SALIVARY GLAND CYTOLOGY IN THE CONTEXT OF THE MILAN SYSTEM

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Salivary gland cytology: a background

Review of the Milan System for Reporting Salivary Gland Cytopathology

Correlations with ancillary studies

Discuss effectiveness of implementing the Milan System

Future Directions
SALIVARY GLAND CYTOLOGY: A BACKGROUND
Utility

- Differentiation of salivary gland lesions helps guide patient management
  - Neoplastic vs. non-neoplastic \rightarrow surgical vs. non-surgical
  - Low vs. high-grade malignancy \rightarrow conservative vs. aggressive surgical management
    - Extent of resection
    - Facial nerve sacrifice
    - Neck dissection

| Table 4. ACCURACY OF FNA TECHNIQUE IN DISTINGUISHING MALIGNANT FROM BENIGN AND NON-NEOPLASTIC LESIONS |
|---------------------------------------------------|-----------------------------------------------|---------------------------------|---------------------------------|-----------------|
| Histology                                         | Malignant (Histology)                        | Benign                         | Non-neoplastic                  | Total           |
| Malignant                                         | 387 (93.25%)                                 | 55 (4.31%)                     | 42 (19%)                       | 484             |
| Benign                                            | 22 (5.3%)                                    | 1,219 (95.46%)                 | 34 (15.38%)                    | 1,275           |
| Non-neoplastic or normal tissue                   | 6 (1.45%)                                    | 3 (0.23%)                      | 145 (65.61%)                   | 154             |
| Total                                             | 415                                          | 1,277                         | 221                            | 1,913           |

SALIVARY GLAND FINE NEEDLE ASPIRATION: DIAGNOSTIC ACCURACY

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range: 33 – 100%</td>
<td>Range: 67 – 100%</td>
</tr>
</tbody>
</table>

Heterogeneous nature of salivary gland lesions
Presence or absence of a cystic component or metaplastic changes
Technical expertise of aspirator (sampling)
Quality of cytologic preparations
Experience/comfort level of pathologist
SALIVARY GLAND CYTOLOGY: LIMITATIONS

Diagnostic

• Subclassification of salivary gland lesions
• Difficulty in distinguishing between different categories of clinically meaningful lesions
• Cellular inflammatory lesion and neoplasms
• Morphologic overlap between benign neoplasms and salivary gland malignancies

