Salivary gland tumors are one of the most heterogeneous groups of neoplasms – So what role is there for FNA?

Salivary Gland Cytology

Salivary gland cytology is especially challenging due to the extensive overlap between many benign and malignant tumors.
Salivary Gland FNA - Solid Adenoid Cystic Carcinoma

- Pleomorphic adenoma
- Adenoid cystic carcinoma
- Basal cell adenoma, tubulotrabecular and solid types
- Warthin tumor
- LG mucoepidermoid carcinoma
- HG mucoepidermoid carcinoma
- Acute and chronic sialadenitis
- Carcinoma ex PA
- Salivary duct carcinoma
- Basal cell adenoma, membranous type
- Metastasis
- Polymorphous low-grade adenocarcinoma
- Reactive lymph node
- Small cell carcinoma
- Lymphoma
- Mucocele
- Epithelial-myoeipithelial carcinoma
- Lymphoepithelial cyst
- Oncocytoma
- MASC
- LESA
- Acinic cell carcinoma

SALIVARY GLAND FNA: How Far Can We Go?

- Depends upon the quality of the specimen
- And the subtype of tumor

SALIVARY GLAND FNA

- CHALLENGING DIAGNOSTIC ISSUES
  - Matrix-containing tumors
  - Oncocytic lesions
  - Basaloid tumors
  - Lymphoid lesions
  - Cystic and mucinous lesions
  - High-grade carcinomas
  - Clear cell tumors
  - Spindle cell lesions
SOME SALIVARY GLAND FACTS

- 3 Major glands:
  - Parotid (serous)
  - Submandibular (mixed seromucinous)
  - Sublingual (mucinous)
- 500-1000 minor glands: Submucosa of oral cavity, nasal cavity, larynx, trachea, and bronchi

SOME SALIVARY GLAND FACTS

- Tumors:
  - 0.4-13.5 per 100,000 people (uncommon)
  - Older adults, females, parotid gland
  - Approx. 75% are benign
  - Risk of malignancy is inversely proportional to the size of the gland (20% in parotid; 50% in submandibular; 80-89% in oral cavity)
Effectiveness of Cytomorphology alone:
- Sensitivity: 86-100%
- Accuracy:
  - Benign/low grade vs HG malignant: 81-98%
  - Specific lesion: 48-94%

Part of the reason for the high accuracy: A majority of SG neoplasms are pleomorphic adenomas, Warthin tumor, or metastatic cancer to parotid LNs.

Rationale for FNA:
- Guide the clinical management/pre-op strategy:
  - Non-neoplastic: Clinical follow-up
  - Benign tumor/low-grade carcinoma: Limited resection
  - Metastatic disease to parotid LNs: LN resection
  - Lymphoma: Home-On referral
  - High-grade primary carcinoma: Radical resection/nerve sacrifice/LN dissection

Why do we need a new reporting system for salivary gland cytology?
Salivary Gland FNA

Diagnostic Terminology

• Current reporting confusion:
  – Diversity of diagnostic categories, vs.
  – Descriptive reports (no categories), vs.
  – Surgical pathology terminology
  – No correlation with ROM or management
• General agreement on the need for a defined set of diagnostic categories for salivary gland FNA
  – Clarity of communication (implicit cancer risk)
  – Exchange of data across institutions
• The Milan System for Reporting Salivary Gland Cytopathology

The Milan System for Reporting Salivary Gland Cytopathology

• Sponsored by the ASC and the IAC
• Over 40 participants from 15 countries
• Goal is to produce a practical classification system that is user-friendly and internationally accepted.
• The system will be evidence-based.
• Will correlate with a management algorithm.
• Print Atlas available in late 2017
• Web-Based Atlas will also be available through the ASC

Participants:
40+ Members from 15 Countries
Cytopathologists, Surgical Pathologists, Molecular Pathologists, ECT Surgeons
The Milan System for Reporting Salivary Gland Cytopathology

Diagnostic Categories

1) Non-Diagnostic
2) Non-Neoplastic
3) Atypia of undetermined significance
4) Neoplastic:
   a) Benign
   b) Uncertain malignant potential
5) Suspicious for Malignancy
6) Malignant

