Salivary Gland Cytology and
The Milan System for Reporting

*Dina R. Mody, MD*

Director of Cytology laboratories
The Ibrahim Ramzy Chair in Pathology
Houston Methodist Hospital
Professor of Pathology and Laboratory Medicine
Weill Cornell Medicine
Conflict of Interest

- None with vendors of cytology equipment or HPV testing
- Amirsys (now Elsevier) and McGraw Hill
  - (Book publishers/Royalties)
Conflict of Interest

- Journal of The American Society of Cytopathology (Editor in Chief)
Conditions Affecting Salivary Glands

- Stones
- Cysts
- Infections/Inflammatory
- Sialadenosis
- Neoplasms
  - Benign
  - Low grade
  - High grade
- Others like intra or adjacent to salivary Lymph nodes
Salivary Gland FNA Diagnosis

- Based on cystic or solid
- Neoplastic or non neoplastic
  - Neoplasms divided into matrix producing or not
  - Basaloid
  - Oncocytic
  - Clear cell
  - Spindle cell cystic and mucinous
- Lymphocytic or not... lymph node/lymphoepithelial cyst
- Other characteristics
Salivary Gland Aspiration Patterns

- **Cystic Lesions**
  - Acellular clear fluid
    - Sialocele
    - Lymphoepithelial cysts
  - Cloudy/mucoid fluid +/- cells
    - Lymphoepithelial cyst
    - Abscess
    - Mucocoele
    - Warthin’s tumor
    - Low Grade Mucoepidermoid ca
    - Acinic cell ca (rare)
    - Cystic degeneration in any neoplasm

- **Inflammatory cells**
  - Abscess
  - Chronic sialadenitis
  - Lymphoepithelial sialadenitis
  - Warthin’s
  - Lymph node
  - Lymphoma (monotonous)

- **Granulomas**
  - Sarcoid
  - TB
  - Fungal
### Salivary Gland Aspiration Patterns

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<thead>
<tr>
<th>Oncocytic cell Pattern</th>
<th>Lymphocytic Cell pattern</th>
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<tr>
<td>Nodular Oncocytic hyperplasia</td>
<td>Chronic sialadenitis</td>
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<td>Oncocytoma</td>
<td>Lymphoepithelial sialadenitis</td>
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<td>Warthin’s</td>
<td>Lymphoepithelial cyst</td>
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<td>Oncocytic carcinoma</td>
<td>Lymph node</td>
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<tr>
<td>Mucoepidermoid ca, oncocytic variant</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>Warthin’s tumor</td>
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<tr>
<td>Salivary duct carcinoma</td>
<td>Acinic cell carcinoma</td>
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<tr>
<td>MASC</td>
<td>Mucoepidermoid carcinoma</td>
</tr>
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</table>
Salivary Gland Aspiration Patterns

- **Basaloid cell Pattern**
  - Basal cell (monomorphic) adenoma/carcinoma
  - Cellular Pleomorphic adenoma
  - Adenoid cystic carcinoma
  - Myoepithelial carcinoma
  - Polymorphous low grade adenocarcinoma
  - Small cell carcinoma, prim/met
  - Cutaneous basal cell ca
  - Sialoblastoma

- **Clear cell Pattern**
  - Normal salivary gland
  - Lipoma
  - Acinic cell carcinoma
  - Mucoepidermoid carcinoma
  - Clear cell myoepithelioma/ca
  - Epithelial/myoepithelial carcinoma
  - Sebaceous lymphadenoma/ca
  - Metastatic clear cell ca/renal/Sq
Salivary Gland Aspiration Patterns

- Neoplasms with stromal pattern
  - Pleomorphic Adenoma: Fibrillary stroma
  - Adenoid Cystic ca: discrete, defined globules
  - Basal cell adenoma/ca: dense membrane like stroma
  - Polymorphous low grade adenoca
  - Myoepithelioma/ca
  - Nodular fasciitis: loose myxoid

- High grade malignant neoplasms
  - High grade Mucoepidermoid ca(MEC)
  - Carcinoma ex Pleomorphic Adenoma
  - Adenocarcinoma NOS
  - Salivary duct ca
  - Mammary Analogue Salivary Carcinoma (MASC)
  - Squamous cell ca
  - Merkel cell ca
  - Melanoma
  - Angiosarcoma
  - Other mets
Salivary Gland Aspiration Patterns

- **Mucinous**
  - Normal submandibular or sub lingual glands
  - Florid adenomatoid hyperplasia
  - Mucocoele
  - Low grade Mucoepidermoid ca

- **Spindle cells**
  - Schwannoma/NF
  - Myoepithelioma
  - PA with predominance of myoep
  - Angiosarcoma
  - Other mets

- **Crystals**
  - Tyrosine: Daisy petals in Pleomorphic adenoma (PA)
  - Amylase: elongated hexagons in chronic sialadenitis/cysts
  - Cholesterol: clear and colorless in Warthin’s and various cysts
  - Asteroid bodies and calcium oxalate in sarcoidosis
  - Calcium crystals: Purple on pap, colorless on DQ: retained products of saliva
  - Psammoma bodies: normal or inflamed salivary gland and neoplasms, Benign and malignant
Problem with old way of reporting...