<table>
<thead>
<tr>
<th>Benign epithelial tumors</th>
<th>Malignant epithelial tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphic adenoma 8940/0</td>
<td>Acinic cell carcinoma 8550/3</td>
</tr>
<tr>
<td>Myoepithelioma 8982/0</td>
<td>Mucoepidermoid carcinoma 8430/3</td>
</tr>
<tr>
<td>Basal cell adenoma 8147/0</td>
<td>Adenoid cystic carcinoma 8200/3</td>
</tr>
<tr>
<td>Warthin tumor 8561/0</td>
<td>Polymorphous adenocarcinoma 8525/3</td>
</tr>
<tr>
<td>Oncocytoma 8290/0</td>
<td>Epithelial-myoepithelial carcinoma 8562/3</td>
</tr>
<tr>
<td>Canalicul ar adenoma and other ductal adenomas 8149/0</td>
<td>Clear cell carcinoma 8310/3</td>
</tr>
<tr>
<td>Sebaceous adenoma 8410/0</td>
<td>Basal cell adenocarcinoma 8147/3</td>
</tr>
<tr>
<td>Lymphadenoma</td>
<td>Sebaceous adenocarcinoma 8410/3</td>
</tr>
<tr>
<td>Sebaceous 8410/0</td>
<td>Secretory carcinoma 8502/3</td>
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<tr>
<td>Non-sebaceous 8410/0</td>
<td>Intraductal carcinoma</td>
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<tr>
<td>Ductal papillomas 8503/0</td>
<td>Oncocytic carcinoma 8290/3</td>
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<td>Sialadenoma papilliferum 8406/0</td>
<td>Salivary duct carcinoma 8500/3</td>
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<tr>
<td>Cystadenoma 8440/0</td>
<td>Adenocarcinoma, NOS 8140/3</td>
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<tr>
<td>Soft tissue tumors</td>
<td>Myoepithelial carcinoma 8982/3</td>
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<tr>
<td>Hemangioma 9120/0</td>
<td>Carcinoma ex pleomorphic adenoma 8941/3</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma 8020/3</td>
<td>Carcinoma ex pleomorphic adenoma 8941/3</td>
</tr>
<tr>
<td>Squamous cell carcinoma 8070/3</td>
<td></td>
</tr>
<tr>
<td>Malignant Neoplasm</td>
<td>Cytological Diagnosis, n</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>58</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>65</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
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<tr>
<td>Epithelial-myoepithelial carcinoma</td>
<td>1</td>
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<tr>
<td>Clear cell adenocarcinoma</td>
<td>2</td>
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<tr>
<td>Basal cell adenocarcinoma</td>
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<tr>
<td>Oncocytic carcinoma</td>
<td>5</td>
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<tr>
<td>Adenocarcinoma NOS</td>
<td>56</td>
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<tr>
<td>Myoepithelial carcinoma</td>
<td>8</td>
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<tr>
<td>Carcinoma ex pleomorphic adenoma</td>
<td>14</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>45</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Lymphoepithelial carcinoma</td>
<td>14</td>
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<tr>
<td>Malignant mesenchymal</td>
<td>3</td>
</tr>
<tr>
<td>Malignant lymphomas</td>
<td>41</td>
</tr>
<tr>
<td>Metastatic tumors</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>415</strong></td>
</tr>
</tbody>
</table>

META ANALYSIS DATA SHOWS VARIABILITY IN DIAGNOSTIC CATEGORIES ACROSS STUDIES

Collection of data from 29 studies with various diagnostic categories:
- 15 non-diagnostic or unsatisfactory
- 23 non-neoplastic
- 28 benign neoplasm
- 8 salivary gland neoplasm or neoplasm
- 13 suspicious for malignancy
- 29 positive for malignancy
# SALIVARY GLAND CYTOLOGY: LIMITATIONS

## Diagnostic

- Subclassification of salivary gland lesions
  - Specific diagnoses rendered in 48-94% of cases
- Difficulty in distinguishing between different categories of clinically meaningful lesions
  - Cellular inflammatory lesion and neoplasms
  - Benign neoplasms and well-differentiated malignancies

## Quality improvement & Data Analysis

- Previous diagnostic framework allows for a high degree of variability of utilization of diagnostic categories for salivary gland FNA
- Difficulty in data analysis for:
  - Cytologic – histologic correlations
  - Comparison of data across pathologists and institutions
- Variability in clinician understanding and subsequent direction for patient management
MOVING TOWARDS A UNIFORM CLASSIFICATION SYSTEM

- Emphasize risk stratification over specific diagnoses
- Providing a categorical risk of malignancy
  - Guide patient management
THE MILAN SYSTEM FOR REPORTING SALIVARY GLAND CYTOLOGY
GOALS OF THE MILAN SYSTEM

- Enhance communication of pathology results between pathologists and clinical counterparts
- Increase concordance between cytology and subsequent surgical resection specimens
- Create uniform diagnostic categories to allow for data analysis and comparison across institutions
MILAN SYSTEM: DIAGNOSTIC CATEGORIES

- Non-diagnostic
- Non-neoplastic
- Atypia of undetermined significance
- Neoplasm
  - Benign
  - Salivary gland neoplasm of uncertain malignant potential
- Suspicious for malignancy
- Malignant
NON-DIAGNOSTIC

Definition & Utility

- An aspirate that for qualitative and/or quantitative reasons provides insufficient diagnostic material to provide an informative interpretation.
- An absolute number of cells needed for salivary gland FNA adequacy has not been established in literature.

