is favored (suggested). Histologic confirmation is needed for a definitive diagnosis.

Pleomorphic Adenoma

- 75% of major salivary gland tumors
- Female, 30-40 years
- Aspirates of thick gelatinous consistency
- Mixture of epithelial and mesenchymal elements
- Epithelial/myoepithelial rich variant (CPA)
Chondroid stroma in PA

Myxoid stroma in PA
Pleomorphic Adenoma

Pleomorphic Adenoma

Biphasic

Cellular/Epithelial

Mesenchymal

Problems in Cytologic Diagnosis of Pleomorphic Adenoma

• Predominance of one component
  • if myxoid stroma predominates, may mistake for cyst fluid or LG mucoepidermoid CA
  • if epithelial cells predominate (CPA) suspect basaloid cell tumor
• Atypical cytologic features

Case #3: Cellular Pleomorphic Adenoma

40 year old female presented with a submandibular mass
LG Mucoepidermoid Carcinoma

Cytology
• Intermediate cells
• Mucus producing cells - resemble macrophages
• Squamous cells - cohesive clusters of cells resembling metaplastic squames. Occasional paranuclear vacuoles
• No fully keratinized epidermoid cells
• Mucin and debris in background
Case #1: Parotid mass, 56 yo male

What is your Diagnosis?

A. Pleomorphic adenoma
B. Basal cell adenoma
C. Adenoid cystic carcinoma
D. LG mucoepidermoid carcinoma
Case #2: Submandibular mass, 37 yo man

Diagnosis?
A. PA
B. Basal cell adenoma
C. Adenoid cystic CA
D. LG mucoepidermoid CA

Mucin vs. Stroma
The Many Cytologic Faces of Pleomorphic Adenoma

• Basal Cell Adenoma
• Adenoid Cystic Carcinoma
• Low grade malignancies such as LG mucoepidermoid CA and acinic cell CA

FNA of Salivary Glands Summary

• Must exclude PA before making a diagnosis of another neoplasm
• Must exclude ACC and low grade malignancies before making a Dx of PA
• FNA can distinguish in most instances between basaloid neoplasms (ACC, BCA) and PA

FNA of Salivary Glands Summary 2

• Cellular neoplasm NOS (LG CA vs. B9)
• Familiarity with variable FNA appearances of SG tumors and awareness of potential pitfalls can prevent many false positive and negative diagnoses
• FNA should be interpreted in context of clinical and radiologic findings