- No consistency
- Salivary gland neoplasms are the most heterogenous group, and hence also the most challenging, even more so on cytology and minute Core needle biopsies
  - Matrix containing tumors
  - Basaloid tumors
  - Oncocytic lesions/tumors
  - Cystic and mucinous lesions/tumors
  - High grade carcinomas
  - Clear cell tumors
  - Spindle cell lesions/neoplasms
Problem with old way of reporting…continued…

- Surgical pathology terminology often used
- Too many DIDGO’s (describe it to death and let it go)…not helpful at all! Clinicians confused

- Agreement for need of defined diagnostic categories
- Clarity of communication
- Exchange of information across institutions
- Uniform management, improvement patient care
Salivary FNA Variances (aka errors)

- **False Positive**
  - Interpretive
  - Monomorphic Adenoma
  - Warthin’s with squamous and mucinous metaplasia with atypia
  - Intraparotid lymph node
  - Oncocytoma
  - Granulomatous sialadenitis

- **False negative**
  - Sampling
  - Interpretive
  - Acinic cell carcinoma
  - Low grade ME carcinoma
  - Lymphoma
  - Adenoid cystic carcinoma
  - Low grade angiosarcoma (cutaneous)
Diagnostic accuracy of fine-needle aspiration cytology of salivary gland lesions: A 6-year retrospective review

Erin N. Consamus, MD, Deborah Smith, CT (ASCP), Sergio Pina Oviedo, MD, Dina R. Mody, MD, Hidehiro Takei, MD

http://doi.org/10.1016/j.jasc.2014.11.003

Introduction
The aim of this study was to evaluate the diagnostic accuracy of salivary gland fine-needle aspiration (FNA) in comparison to histologic examination and to recognize possible pitfalls in diagnosis.

Materials and methods
The diagnoses and demographics of all cases of salivary gland FNAs with concurrent or subsequent histologic correlation at our institution over a 6-year period (2006-2011) were retrospectively reviewed and compared for discrepancies. Discrepancies were categorized as either major or minor and due to sampling or interpretive variance.

Results
Overall, the following values were calculated: sensitivity 80.6%, specificity 97.5%, positive predictive value 92.6%, negative predictive value 92.8%, accuracy 92.7%, and concordance rate 90.9%. In addition, concordance rates were calculated for the 2 most common diagnoses: pleomorphic adenoma (97.1%, n = 35) and Warthin tumor (88.9%, n
## Salivary Glands Statistics

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<tr>
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<th>Our results (%)</th>
<th>Literature results (%)</th>
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<tr>
<td>Sensitivity</td>
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<td>86-100</td>
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<tr>
<td>Specificity</td>
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<td>81-100</td>
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<tr>
<td>Positive Predictive value</td>
<td>92.6</td>
<td></td>
</tr>
<tr>
<td>Negative Predictive value</td>
<td>92.8</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>92.7</td>
<td>48-94 (specific neoplasm), (B9vs Malig) 81-100</td>
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<td>Pleomorphic Adenoma</td>
<td>97.1 (concordance)</td>
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### Salivary Gland Lesions/Neoplasms

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<th>Descriptive ..I call them DIDGOs</th>
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<td>Mucocoele</td>
<td>Basal adenoma (other than membranous)</td>
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<td>Adenoid cystic carcinoma</td>
<td>Basal cell adenocarcinoma</td>
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<td>Lympho epithelial cyst</td>
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<tr>
<td>Basal cell adenoma, membranous type</td>
<td>Carcinoma ex PA</td>
<td>Epithelial-myoepithelial carcinoma</td>
</tr>
<tr>
<td></td>
<td>Small cell carcinoma</td>
<td>(Mammary analogue) Secretory carcinoma</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td></td>
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Management of Salivary Gland Lesions/conditions

- If inflammatory: Medical management
- Lymphoma: Heme Onc referral
- Metastasis: Resection or radical neck dissect
- Benign or low grade primary neoplasm: Limited resection
- High grade carcinoma: Radical resection
The Milan System for Salivary Gland Cytopathology

- ASC and IAC co sponsors
- Over 40 participants, 14 countries
- Evidence based
- Print atlas in early 2018...already out!
- Web based atlas also available through ASC
- Co chairs Drs Bill Faquin and Diana Rossi
- Others include Drs Baloch, Barkan, Foschini, Kurtyz, Pusztaszeri, Vielh
- Online survey data..49 questions, 515 participants, 54% academic
  >95% agreed with new reporting structure
- Both Romanowsky and pap staining essential
The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC): an ASC-IAC-sponsored system for reporting salivary gland fine-needle aspiration