Criteria

- Rare or absent cells (<60)
- Poor preservation with artifacts
- Non-mucinous cyst fluid with no epithelial component
- Debris only
- Sampling of normal elements in light of a clinically or radiologically defined mass
EXCEPTIONS

- The presence of any atypia
- **Mucinous** cyst fluid contents without epithelial component
- Presence of abundant inflammatory infiltrate without epithelial component
- Presence of matrix component suggestive of a neoplasm
NON-NEOPLASTIC

General Definition

- Specimens showing benign non-neoplastic changes
  - Acute or chronic inflammatory response to:
    - Obstruction
    - Structural alterations
    - Autoimmune disease
    - Infection

Requires correlation (and explanations)

- Clinical and radiologic correlation required to avoid false negative
Non-Neoplastic

General Definition

- Specimens showing benign non-neoplastic changes
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  - Autoimmune disease
  - Infection

Requires correlation (and explanations)

- Clinical and radiologic correlation required to avoid false negative
- Pitfalls associated with false positive
  - Sialolithiasis
    - Can induce metaplastic changes
  - Acute sialadenitis
    - Can induce reactive changes
    - Rule out tumor diathesis
  - Chronic sialadenitis
    - Can induce atrophy and metaplastic changes
    - Granulomatous inflammation
NON-NEOPLASTIC (?): LYMPHOCYTES

And what about lymphoma?
NON-NEOPLASTIC GREY ZONES

SIALADENOSIS

ONCOCYCTOSIS
DIAGNOSTIC CONSIDERATIONS

Bland salivary gland acini
- Non-diagnostic vs. diagnostic
- Non-neoplastic
  - Accessory salivary gland
  - Hamartoma
  - Sialolithiasis
- Neoplastic
  - Acinic cell carcinoma

Oncocytes
- Oncocytic lesions
  - Benign lesions
    - Warthin tumor
    - Oncocytoma
  - More to come…

<table>
<thead>
<tr>
<th>MILAN SYSTEM: DIAGNOSTIC CATEGORIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diagnostic</td>
</tr>
<tr>
<td>Non-neoplastic</td>
</tr>
<tr>
<td>Atypia of undetermined significance</td>
</tr>
<tr>
<td>Neoplasm</td>
</tr>
<tr>
<td>• Benign</td>
</tr>
<tr>
<td>• Salivary gland neoplasm of uncertain malignant potential</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
</tr>
<tr>
<td>Malignant</td>
</tr>
</tbody>
</table>
ATYPIA OF UNDETERMINED SIGNIFICANCE (AUS)

Definition & Utility

- Salivary gland aspirates that lack qualitative or quantitative cytomorphologic features to be diagnosed confidently as either non-neoplastic or neoplastic
- Has sufficient atypical features precluding a “non-diagnostic” categorization.
- Heterogeneous category
  - Reactive atypia
  - Poorly sampled neoplasms
**Definition & Utility**

- Salivary gland aspirates that lack qualitative or quantitative cytomorphologic features to be diagnosed confidently as either non-neoplastic or neoplastic.
- Has sufficient atypical features precluding a “non-diagnostic” categorization.
- Heterogeneous category
  - Reactive atypia
  - Poorly sampled neoplasms

**Criteria**

- Atypia that cannot be definitively categorized
  - Squamous, oncocytic, or other metaplastic changes indeterminate for neoplasm
- Low cellularity specimens suggestive of, but not diagnostic for neoplasm
- Mucinous cystic lesions with few epithelial cells
- Lymphocyte-rich aspirates indefinite for lymphoproliferative disorders
AUS APPLICATIONS

Basaloid Cells

Spindled Cells

Cystic Lesions
GOALS OF AUS

- Providing a category for uncertainty in distinguishing non-neoplastic from neoplastic
  - Reduce false negative rate in non-neoplastic category
  - Reduce false positive rate in neoplasm category
- Benchmark: <10% of all salivary gland FNA
  - Encouragement for cytopathologists to classify specimens using other categories
FNA specimen has characteristic cytomorphologic features of a specific benign epithelial or mesenchymal neoplasm of the salivary gland.
NEOPLASM: BENIGN