The Milan System for Reporting Salivary Gland Cytopathology

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>ROM (%)</th>
<th>Management</th>
</tr>
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<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>10-25</td>
<td>Clinical and radiologic correlation/repeat FNA with U/S guidance</td>
</tr>
<tr>
<td>Non-Neoplastic</td>
<td>0-10</td>
<td>Clinical follow-up and radiologic correlation; surgical resection in some cases</td>
</tr>
<tr>
<td>Atypia of undetermined significance</td>
<td>TBD</td>
<td>Repeat FNA or surgery</td>
</tr>
<tr>
<td>Neoplastic:</td>
<td></td>
<td>Conservative surgery or clinical follow-up</td>
</tr>
<tr>
<td>- Benign:</td>
<td></td>
<td>Conservative surgery</td>
</tr>
<tr>
<td>- Uncertain malignant potential (SUMP)</td>
<td>20-40</td>
<td>Surgical intervention based on clinical and radiologic features</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>60-70</td>
<td>Surgery: Correlate with clinical and radiologic features</td>
</tr>
<tr>
<td>Malignant:</td>
<td>85-95</td>
<td>Surgery: Correlate LG vs HG to determine extent</td>
</tr>
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The ROM will depend upon the nature of the specimen and the salivary gland site.

Non-Diagnostic

- Insufficient quantitative and/or qualitative cellular material to make a cytologic diagnosis.
- 10% would be a target maximum rate
- Includes aspirates with benign elements only
- Includes non-mucinous cyst contents
FNA OF THE NORMAL SALIVARY GLAND

Cytologic Features of the Normal Aspirate
- Serous and mucinous-type acinar cells in lobules
- Background naked acinar cell nuclei
- Few admixed small sheets and tubules of ductal epithelium
  - Intercalated ducts
  - Striated ducts
  - Excretory duct
- Adipose tissue

Non-Diagnostic:
Benign salivary gland elements only

FNA OF THE NORMAL SALIVARY GLAND
Polarized grape-like clusters of acinar cells
For aspirates containing only normal salivary gland elements, a cautionary note is recommended. Note: Clinical and radiologic correlations are recommended to ensure that the aspirate is representative of the lesion; the findings in this aspirate do not explain the presence of a salivary gland mass.

Non-Diagnostic:
Non-mucinous cyst contents

DDX: Ductal cyst, pseudocyst, cystic neoplasm
Non-Neoplastic

- Specimens lacking evidence of a neoplastic process:
  - Inflammatory, metaplastic, and reactive (includes acute, chronic, and granulomatous sialadenitis, sialadenosis, etc.)
  - Reactive lymph nodes (flow cytometry is needed)
  - Clinico-radiological correlation is essential to ensure that the specimen is representative of the lesion.
  - A subset will need surgical excision to exclude a poorly sampled neoplasm.

Non-Neoplastic: Reactive Lymph Node

- Mixed population of lymphs, tingible body macrophages, germinal center fragments

REACTIVE PROCESS VS LYMPHOMA

- IMMUNOPHENOTYPING combined with cytomorphology is the key to diagnosing and subtyping reactive conditions vs lymphoma.
Non-Neoplastic: Chronic Sialadenitis

Hypocellular, cohesive basaloid groups, inflammation

Non-Neoplastic: Granulomatous Sialadenitis

DDX includes infection, sarcoidosis, and neoplasm

Non-Neoplastic: Sialolithiasis
Atypia of Undetermined Significance (AUS)

- Cannot entirely exclude a neoplasm.
- Heterogeneous category
- A majority will be reactive atypia or poorly sampled neoplasms.
- Specimens are often compromised (e.g., air-drying, blood clot).
- Should be used rarely (<10% of all salivary gland FNAs).
- Most cases will require tissue biopsy or resection

Atypia of Undetermined Significance (AUS): Mucinous Cyst Contents Only - Cannot exclude MEC

Atypia of Undetermined Significance (AUS): Oncocytic metaplasia vs Neoplasm
i) **Benign Neoplasm:**
Reserved for clear-cut benign neoplasms
This category will include classic cases of PA, WT, lipoma, etc...

ii) **Salivary Gland Neoplasm of Uncertain Malignant Potential:**
Diagnostic of a neoplasm; however, a diagnosis of a specific entity cannot be made.
A malignant neoplasm cannot be excluded.