Esther Diana Rossi, MD, PhD, MIAC*,**, Zubair Baloch, MD, PhD***, Marc Pusztaszeri, MD, William C. Faquin, MD, PhD****

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The diagnostic role of salivary gland fine-needle aspiration (SG-FNA) is well established in the preoperative evaluation of patients with salivary gland lesions. At present, most salivary SG-FNA specimens are diagnosed based on conventional diagnostic criteria. Nevertheless, there exists a lack of uniform reporting for these specimens to guide the clinical management of patients. This has motivated a group of experienced cytopathologists to spearhead the development of a uniform reporting system. This international panel, under the sponsorship of the American Society of Cytopathology (ASC) and the International Academy of Cytology (IAC), gathered in September 2015 at the European Congress of Cytology, held in Milan, Italy, to propose “The Milan System for Reporting Salivary Gland Cytopathology.” This effort sparked the interest of many and brought forth an agreement to develop an evidence-based tiered classification consisting of 6 diagnostic categories. We hope that this standard reporting system will enhance the overall effectiveness of SG-FNA reporting across institutions, with the ultimate result being better communication and improved patient care.

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KEYWORDS
Fine-needle aspiration, Salivary gland, Benign lesions, Standardized reporting, Malignant lesions

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E-mail address: esther.rossi@policlinicogemelli.it (E.D. Rossi).
The Milan System for Reporting Salivary Gland Cytopathology

Milan System Interobserver Reproducibility Study
Posted on January 22, 2018  By Milan System

We invite you to evaluate a series of images from the new Milan System for Reporting Salivary Gland Cytopathology Atlas. The responses will be recorded...

Classification System
Posted on December 8, 2017  By Milan System
The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)

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<th>Management</th>
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<td>~25%</td>
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ROM depends on salivary gland site and nature of specimen
Salivary Gland Mass sampling

- Palpation or Ultrasound guidance
- FNA preferred
- Both Romanowsky and Pap/H&E stains preferred
- Cell block preparation encouraged
- Core needle biopsies an option but….
  - Tracking
  - Facial nerve injury, especially with larger cores
Non Diagnostic

- Currently no validated criteria in literature
- Call non diagnostic after everything is processed and examined and correlated clinically and radiologically
- Insufficient material qualitative or quantitative for a diagnosis
- 10% or less targeted reporting rate (hopefully!)
- Exceptions: matrix material, mucinous cyst contents, acute inflammation, any atypia
- ? Minimum of 60 lesional cells for adequacy…like thyroid?
- Repeat Sampling using US or CT guidance
Normal Salivary Gland Cytology
Non Diagnostic..continued

- E.g. Salivary duct stone with cyst...aspiration yields clear fluid, no more mass...then adequate as it explains the scenario
- Bilateral enlarged salivary glands with no definite mass, then adequate
- However, if mass, and all you get is normal salivary gland tissue...
  - Then non diagnostic as it does not explain the mass/"it"
## The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)

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ROM depends on salivary gland site and nature of specimen  Personal communication Drs Faquin and Rossi and presentation at ASC meeting in Phoenix, Nov 2017
Non Neoplastic

- Chronic and granulomatous Sialadenitis
- Sialolithiasis
- Lymph nodes (reactive) within or adjacent to salivary gland
  - Recommend flow if clinically and cytologically worrisome, older age
- Other benign conditions like cysts etc..
- Risks of malignancy should be low if adequately sampled
- A subset will need surgical excision to exclude a poorly sampled neoplasm
Non Neoplastic

- Chronic Sialadenitis
- Granulomatous Sialadenitis
Lymph nodes on Salivary FNA

- Reactive lymph nodes
  - Polymorphous population
  - Age, usually <50
  - Tingible body macrophages
  - But all of above can be seen in lymphomas
- Flow cytometry if worrisome or older patient
  - Even that may be problematic as some large cell lymphomas, Hodgkin’s, T cell rich B cell Lymphomas negative by flow
  - Recommend excision if uncertain
  - Make sure representative of lesion
  - Note of caution, consider follow up/excision if does not go away
Milan 3. Atypia of Undetermined Significance

- Heterogeneous category with majority being due to poor sampling or preparation/compromised specimen (air drying, blood clot, artefact)
- Cannot entirely exclude a neoplasm
- <10% reporting rate (hopefully!)
- Example is mucinous cyst contents only (cannot exclude a low grade Mucoepidermoid carcinoma)
Atypia of Undetermined Significance..Scenarios

- Oncocytic metaplasia (vs neoplasm)
- Reactive/reparative atypia, cannot rule out a neoplasm
- Low cellularity specimen, worrisome for but not diagnostic of a neoplasm
- Salivary gland lymph nodes, indefinite for a lymphoma on morphology alone (do flow or excise)
- Sclerosing polycystic adenosis
- Lymphoepithelial cyst with squamous atypia in cyst lining
Cystic salivary Gland Aspirates ..Intrinsic