Mesenchymal

- Lipoma
- Schwannoma
- Lymphangioma
- Hemangioma

NEOPLASM: BENIGN

Epithelial

- Pleomorphic adenoma
- Warthin Tumor
- Oncocytoma

- Classic
- Not so classic
THE CLASSIC: PLEOMORPHIC ADENOMA
IT’S NOT ALWAYS THAT EASY…

Atypia

Matrix-poor lesions
TRICKY MATRIX:
ADENOID CYSTIC CARCINOMA

TRICKY MATRIX:
ACINIC CELL CARCINOMA
THE CLASSIC: WARTHIN TUMOR

Lymphocytes & Oncocytes
DIAGNOSTIC CONSIDERATIONS: METAPLASIA IN WARTHIN TUMOR
DIAGNOSTIC CONSIDERATIONS:
ONCOCYTES, ONCOCYTES, & “ONCOCYTES”: IS THERE EVEN A “CLASSIC” ONCOCYTOMA?

Secretory Carcinoma  Sclerosing Polycystic Adenosis
DIAGNOSTIC CONSIDERATIONS

- Non-neoplastic
  - Nodular oncocytosis
  - Oncocytic cyst

- Benign neoplasms
  - Oncocytoma
  - Warthin Tumor
  - Myoepithelioma

- Malignant Neoplasms
  - Acinic cell carcinoma
  - Secretory carcinoma
  - Salivary duct carcinoma
  - Oncocytic carcinoma
  - Intraductal carcinoma, low-grade
  - Other low-grade salivary gland neoplasms with oncocytic features
FNA specimen has characteristic cytomorphologic features of specific benign epithelial or mesenchymal neoplasm of the salivary gland
SALIVARY GLAND NEOPLASM OF UNCERTAIN MALIGNANT POTENTIAL (SUMP)

- Diagnostic category reserved for specimens diagnostic of a neoplasm for which specific categorization is not feasible
  - A malignant neoplasm cannot be exclusion
SUMP:
SUBCATEGORIZATION

BASALOID NEOPLASM
ONOCYTIC/ONOCYTOID NEOPLASM
NEOPLASM WITH CLEAR CELL FEATURES
SUMP:
SUBCATEGORIZATION

**Basaloid**

- Cellular pleomorphic adenoma
- Basal cell adenoma/adenocarcinoma
- Adenoid cystic carcinoma
- Basal cell carcinoma
- Myoepithelioma/myoepithelial carcinoma
- Epithelial – myoepithelial carcinoma
- Polymorphous adenocarcinoma
BASALOID TUMORS
AND THINGS CAN GET EVEN WEIRDER…

Eccrine carcinoma

Pilomatrixoma

Oncocytic

- Warthin Tumor
- Oncocytoma
- Myoepithelioma
- Sclerosing polycystic adenosis
- Oncocytic carcinoma
- Acinic cell carcinoma
- Secretory carcinoma
- Salivary duct carcinoma
- Intraductal carcinoma
- Oncocytic features of:
  - Mucoepidermoid carcinoma
  - Metastasis
Intraductal carcinoma, low grade

Sclerosing polycystic adenosis

Benign oncocytic cyst

Secretory carcinoma
Clear cell

- Cytoplasmic clearing
  - Intracytoplasmic lipid, mucin, or glycogen
  - Intracellular edema
  - Paucity of intracellular organelles

- Clear cell features
  - Myoepithelioma
  - Epithelial-myoepithelial carcinoma
  - Pleomorphic adenoma
MILAN SYSTEM: DIAGNOSTIC CATEGORIES

- Non-diagnostic
- Non-neoplastic
- Atypia of undetermined significance
- Neoplasm
  - Benign
  - Salivary gland neoplasm of uncertain malignant potential
- Suspicious for malignancy
- Malignant
ANOTHER INDETERMINATE CATEGORY?