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**Neoplastic: Benign Warthin Tumor**

Oncocytes, chronic inflammation, and cystic debris

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**Neoplastic: Benign Pleomorphic Adenoma**

Matrix-rich types of PA are the easiest.
Neoplastic: SUMP
Myoepithelioma

Suspicious for Malignancy

- Aspirates which are highly suggestive of malignancy but not definitive.
- Often high grade carcinomas with limited sampling or other limitation.
Suspicious for Malignancy
AdCC with Artifact

Aspirates which are diagnostic of malignancy.
- Sub-classify into specific types and grades of carcinoma: e.g. low grade vs high grade.
- Grading is important for clinical management
- "Other" malignancies such as lymphomas, sarcomas and metastases are also included in this category and should be specifically designated.

Malignant

Salivary Duct Carcinoma
Undifferentiated Carcinoma
Salivary Gland FNA

- Improvements in IHC and molecular testing will make the Milan System and salivary gland FNA in general more effective.
- It is critical that the FNA specimen include adequate material for ancillary studies in difficult cases (i.e. cell block).

Ancillary Studies to Improve the FNA Diagnosis of Head and Neck Tumors

- Immunocytochemistry
  - LBP
  - NDRG1
  - Cell block
- FISH
- RT-PCR
- Next Generation Sequencing

Increasing Availability of Molecular Markers For Salivary Gland Tumors

- Mammary analogue secretory carcinoma
  - ETV6-NTRK2; t(12;15)
- Pleomorphic adenoma
  - PLAG1; t(3;8)
- Clear cell carcinoma
  - BRAF V600E
- Ewing sarcoma
  - EWSR1-ATF1; t(12;22)
- Mucoepidermoid carcinoma
  - MECT1/MAML2; t(11;19)
- Cribriform Adenocarcinoma
  - PRKDC rearrangement
- Adenoid cystic carcinoma
  - MYB-NFIB; t(6;9)
- Basal cell adenoma
  - CTNNB1 mutations
NGS-SNaPshot Panel at MGH

- Anchored Multiplex PCR (AMP)
- ~190 target amplicons across 39 genes and 50+ rearrangements
- High-quality sequence:
  - Staggered start sites
  - >1,000X target coverage
  - Molecular indexing
  - Bi-template coverage
  - ~2% analytical sensitivity
- Fast turn-around (~2 weeks)
- Cost-effective (<$500)
- Small tissue amounts (5-10 ng)

Molecular Analysis at MGH
NGS-Translocation Panel

NATIONAL SALIVARY GLAND
 Immunohistochemical Markers

Ker 7, 19, CAM 5.2, EMA
Ker 7, 5/6, p63, calponin, SM actin
Ker 5/6, p63, CAM 5.2, DOG1
Matrix-Containing Salivary Gland Tumors

Case History:
A 36 year-old female presented with a 1.0 cm mass in the right cheek that had been slowly enlarging for one year. An FNA was performed.
CYTOLOGIC DIAGNOSIS:
Pleomorphic adenoma arising in accessory parotid tissue of cheek.
Pleomorphic Adenoma (Benign Mixed Tumor)

- Most common salivary gland tumor in both children and adults
- 75-80% of parotid tumors & 50% of all salivary tumors
- Superficial parotid gland...esp. the tail of the parotid at jaw angle
- 5-10% risk of malignant transformation
- PLAG1 gene rearrangement is most common molecular change

Most cases diagnosed in the Milan System as “Neoplastic: Benign”

Pleomorphic Adenoma

Cytologic Features:

- **Cells:**
  - Epithelial cells in cohesive, honeycomb groups
  - Myoepithelial cells singly and clusters:
    - plasmacytoid, epithelioid, spindled, or clear
- **Matrix:**
  - Fibrillar with frayed, indistinct margins, embedded cells, metachromatic
Pleomorphic Adenoma with Chondroid Matrix