- Non neoplastic
  - Salivary duct cyst
  - Lymphoepithelial cyst
  - Polycystic disease

- Neoplastic
  - Warthin’s, Pleomorphic adenoma
  - Muco epidermoid ca, Acinic cell ca
  - Cystadenoma/ca
  - Secretory carcinoma
Atypia of Undetermined Significance...Examples
Cystic salivary Gland Aspirates ..Extrinsic

- Non neoplastic
  - Branchial cleft cyst
- Neoplastic
  - Metastatic carcinoma (with cystic degeneration/necrosis to an intra or peri salivary gland lymph node (especially parotid and sometimes sub mandibular)
# The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)

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ROM depends on salivary gland site and nature of specimen. Personal communication Drs Faquin and Rossi and presentation at ASC meeting in Phoenix AZ NOV 2017.
Atypia of Undetermined Significance

Evaluation limited by scant cellularity

Atypia of Undetermined Significance

Histiocytes with scant epithelial cells in an abundant mucinous background. Differential includes a mucocele, mucus retention cyst and low grade mucoepidermoid carcinoma

Satisfactory for evaluation

Atypia of Undetermined Significance

Aspirate is suggestive of a reactive lymph node. However, in absence of flow cytometry, a low grade lymphoma cannot be ruled out. Clinical and radiological considerations recommended.

From The Milan System for reporting salivary gland cytopathology by Faquin and Rossi, Springer 2018
Milan 4. Neoplasm

- Benign Neoplasm
- Salivary gland neoplasm of uncertain malignant potential
Neoplasm: Benign

- Pleomorphic Adenomas
- Warthin’s
- Lipomas
- Schwannomas
Pleomorphic Adenoma (Benign Mixed Tumor)

- Most common salivary neoplasm
- Parotid most common site
- Fibrillary chondromyxoid stroma
  - Metachromatic on DQ
  - Greyish blue on Pap
- Ductal cells
  - Small cuboidal to polygonal cells
- Myoepithelial cells
  - Plasmacytoid, dyshesive, bland
KEEP CALM AND GO ROGUE
Carcinoma ex Pleomorphic Adenoma

- Carcinoma arising from Pleomorphic adenoma
- Requires concurrent PA or history of PA at same site
- 80% occur in major salivary glands especially Parotid
  - 7-10% of PAs (especially long standing), 6th to 8th decade (20 yrs later than PA)
  - 6th most common salivary gland malignancy in adults
- Cellular specimen with predominantly epithelial cells
- 2 distinct patterns, benign PA with malignant or equivocally malignant cells with or without necrosis, mitosis
  - Malignant component could be adenocarcinoma, salivary duct carcinoma, ACC, MEC, PLGA, epi-myoidepithelial carcinoma
Warthin’s Tumor

- 2nd most common benign salivary gland tumor
- Smokers, typically 5th - 7th decade, M>F
- Almost exclusively Parotid, superficial lobe in the tail
- Painless mass with a doughy feel
- Aspiration usually yields a drop or two of thick, tan brown fluid (looks like motor oil)
- Bimorphic population of lymphocytes and oncocytes, some papillary configuration
- DD MECarcinoma, Oncocytoma, lymphnode, Sq ca
A sheet or honeycomb of oncocytic cells. Note the well defined borders and a suggestion of papillary architecture.

This air dried material shows the remarkable oncocytic nature of the proliferation. There is a monotony to the cells. Note the lymphoid cells in the background.
Warthin’s with atypical Squamous metaplasia
Myoepithelioma

- Benign myoepithelial tumor, 2% of salivary gland neoplasms, 6% of minor salivary gland tumors
- 3\textsuperscript{rd}-5\textsuperscript{th} decade, M:F (1:1), slowly growing, painless mass
- Two cell types, spindle or plasmacytoid or may be mixed.
- Collagenized stroma, chondroid or chondromyxoid areas
- Looks like PA but without ductal cells
- DD PA, Plasmacytoma, myoepithelial carcinoma (has necrosis, atypical mitosis, invasion into surrounding)
Neoplasm: Uncertain Malignant Potential (SUMP)

- Can diagnose as a neoplasm but cannot tell what type/specific diagnosis
- Malignancy cannot be excluded
- Majority will consist of cellular benign neoplasms with atypical/confusing features or low grade carcinomas
  - Myoepithelioma
  - Stroma poor Pleomorphic adenoma
  - Warthin’s with atypical metaplasia
  - Basiloid tumors(adenoma vs carcinoma)
Neoplasm: Uncertain Malignant Potential (SUMP)

Subgroups include:

- Cellular Basaloid neoplasm
  - (fibrillary stroma) Pleomorphic adenoma (PA), Myoepithelioma/ca, Basal cell adenoma/ca
  - (Hyaline stroma) Basal cell adenoma/ca, Adenoid cystic ca (Adcc), Polymorphous ACA, epi-myo epithelial carcinoma
  - (Mixed/other stroma) Adenoid cystic ca, Polymorphous ca
  - Scant Stroma: Cellular PA, Adcc, canalicular adenoma, myoepith ca