SUSPICIOUS FOR MALIGNANT CELLS
## MILAN SYSTEM: RISK OF MALIGNANCY

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>ROM (%)</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Non-diagnostic</td>
<td>25</td>
<td>Clinical and radiologic correlation/repeat FNA</td>
</tr>
<tr>
<td>II. Non-neoplastic</td>
<td>10</td>
<td>Clinical and radiologic correlation/clinical follow-up</td>
</tr>
<tr>
<td>III. Atypia of undetermined significance</td>
<td>20</td>
<td>Repeat FNA or surgery</td>
</tr>
<tr>
<td>IV. Neoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Benign</td>
<td>&lt;5</td>
<td>Clinical follow-up or surgery</td>
</tr>
<tr>
<td>B. SUMP</td>
<td>35</td>
<td>Surgery</td>
</tr>
<tr>
<td>V. Suspicious for malignancy</td>
<td>60</td>
<td>Surgery</td>
</tr>
<tr>
<td>VI. Malignant</td>
<td>90</td>
<td>Surgery</td>
</tr>
</tbody>
</table>
SUSPICIOUS FOR MALIGNANT CELLS

Definition

- Some but not all criteria for a diagnosis of malignancy are present
- BUT overall cytologic features are suggestive of malignancy
- Suspicious for:
  - primary salivary gland malignancy
  - lymphoma
  - metastasis

Criteria

- Markedly atypical cells with poor preservation/visualization
- Limited cytologic features of a specific malignant lesion
- Markedly atypical and/or suspicious cytologic features in a subset of cells in the background of benign salivary gland lesion
  - Prominent/macro-nucleoli
  - Anisonucleosis
  - Nuclear molding
  - Pleomorphism
  - Atypical mitoses

CHARACTERIZED BY HIGHER DEGREE OF ATYPIA THAN IN AUS AND SUMP CATEGORIES
MALIGNANT

Definition

- Combination of cytomorphologic features +/- ancillary studies that is diagnostic for malignancy

When possible

- Grade of the tumor
  - Low
  - High
- Subtype
MALIGNANT: SUBCATEGORIZATION

Low-grade

- Adenoid cystic carcinoma
- Acinic cell carcinoma
- Secretory carcinoma
- Epithelial-myoepithelial carcinoma
- Low-grade mucoepidermoid carcinoma

High-grade
THE CLASSIC: ADENOID CYSTIC CARCINOMA
DIAGNOSTIC CONSIDERATIONS

- Non-specific features
  - Basaloid morphology
  - Hyaline globules

- Pleomorphic adenoma
  (can be diffuse in cylindromatous variant)
- Basal cell adenoma
- Basal cell adenocarcinoma
- Epithelial myoepithelial carcinoma
- Metastatic basal cell carcinoma
THE CLASSIC: ACINIC CELL CARCINOMA
DIAGNOSTIC CONSIDERATIONS

- Differential diagnosis:
  - Non-neoplastic
    - Benign salivary gland elements
    - Sialadenosis
DIAGNOSTIC CONSIDERATIONS

- **Differential diagnosis:**
  - **Non-neoplastic**
    - Benign salivary gland elements
    - Sialadenosis
  - **Neoplastic**
    - Low-grade mucoepidermoid carcinoma
    - Sebaceous tumor
    - Epithelial – myoepithelial carcinoma
    - Secretory carcinoma
    - Warthin tumor
    - Metastasis (*renal cell carcinoma)

*Sclerosing polycystic adenosis*
DIAGNOSTIC CONSIDERATIONS
DIAGNOSTIC CONSIDERATION: MUCOEPIDERMIDOID CARCINOMA
THE CLASSIC: SECRETORY CARCINOMA

- Differential
  - Acinic cell carcinoma
  - Oncocytic lesions
  - Low-grade mucoepidermoid carcinoma
SECRETORY CARCINOMA
THE CLASSIC:
EPITHELIAL-MYOEPITHELIAL CARCINOMA