Spectrum of Pleomorphic Adenoma

Matrix-Rich Pleomorphic Adenoma

Cellular Pleomorphic Adenoma

Myoepithelioma/Myoepithelial-Predominant

Basal Cell Adenoma

CLASSIC PLEOMORPHIC ADENOMA
Fibrillar Matrix

Stellate Myoepithelial Cells

GROUPS OF BLAND MYOEPIHELIAL CELLS

METACHROMATIC MATRIX ON DIFF-QUIK
Cluster of Ductal Cells

Different Appearances of Myoepithelial Cells
- Plasmacytoid
- Spindled

Rare Diagnostic Problems Arise from Variants of PA
- Cellular PA with sparse matrix
- Focal adenoid cystic–like areas
- Cytologic atypia
- Metaplasia
  - Squamous
  - Mucinous
  - Sebaceous
  - Oncocytic
Focal Cytologic Atypia

Carcinoma Ex Pleomorphic Adenoma

Malignant Cells

Pitfall:
PA with Squamous Metaplasia
Pleomorphic Adenoma with Sebaceous Metaplasia

Pleomorphic Adenoma with Tyrosine Crystalloids: Often associated with benign tumors and cysts

Pleomorphic Adenoma with Tyrosine Crystalloids
Pleomorphic Adenoma

PLAG1 rearrangement can be detected by molecular testing but an antibody is also available (less specific)

PLAG-1 Immunoreactivity:
Overexpressed in 94% of PA

Does not distinguish benign from malignant

How does adenoid cystic carcinoma compare to pleomorphic adenoma?
Most cases diagnosed in the Milan System as “Neoplastic: SUMP” or “Suspicious for Malignancy”

Adenoid Cystic Carcinoma
- 4-10% of all salivary gland neoplasms
- Indolent behavior but very poor long-term survival (<20% survival at 10 years)
- Submandibular gland, palate, and parotid
FNA of Adenoid Cystic Carcinoma

3 Subtypes:
- **Cribriform** – Most easily recognized
  - Cylindromatous pattern of pseudocystic spaces
- **Tubular**
  - Ducts and tubules with central lumens
- **Solid** – Difficult to recognize
  - Lacks matrix
  - More atypical with mitotic activity

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Adenoid Cystic Carcinoma, Cribriform Subtype

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Adenoid Cystic Carcinoma, Tubular Subtype
Adenoid Cystic Carcinoma:
Solid Subtype is more aggressive and more difficult to diagnose

Marked Neurotropism:
FNA is Painful!

**Adenoid Cystic Carcinoma**

**Cytologic Features:**

**Cells:**
- Basaloid cells with dark angulated nuclei
- Variable atypia

**Matrix:**
- 3-D spheres and branching tubules
- Acellular
- Metachromatic with sharp borders
Adenoid Cystic Carcinoma: Classic Cribriform ("Swiss Cheese") Pattern

Classic Adenoid Cystic Carcinoma

Classic Adenoid Cystic Carcinoma
Adenoid Cystic Carcinoma

**Immunohistochemistry:**
- Over 90% are strongly positive for CD117 (KIT)
- Useful for all variants including solid forms
- IHC can be performed on cytologic and histologic samples

**Immunoreactivity for CD117 (KIT):**
Overexpressed but mutations in KIT have not been detected

**Cytogenetics:**
- t(6:9) MYB oncogene-NFIB transcription factor
- 86% MYB-NFIB fusion transcripts
- Detected by FISH

MYB immunostaining is a useful ancillary test for distinguishing adenoid cystic carcinoma from pleomorphic adenoma in FNAB specimens.

Pusztaszeri M, Sadow M, Faquin W. Cancer Cytopath 2014

Adenoid Cystic Carcinoma
With High Grade Transformation

- First described in 1988 for acinic cell carcinoma
- “Undifferentiated” appearance
- Very aggressive clinical course
- Can occur in many different types of salivary gland carcinoma
**Pitfall: FNA Sample Preparation**

Both alcohol-fixed and air-dried preparations are essential in the evaluation of matrix-containing tumors!