- Cellular oncocytic/Oncocytoid neoplasm
  - Warthin’s, Oncocytoma, acinic cell ca, MASC, meta RCC, MEC, myoepithelioma

- Cellular neoplasm with clear cell features
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Neoplasm: SUMP

- Pleomorphic Adenoma with myoepithelial cell prominence
- PA with focal areas questionable for Adenoid cystic carcinoma
Sample Reports

Neoplasm, Benign

Satisfactory for evaluation

Neoplasm: Benign

Pleomorphic Adenoma

SUMP

Satisfactory for evaluation

Neoplasm: Salivary Gland
neoplasm of Uncertain Malignant Potential

Cellular basaloid neoplasm. See note: Specimen consists of mostly basaloid cells with minimal nuclear atypia, lack of necrosis or mitosis. Although a diagnosis of pleomorphic adenoma is favored, a basal cell adenoma/carcinoma cannot be ruled out.

From: The Milan System for reporting salivary gland cytopathology by Faquin and Rossi, Springer 2018
Suspicious for Malignancy

- Aspirates with features highly suggestive of carcinoma but qualitatively or quantitatively fall short of a definitive diagnosis
- An attempt should be made to subcategorize if worried about low or high grade malignancy
- Majority (but not all) will be cases of high grade carcinomas with compromised sampling/preparation
Suspicious for malignancy...sample reports

Example 1
Satisfactory for evaluation
Suspicious for malignancy
Rare highly malignant cells, suspicious for high grade carcinoma

Example 2
Evaluation limited by scant cellularity
Suspicious for malignancy
Atypical cells in a mucinous background, suspicious for low grade muco epidermoid carcinoma
### The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Malignancy (ROM)</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Non-Diagnostic</td>
<td>~25%</td>
<td>Clinical/radiologic correlation, repeat FNA</td>
</tr>
<tr>
<td>II. Non - Neoplastic</td>
<td>~10%</td>
<td>Clinical follow up, radiologic correlation</td>
</tr>
<tr>
<td>III. Atypia of Undetermined Significance (AUS)</td>
<td>~20%</td>
<td>Repeat FNA or surgery</td>
</tr>
<tr>
<td>IVa. Neoplasm: Benign</td>
<td>&lt;5%</td>
<td>Follow or conservative surgery</td>
</tr>
<tr>
<td>IVb. Salivary gland Neoplasm of Uncertain malignant potential (SUMP)</td>
<td>~35%</td>
<td>Conservative surgery</td>
</tr>
<tr>
<td>V. Suspicious for malignancy</td>
<td>~60% (range 0-100%)</td>
<td>Surgery, decide if low grade or High grade and manage accordingly</td>
</tr>
<tr>
<td>VI. Malignant (low vs High grade)</td>
<td>~90% (57-100%)</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

*ROM depends on salivary gland site and nature of specimen  Personal communication Drs Faquin and Rossi and presentation at ASC meeting in Phoenix AZ Nov 2017*
Malignant

- Aspirates diagnostic of malignancy
- Every attempt should be made to classify into specific type/grade when possible as grading is critical for management
  - Low grade (low grade mucoepidermoid carcinoma)
  - High Grade (Salivary duct carcinoma)
- Other malignancies like Metastasis, Lymphomas and Sarcomas also belong here but should be specified as to type etc.
Mucoepidermoid Carcinoma

- Most common malignant salivary gland tumor in children and adults, wide age range
- Major and minor glands affected, size varies
- Low and High grade types
- Low grade, 98% Disease Specific Survival
- High grade metastatise, 65% DSS
- 3 cell types, mucus, intermediate and epidermoid cell
- High grade difficult to diagnose on cytology, often call high grade carcinoma/squamous ca
Mucoepidermoid Carcinoma…continued

- Low grade may have abundant Mucin and few cells
- MEC has 3 cell types: Clusters of bland intermediate, epithelial cells and mucocytes
- Mucin producing cells may be columnar, cuboidal or histiocyte like
- Intermediate cells often found in nests or sheets, can be polygonal/epidermoid in appearance
- Epidermoid cells are polygonal and appear in nests or scattered
Mucoepidermoid Carcinoma

- **IHC:** Her 2+ in 60% of high grade MECs
- **EGFR** high copy numbers in high grade
- **P16 +** in 60%...**NOT** HPV related
- **Molecular:** t(11;19)(q21;p13) seen in 55-65% of MEC
  - This translocation fuses CREB-regulated transcription coactivator 1 (CRTC1, formerly MECP1) (exon 1 of gene at 19p13) with Mastermind-like gene family (MAML2) (exons 2–5 of gene at 11q21)
  - Identified in low- to intermediate-grade tumors usually
  - Tumors with few copy number alterations (usually CRTC1-MAML2) seem to have better prognosis
Acinic Cell carcinoma