DIAGNOSTIC CONSIDERATIONS

- Tricky matrix material
  - Pleomorphic adenoma
  - Adenoid cystic carcinoma
- Myoepithelial rich tumors
  (myoepithelioma/carcinoma, PA)
- Clear cell tumors
  - Clear cell carcinoma of minor salivary glands
  - Metastatic RCC
THE CLASSIC:
LOW-GRADE MUCOEPIDERMOID CARCINOMA
DIAGNOSTIC CONSIDERATIONS:
ACINIC CELL CARCINOMA & WARTHIN TUMOR
MALIGNANT: SUBCATEGORIZATION

Low-grade
- Adenoid cystic carcinoma
- Acinic cell carcinoma
- Secretory carcinoma
- Epithelial-myoepithelial carcinoma
- Low-grade mucoepidermoid carcinoma

High-grade
- Salivary duct carcinoma
- Squamous cell carcinoma
- High-grade mucoepidermoid carcinoma
- Carcinoma ex-PA
- Lymphoepithelial carcinoma
- Small cell neuroendocrine carcinoma of salivary gland
- Other:
  - Metastasis
HIGH-GRADE SALIVARY GLAND MALIGNANCIES
ANCILLARY TESTS

IMMUNOHISTOCHEMISTRY

MOLECULAR ASSAYS
ANCILLARY TESTING

Available Options

- Molecular/genetic alterations
  - Karyotype analysis
  - Fluorescence in situ hybridization (FISH)
- Immunohistochemistry
- Flow Cytometry

When to use them?

- When it can alter clinical management or effect clinical risk/prognosis
- Non-neoplastic → not indicated (except infection work-up)
- AUS → if helps resolve diagnostic uncertainty
- Neoplasm
  - Definitively benign or high-grade malignancy → not indicated
  - SUMP or SM → can help refine diagnosis
ANCILLARY TESTING

TABLE 4. Ancillary Testing for Basaloid Neoplasms

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Useful Immunohistochemical Features</th>
<th>Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>MYB</td>
<td>MYB FISH</td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>PLAG1 or HMGA2 positive</td>
<td>PLAG1 or HMGA2 FISH</td>
</tr>
<tr>
<td>Basal cell adenoma/adenocarcinoma</td>
<td>β-catenin</td>
<td>CTNNB1 mutation analysis (basal cell adenoma)</td>
</tr>
<tr>
<td>Carcinoma ex pleomorphic adenoma, including epithelial-myoepithelial carcinoma</td>
<td>PLAG1, HMGA2 Epithelial-myoepithelial carcinoma: EMA and myoepithelial markers(^a)</td>
<td>PLAG1 or HMGA2 FISH</td>
</tr>
</tbody>
</table>

\(^a\)Myoepithelial markers include p63/p40, S100, SMA, and calponin.

TABLE 5. Ancillary Testing for Oncocytic Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Useful Immunohistochemical Features</th>
<th>Additional Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warthin tumor</td>
<td>p63/p40 negative</td>
<td></td>
</tr>
<tr>
<td>Oncocytoma/oncocytic carcinoma</td>
<td>p63/p40 negative</td>
<td>PLAG1 or HMGA2 FISH</td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>PLAG1 or HMGA2 positive</td>
<td>PAS with diastase</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>DOG1 positive</td>
<td>ETV6 FISH</td>
</tr>
<tr>
<td>Secretery carcinoma</td>
<td>S100 and mammaglobin coexpression</td>
<td></td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>p63/p40 positive, SOX10 negative</td>
<td>MAML2 FISH</td>
</tr>
<tr>
<td>Salivary duct carcinoma (including ex pleomorphic adenoma)</td>
<td>AR positive, HER2 (30%), PLAG1 or HMGA2 for salivary duct carcinoma ex pleomorphic adenoma</td>
<td>PLAG1 or HMGA2 FISH for salivary duct carcinoma ex pleomorphic adenoma</td>
</tr>
</tbody>
</table>