**Pitfall: Adenoid Cystic Carcinoma vs. Pleomorphic Adenoma?**

Pleomorphic adenoma

Adenoid cystic carcinoma

**Pitfall: Adenoid Cystic Carcinoma vs. Pleomorphic Adenoma?**

Pleomorphic adenoma

Adenoid cystic carcinoma
Classic Pleomorphic Adenoma vs Classic Adenoid Cystic Carcinoma

<table>
<thead>
<tr>
<th>Pleomorphic Adenoma</th>
<th>Adenoid Cystic Ca</th>
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</thead>
<tbody>
<tr>
<td>- predominance of myoepithelial cells</td>
<td>- Basaloid cells</td>
</tr>
<tr>
<td>- minimal atypia</td>
<td>- variable atypia</td>
</tr>
<tr>
<td>- matrix: fibrillar with ragged edges and embedded myoepithelial cells</td>
<td>- matrix: acellular, smooth edges, homogeneous</td>
</tr>
<tr>
<td>- PLAG1 rearrangement</td>
<td>- MYB+, CD117+</td>
</tr>
</tbody>
</table>

Pitfall: The solid variant of adenoid cystic carcinoma lacks matrix.

Beware: Solid Variant of Adenoid Cystic Carcinoma vs. Cellular Pleomorphic Adenoma
Overlapping Features:
Solid Basal Cell Adenoma vs Solid Adenoid Cystic Carcinoma

Because of significant cytologic overlap, most basaloid cases will receive a descriptive diagnosis and DDX.

Basal Cell Adenoma:
Solid Type: Squamous morules are a clue

Basaloid Tumors
Metastatic Basal Cell Carcinoma & Basaloid Squamous Carcinoma: Overtly malignant and patient has clinical history

Basal cell carcinoma Basaloid squamous carcinoma

BASAL CELL ADENOCARCINOMA
• Low-grade salivary gland neoplasm
  ° 2% of malignant salivary gland tumors
  ° Malignant counterpart of basal cell adenoma
  ° Parotid gland, rarely in submandibular gland
  ° Average age: 60 years (range: 27-92 years)
• Good prognosis:
  – Local recurrence (35%), infrequent metastatic disease (10%), and low mortality (3%)
• Complete surgical excision with disease-free margins

Immunohistochemistry:
Positive for keratin 7, CEA, EMA
Positive for myoepithelial markers
Nuclear beta-catenin +
**Basal Cell Adenoma & Adenocarcinoma**

- CTNNB1 mutation – 3p21
- Beta-Catenin overexpression
  - Present at cell junctions
  - Part of WNT signaling pathway

**Polymorphous Low-Grade Adenocarcinoma**

- Minor salivary glands, esp. palate, where it is more common than adenoid cystic carcinoma
- Circumscribed unencapsulated subepithelial mass
- Few myoepithelial cells
- Diffuse strong S-100 positivity

**Nuclear Beta-Catenin in Basal Cell Adenoma**

*Jo et al. AJSP 2016;40:1143-1150.*
Polymorphous Low-Grade Adenocarcinoma

Cytologic features of PLGA:
- Monomorphic cells
- Plump oval to fusiform nuclei
- Fine stippled chromatin
- Variable architectural patterns including papillary
- Scant matrix

Polymorphous Low-Grade Adenocarcinoma:
No specific molecular features identified

Polymorphous Low-Grade Adenocarcinoma:
Open chromatin and small nucleolus
**Polymorphous Low-Grade Adenocarcinoma:**

Varied Patterns

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**Features Favoring PLGA:**
- Palatal lesion
- Monomorphic basaloid cells
- Varied architecture

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**Summary**

- Salivary gland cytology is challenging due to many subtypes of tumors with overlapping features
- The Milan System offers an opportunity for a practical and uniform international reporting system
- Keys to accurate FNA diagnosis depend upon having an adequate sample and material for ancillary studies (IHC & molecular).
Thank You!