- 80% occur in Parotid gland, 2nd most common malignant salivary gland tumor (10-12%), 6% of all salivary tumors
- F:M=3:2, wide age range, mean mid 40s
- 2nd most common malignant salivary tumor in kids
- Slowly growing, may have pain or facial nerve paralysis
- High cellularity, loose or tight acinar structures
- Many stripped bare tumor nuclei in background
- Ample granular vacuolated fragile cytoplasm
Acinic Cell carcinoma

- High false negative rate
- Mistaken for normal salivary gland (note ducts and adipocytes missing, acini tight in normal)
- Other DD Warthin’s, MEC, other clear cell tumors
- PAS+ diastase resistant granules (not useful in cytology)
- No specific IHC profile
- No specific genetic/molecular test
- 5 yr survival around 90%, local recurrence in 35%
Adenoid Cystic Carcinoma

- 4th most common malignant salivary gland tumor, F:M=3:2, peak age 6th decade
- Major and minor salivary glands, Parotid most frequent
- Mass, pain, tenderness, facial nerve paralysis
- Treated with radical excision
- Poorly circumscribed infiltrative tumor with multiple patterns
- Survival based on stage, stage I 75%, II 43%, III & IV 15%
Adenoid Cystic Carcinoma

- Variety of patterns (cribiform, tubular, solid, combination)
- Small to medium cells with clear to eosinophilic cytoplasm
- Cohesive cellular clusters surrounding balls of metachromatic material (distinct cells and stroma)
- High N:C ratio, dark nuclei with scant cytoplasm
- Difficult to distinguish from other salivary neoplasms in absence of metachromatic stroma
- DD PA, other basaloid salivary tumors, B9 or malignant
Adenoid Cystic Carcinoma...Ancillary tests

- Immunohistochemistry of limited practical use as tumors in DD react similarly
- Ckit
- Molecular testing:
  - $MYB$-$NFIB$ fusion protein up-regulates $MYB$ protein expression
  - Rare cases do not rely on $MYB$ overexpression for tumorigenesis
Salivary Duct Carcinoma

- High grade carcinoma resembling breast ca
- 7th decade, parotid, M:F-2-4:1
- Rapid growth with facial nerve involvement
- Cytology shows features of high grade adenocarcinoma with necrosis and mitosis
- Cribiform, papillary, sheets and single cells
- Immunoreactive for epithelial markers, Androgen receptor and Her 2
Carcinoma ex Pleomorphic Adenoma

- Carcinoma arising from Pleomorphic adenoma
- Requires concurrent PA or history of PA at same site
- 80% occur in major salivary glands especially Parotid
  - 7-10% of PAs (especially long standing), 6th to 8th decade (20 yrs later than PA)
  - 6th most common salivary gland malignancy in adults
- Cellular specimen with predominantly epithelial cells
- 2 distinct patterns, benign PA with malignant or equivocally malignant cells with or without necrosis, mitosis
  - Malignant component could be adenocarcinoma, salivary duct carcinoma, ACC, MEC, PLGA, epi-myoepithelial carcinoma
Approach to Interpretation of Salivary Gland Aspiration Biopsies and Reporting Terminology (Milan System)

PATTERNS OF SALIVARY GLAND ASPRATES

Cystic Lesions
- Acinar clear fluid
- Normal gland
- Salivary duct obstruction
- Duct obstruction
- Abscess
- Mucocele
- LEC
- Warthin tumor
- Low-grade mucoepidermoid carcinoma (MEC)
- Acinar cell carcinoma (ACC) (rare)
- Cystic degeneration in any neoplasm, benign or malignant

Inflammatory Cells
- Abscess

Lymphocytic Cell Pattern
- Chronic salivary glands
- Lymphoepithelial salivary glands
- LEC
- Lymph node
- Lymphoma (mononuclear cells)
- Warthin tumor

Basaloid Cell Pattern
- Basal cell (monomorphic) adenoma or carcinoma
- Adenoid cystic carcinoma (ACC)
- Myoepithelial carcinoma
- Polymorphous low-grade adenocarcinoma
- Small cell carcinoma, primary or metastatic
- Secondary involvement by cutaneous basal cell carcinoma
- Salivary duct carcinoma

Stroma Patterns in Neoplasms

Cribiform Adenocarcinoma

TERM INOLOGY
- Known as cribriform adenocarcinoma of minor salivary gland (CSAMG) or tubular (T)
- May be subtype of polymorphous adenocarcinoma but has different clinicopathologic features

CLINICAL ISSUES
- Very rare with no gender predilection
- Arises in minor salivary gland sites, primarily base of tongue
- Usually develops slowly and remains localized for a prolonged period

CYTOPATHOLOGY
- Sheets and clusters of cells with irregular outlines; focal papillary patterns may be seen
- Cribriform pattern in sheets is characteristic
- Very few desmosomes; cells imparting clean background
- Nuclei resemble papillary carcinoma of thyroid
- Overlapping, rounded cell appearance with grooves and pseudofollicles

Irregular Branching Sheets

Monomorphic Tumor Cells

(Left) Low-power view of this Pap-stained thyroid shows several large clusters of tumor cells forming sheets of cells with irregular branching projections. Right: Higher power of the same Pap-stained smear, showing the uniformity of the nuclei.