### TABLE 6. Ancillary Testing for Clear Cell Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Useful Immunohistochemical Features</th>
<th>Additional Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncocytoma/oncocytic carcinoma</td>
<td>p63/p40 negative</td>
<td></td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>PLAG1 or HMGA2 positive</td>
<td>PLAG1 or HMGA2 FISH</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>Positive for p63 and CK7</td>
<td>EWSR1 FISH</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>DOG1 positive</td>
<td>PAS with diastase</td>
</tr>
<tr>
<td>Secretory carcinoma</td>
<td>S100 and mammaglobin coexpression</td>
<td>ETV6 FISH</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>p63/p40 positive, SOX10 negative</td>
<td>MAML2 FISH</td>
</tr>
<tr>
<td>Epithelial-myoepithelial carcinoma (including ex</td>
<td>EMA and myoepithelial markers&lt;sup&gt;a&lt;/sup&gt;; PLAG1 or HMGA2 for ex</td>
<td>PLAG1 or HMGA2 FISH for ex</td>
</tr>
<tr>
<td>pleomorphic adenoma)</td>
<td>pleomorphic adenoma</td>
<td>pleomorphic adenoma</td>
</tr>
<tr>
<td>Salivary duct carcinoma (including ex pleomorphic adenoma)</td>
<td>AR positive, HER2 (30%); PLAG1 or HMGA2 for salivary duct carcinoma ex pleomorphic adenoma</td>
<td>PLAG1 or HMGA2 FISH for salivary duct carcinoma ex pleomorphic adenoma</td>
</tr>
</tbody>
</table>

<sup>a</sup>Myoepithelial markers include p63/p40, S100, SMA, and calponin.
ANCILLARY TESTS:
HIGH-GRADE SALIVARY GLAND MALIGNANCIES

- Often unhelpful EXCEPT in consideration of:
  - Salivary duct carcinoma
    - Androgen receptor
    - GCDFP-15
    - Her-2
  - Differentiating from secretory carcinoma
    - Mammaglobin
    - S100
    - ETV6/NTRK3 translocation
AFTER ALL OF THIS: IS THE MILAN SYSTEM HELPFUL?

GREY ZONES
EFFECTIVENESS AFTER INSTITUTIONAL IMPLEMENTATION
GREY ZONES OF THE MILAN SYSTEM

Appropriate use of the non-neoplastic category

- Lymphoid infiltrate
- Oncocytic lesions
- Overall comfort level in absolving neoplastic etiology (clinical/radiologic interpretation)

Neoplasm category

- Broad differential, morphologic pitfalls, lots of possibilities especially with PA

Difficulties in utilizing indeterminate categories

- When to use AUS vs. ”stronger” indeterminate categories
- SUMP vs. Suspicious for malignant cells
  - Both consider the possibility of sampling of low-grade salivary gland malignancy
  - Added difficulty of basaloid and oncocytic neoplasms
GREY ZONES OF THE MILAN SYSTEM

Appropriate use of the non-neoplastic category

- Lymphoid infiltrate
- Oncocytic lesions
- Overall comfort level in absolving neoplastic etiology (clinical/radiologic interpretation)

Neoplasm category

- Broad differential, morphologic pitfalls, lots of possibilities especially with PA

Difficulties in utilizing indeterminate categories

- When to use AUS vs. “stronger” indeterminate categories
- SUMP vs. Suspicious for malignant cells
  - Both consider the possibility of sampling of low-grade salivary gland malignancy
  - Added difficulty of basaloid and oncocytic neoplasms
SO SHOULD WE USE IT?
### TABLE 9  Comparison of ROM of the present study with other current publications