Secretory Carcinoma

TERM INOLOGY
- Also known as mammary analogue secretory carcinoma
- Previously considered “zymogen-poor acinar cell carcinoma”

CLINICAL ISSUES
- Usually slow-growing, painless mass
- Uncommon high-grade variants may present with faster growth and nerve injury
- Most (70%) are in parotid
- Can also arise in other major and minor salivary glands and in thyroid

CYTOPATHOLOGY
- Diverse architecture
- Papillary configurations with transgressing vessels
- Acinar-like cell clusters
- Sheets of cells
- Cystic tumors may have abundant secretory material
- “Histiocytoid” cells are characteristic

Top Differential Diagnoses
- Acinic cell carcinoma
- Low-grade salivary duct carcinoma

KEY FACTS
- Abundant cytoplasm with vacuoles, mostly small but may be signet ring-like
- Nuclei are typically uniform and bland

MOLECULAR
- ER/PR translocations are characteristic, usually detected by FISH break-apart probes
- t(12,15)(p13;q25) ETV6-NTRK3 translocation is typical
- Specific to secretory carcinoma (in salivary gland)
- ETV6 can rarely have other translocation partners

ANCILLARY TESTS
- Mammaglobin and S100 positive; not specific to secretory carcinoma among salivary gland tumors
- Mucin vacuoles stain with mucicarmine, alcin blue, and PAS without diastase

TOP DIFFERENTIAL DIAGNOSES
- Acinic cell carcinoma
- Low-grade salivary duct carcinoma

(Left) Pap-stained smear contains numerous tumor cells with focal cohesive sheets but predominantly dispersed individual cells. Note that occasional binucleated cells are present in this example. (Right) Higher power shows that most of the cells have obvious cytoplasmic vacuoles visible by Pan. Most cells have single large vacuole but occasional cells have a single large vacuole creating a signet ring-like cell configuration with nuclear displacement. (Courtesy S. Ali, MD.)

Histiocytoid Appearance of Tumor Cells

(Left) Diff-Quik-stained smear demonstrates a fairly uniform population of cells with round nuclei, having smooth contours. Abundant secretory.
Other Primary and Metastatic Malignancies in Salivary Gland

- Squamous cell carcinoma, primary or mets/direct extension
- Lymphomas primary or secondary
- Melanomas usually mets or direct extension
- Small cell carcinoma/Merkel cell ca
- Angiosarcomas, usually direct extension from cutaneous
- Other metastasis
- Parotid most frequent recipient of mets
Milan System Reporting

- Statement on adequacy
- Brief description of cytological features
- Specific diagnosis as to nature of process (neoplastic or non-neoplastic)
- If above not possible, then reason for categorization
- Do not use category numbers without the category name
- Optional to report ROM, depends on laboratory
Molecular Testing specific to Salivary Gland Tumors

- **Pleomorphic Adenoma & ca ex PA**
  - Most show overexpression of PLAG1 protein
  - Some have *HMGA2* gene amplification or rearrangement
  - Some show neither PLAG1 protein overexpression nor *HMGA2* gene

- **Mucoepidermoid Carcinoma**
  - *CRTC1-MAML2* or *CRTC3-MAML2* fusion proteins disrupt Notch signaling pathway
  - By FISH or NGS

- **Adenoid Cystic Carcinoma**
  - *MYB-NFIB* fusion protein up regulates MYB protein expression
  - Rare cases do not rely on MYB overexpression for tumorigenesis

- **(Mammary Analog) Secretory Carcinoma**
  - *ETV6-NTRK3* gene fusion
    - *ETV6*: Transcriptional regulator
    - *NTRK3*: Membrane receptor kinase

- **Hyalinizing Clear Cell Carcinoma**
  - *EWSR1-ATF1* gene fusion
Additional Molecular Testing for Salivary Gland Tumors

- Basal cell adenoma
  - CTNNB1 mutations
- Cribriform adenocarcinoma
  - PRKD rearrangement
## Immunocytochemistry for Salivary Gland Neoplasms