<table>
<thead>
<tr>
<th>Category</th>
<th>Savant et al.</th>
<th>Rohilla et al.</th>
<th>Milan system</th>
<th>Wei et al.</th>
<th>Akhtar et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Nondiagnostic</td>
<td>0.00% (0/18)</td>
<td>0% (0/1)</td>
<td>25% (0-67%)</td>
<td>25.0% ± 16.7%</td>
<td>9% (2/22)</td>
</tr>
<tr>
<td>II. Nonneoplastic</td>
<td>0.00% (0/4)</td>
<td>17.4% (4/23)</td>
<td>10% (0-20%)</td>
<td>10.2% ± 5.5%</td>
<td>0.8% (1/117)</td>
</tr>
<tr>
<td>III. AUS</td>
<td>33.33% (4/12)</td>
<td>100% (2/2)</td>
<td>20% (10-35%)</td>
<td>No cases</td>
<td>0.4% (2/5)</td>
</tr>
<tr>
<td>IVA. Benign</td>
<td>0.84% (1/118)</td>
<td>7.3% (3/41)</td>
<td>&lt;5% (0-13%)</td>
<td>3.4% ± 1.3%</td>
<td>0% (0/120)</td>
</tr>
<tr>
<td>IVB. SUMP</td>
<td>40.90% (9/22)</td>
<td>50% (1/2)</td>
<td>35% (0-100%)</td>
<td>37.5% ± 24.7%</td>
<td>47% (8/17)</td>
</tr>
<tr>
<td>V. Suspicious for malignancy</td>
<td>100.00% (3/3)</td>
<td>0% (0/0)</td>
<td>60% (0-100%)</td>
<td>58.6% ± 19.5%</td>
<td>62.5% (5/8)</td>
</tr>
<tr>
<td>VI. Positive for malignancy</td>
<td>100.00% (22/22)</td>
<td>96% (24/25)</td>
<td>90% (57-100%)</td>
<td>91.9% ± 3.5%</td>
<td>81.8% (27/33)</td>
</tr>
</tbody>
</table>

There are main takeaways in the practice of salivary gland cytology:

- Clinically meaningful diagnoses that impact management (a practice that focuses on risk stratification)
  - Non-neoplastic vs. neoplastic
  - Low-grade neoplasms vs. high-grade neoplasms
    - Determines the extent and urgency of treatment

- Clear communication with clinicians
  - If you choose to use indeterminate categories:
    - Make clear what your considerations are
    - List out potential etiologies in the order you are concerned

Standardization of practice:
a helpful tool in the evaluation of data across institutions  ➔ modification to classification system
WHAT’S NEXT?
FUTURE DIRECTIONS
NEW MARKERS ON THE RISE

NR4A3:
- Transcription factor *Nuclear Receptor Subfamily 4 Group A Member 3* upregulated through enhancer hijacking as the oncogenic driver event in acinic cell carcinoma
- Can be utilized in FISH or immunohistochemical (nuclear) evaluation

Pan-TRK:
- Nuclear immunohistochemical marker for identification of tumors with NTRK fusions (secretory carcinoma)
  - Membranous/cytoplasmic immunoreactivity can be seen in other benign salivary gland neoplasms and low-grade salivary gland carcinomas

Next-generation RNA sequencing (RNAseq) can assay expression signatures and gene fusions of salivary gland tumors

- University of Michigan:
  - Designed and validated a novel RNAseq assay for salivary gland carcinomas using a custom AmpliSeq panel and Ion Torrent Next-Generation Sequencing system for 55 salivary gland resection specimens (AdCC, AiCC, MEC, SDC)
  - Can detect expected gene fusions, but also showed gene expression-based clustering of subtyping, cellular proliferation, and immunoncology genes in tumor samples correspond to discrete histopathologic subtypes
  - Current work underway to utilize this technology for FNA specimens
    - Effective multiplexing for analysis of gene fusions and alterations
- Reviewed of diagnostic accuracy, utility, and shortcomings of salivary gland FNA
-The benefit of a uniform classification system for quality improvement

-Reviewed the definitions and criteria for diagnostic categories of the Milan System
-Discussed diagnostic pitfalls and grey zones of the Milan System categories

Correlations with ancillary studies in the context of morphologic patterns of salivary gland neoplasm

Discussed effectiveness of implementing the Milan System: variable distribution of indeterminate categories, but utility in combining data between institutions

Future Directions: new immunohistochemical markers and high throughput sequences
THANK YOU FOR YOUR ATTENTION!

QUESTIONS/COMMENTS?