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Positive Markers</th>
<th>Negative Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphic Adenoma</td>
<td>(Epith) CK7, CEA, EMA, SOX10</td>
<td>MYB</td>
</tr>
<tr>
<td></td>
<td>(Myoep) SMA, S-100, Calponin, CK5/6, P63, GFAP, PLAG1, HMGA2</td>
<td></td>
</tr>
<tr>
<td>Adenoid Cystic carcinoma</td>
<td>(EPITH) CK7, CEA, EMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Myoep) SMA, calponin, S-100, CK5/6, P63, SOX10 CD117(&gt;90%+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MYB+, MYB translocation by FISH is specific for ACC</td>
<td></td>
</tr>
<tr>
<td>Basal cell adenoma/ca</td>
<td>CK7, CEA, EMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ for myoep markers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta catenin overexpression, LEF-1+</td>
<td></td>
</tr>
<tr>
<td>Acinic cell carcinoma</td>
<td>DOG1 strong diffuse staining</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SOX10, strong diffuse nuclear staining in most ACC, PAS-D</td>
<td></td>
</tr>
<tr>
<td>Oncocytoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DOG1, SOX10, PAS-D</td>
<td></td>
</tr>
</tbody>
</table>
# IHC and Molecular profiles of Salivary Gland Neoplasms (that we know of now...)

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Genetic Alteration</th>
<th>Genes</th>
<th>FISH probe</th>
<th>IHC markers +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphic adenoma (&amp; ca ex)</td>
<td>Translocation 8q12</td>
<td>PLAG1</td>
<td>PLAG1</td>
<td>PLAG1 HMGA2</td>
</tr>
<tr>
<td></td>
<td>Translocation 12q13-15</td>
<td>HMGA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cell adenoma</td>
<td>3p22.1 mutation</td>
<td>CTNNB1, CYLD</td>
<td></td>
<td>Beta catenin, LEF-1</td>
</tr>
<tr>
<td>Adenoid Cystic carcinoma</td>
<td>T(6;9)(q21-23;p23-24)</td>
<td>MYB-NFIB</td>
<td>MYB</td>
<td>MYB (82% test +)*</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>T((11;19)(q21;p13)</td>
<td>CRTC1-MAML2</td>
<td>MAML2</td>
<td>P63/p40</td>
</tr>
<tr>
<td></td>
<td>T(11;15)(q21;q26)</td>
<td>CRTC3-MAML2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretory carcinoma (MASC)</td>
<td>T(12;15)(p13;q25)</td>
<td>ETV6-NTRK3</td>
<td>ETV6</td>
<td>S-100, Mammoglobin</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>T(12;22)(q13;q12)</td>
<td>EWSR1-ATF1</td>
<td>EWSR1</td>
<td></td>
</tr>
</tbody>
</table>

*From presentation by Dr Krane at American society of cytopathology meeting, Phoenix AZ, Nov 2017
*Basiloid squamous cells may test positive*
<table>
<thead>
<tr>
<th>Tumor</th>
<th>Immunohistochemistry (useful positive markers)</th>
<th>Molecular, Genes Involved (Prevalence in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinic cell carcinoma (ACC)</td>
<td>DOG1, SOX10</td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic carcinoma (AdCC)</td>
<td>MYB, DOG-1, CD117, Sox10, S100, calponin, CK5/6, p63, p40, CK7</td>
<td>MYB–NRB translocation (25-64)</td>
</tr>
<tr>
<td>Basal cell adenoma/carcinoma</td>
<td>β Catenin overexpression, CK7, myoep markers, GATA-3+, PLAG-1+</td>
<td>C7NNB1 mutations (60-70), CYLD loss (75-80)</td>
</tr>
<tr>
<td>Hyalinizing Clear cell carcinoma</td>
<td>Pan CK, Low and HMW keratins, p63+</td>
<td>EWSR1-ATF gene fusion (35)</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>P63, p40, GATA-3+, CD117+</td>
<td>CRTC1-MAML2 fusion (40-80) or CTRC3-MAML2 fusion (~5)</td>
</tr>
<tr>
<td>Pleomorphic Adenoma</td>
<td>PLAG1, SMA, calponin, p63, p40, SOX10, GFAP, CK7, S100, GATA-3+</td>
<td>PLAG1, CTNNB1, LIFR (50-60), HMG A2 amplification or fusion</td>
</tr>
<tr>
<td>Salivary Duct carcinoma</td>
<td>GATA-3, Androgen receptor, Her-2+</td>
<td>ERBB2 ampl (~40), PI3KCA mutation (~20)</td>
</tr>
<tr>
<td>(Mammary Analogue) Secretory carcinoma</td>
<td>GATA-3, S100, keratins, Mammoglobin</td>
<td>ETV6-NTRK3 gene fusion (90-100)</td>
</tr>
</tbody>
</table>

*variable positivity, ampl=amplification
## SOX10 in Salivary Gland neoplasms

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinic cell carcinoma</td>
<td>Salivary duct carcinoma</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>Mucoepidermoid carcinoma</td>
</tr>
<tr>
<td>Epithelial-Myoepithelial carcinoma</td>
<td>Warthin’s</td>
</tr>
<tr>
<td>Myoepithelial carcinoma</td>
<td>Oncocytoma</td>
</tr>
<tr>
<td>Pleomorphic Adenoma</td>
<td></td>
</tr>
</tbody>
</table>