THE MILAN SYSTEM AND MOLECULAR ADVANCES IN THE CYTOLOGIC DIAGNOSIS OF SALIVARY GLAND TUMORS

William C. Faquin, MD, PhD
Director, Head & Neck Pathology
Professor of Pathology
Massachusetts Eye & Ear
Massachusetts General Hospital
Harvard Medical School
Even if you do not adopt the Milan System in your practice, reviewing the structure of a reporting system will provide insight to salivary gland FNA!
Salivary gland tumors are one of the most heterogeneous groups of neoplasms – So what role is there for FNA?
Salivary Gland FNA - Pleomorphic Adenoma
Salivary Gland FNA – Pleomorphic Adenoma
Salivary Gland FNA - Adenoid Cystic Carcinoma
Salivary Gland FNA - Solid Adenoid Cystic Carcinoma
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**SALIVARY GLAND FNA:**

**How Far Can We Go?**

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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; 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.
SALIVARY GLAND FNA

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: Heme-Onc referral
- High-grade primary carcinoma: Radical resection
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

- 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
Sponsored by the ASC and the IAC
Over 40 participants from 14 countries
Goal is to produce a practical classification system that is user-friendly and internationally accepted.
The system will be evidence-based.
Print Atlas available in 2017
Web-Based Atlas will also be available through the ASC
Participants:
40+ Members from 14 Countries
Cytopathologists, Surgical Pathologists, Molecular Pathologists, ENT Surgeons

1. Overview of Diagnostic Terminology and Reporting:
   - Core group, Bruce Wenig, Raja Seethala, Andrew Field, Nora Katabi

2. Non-diagnostic:
   - Mariapia Foschini (lead), Laszlo Vass, Esther Diana Rossi, Jhala Nirag, Philippe Vielh, Kayoko Higuchi, Ivana Kholova, Makato Urano

3. Non-neoplastic:
   - Bill Faquin (lead), Massimo Bongiovanni, Sule Canberk, Marc Pusztaszeri, Tarik Elsheik, Dan Kurtycz, Fabiano Callegari, Oscar Lin

4. AUS:
   - Marc Pusztaszeri (lead), Zubair Baloch, Bill Faquin, Diana Rossi

5. Neoplastic (benign & SUMP):
   - Zubair Baloch (lead), Jeff Krane, Lester Layfield, Marc Pusztaszeri, Jerzey Klijanienko, Ritu Nayar, Celeste Powers, Pinar Firat, Guido Fadda

6. Suspicious for malignancy:
   - Diana Rossi (lead), Syed Ali, Ashish Chandra, Zarha Maleki, Bo Ping, He Wang, Andrew Field, Yun Gong

7. Malignant:
   - Güliz Barkan (lead), He Wang, Philippe Vielh, Stefan E. Pambuccian, Swati Mehrotra, Mousa Al-Abbadi, Eva Wojcik

8. Ancillary Studies:
   - Mark Pusztaszeri (lead), Jorge Reis-Filho, Fernando Schmitt, Raja Seethala

9. Clinical Management:
   - Mark Varvares (lead – MGH), Piero Nicolai (Italy), Mandeep Bajwa
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 ROM will depend upon the nature of the Specimen and the salivary gland site.

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<th>ROM*</th>
<th>Management</th>
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<td>Non-Diagnostic</td>
<td>10-20%</td>
<td>Clinical and radiologic correlation/ repeat FNA</td>
</tr>
<tr>
<td>Non-Neoplastic</td>
<td>TBD (0-20%)</td>
<td>Clinical follow-up and radiologic correlation</td>
</tr>
<tr>
<td>Atypia of Undetermined</td>
<td>TBD</td>
<td>Repeat FNA or surgery</td>
</tr>
<tr>
<td>ii. Uncertain Malignant Potential (SUMP)</td>
<td>20-40%</td>
<td>Conservative surgery</td>
</tr>
<tr>
<td>Suspicious for Malignancy (Low grade vs High grade)</td>
<td>70-80%</td>
<td>Surgery: Correlate LG vs HG</td>
</tr>
<tr>
<td>Malignant (Low grade vs High grade)</td>
<td>85-95%</td>
<td>Surgery: Correlate LG vs HG</td>
</tr>
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*Based on literature review, criteria have not been validated; SUMP-Salivary gland neoplasm of uncertain malignant potential; TBD: Needs further literature review and data.
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
Non-Diagnostic:
Benign salivary gland elements only
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

Absence of an epithelial component
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.
Non-Neoplastic:
Reactive Lymph Node

Mixed population of lymphs, Tingible body macrophages, germ center frags
IMMUNOPHENOTYPING combined with cytomorphology is the key to diagnosing and subtyping reactive conditions vs lymphoma.
For negative lymph nodes, caution is warranted:
A note suggesting repeat FNA or tissue biopsy if lymphadenopathy persists.
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 (eg, air-drying, blood clot).
- Should be used rarely (<10 % of all salivary gland FNAs).
Atypia of Undetermined Significance (AUS):
Mucinous Cyst Contents Only - Cannot exclude MEC
Atypia of Undetermined Significance (AUS):

Oncocytic metaplasia vs Neoplasm
Atypia of Undetermined Significance (AUS): *Reactive vs basaloid neoplasm*

Sclerosing polycystic adenosis
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.
Neoplastic: Benign
Warthin Tumor

Oncocytes, chronic inflammation, and cystic debris
Neoplastic: Benign
Pleomorphic Adenoma

Matrix-rich types of PA are the easiest.
Neoplastic: SUMP
Pleomorphic Adenoma
*With Atypia or Metaplasia*

**Squamous metaplasia**

**Focal atypia**
Neoplastic: SUMP
Myoepithelioma
Neoplastic: SUMP
Basal Cell Neoplasm-
DDX basal cell adenoma, cellular PA, AdCC
• 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
Malignant

- Aspirates which are diagnostic of malignancy.
- Sub-classify into specific types and grades of carcinoma: e.g. low grade vs high grade.
- "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
Malignant:
Adenoid Cystic Carcinoma
Malignant: Mucoepidermoid Carcinoma
Malignant: HG B-Cell Lymphoma
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.
Ancillary Studies to Improve the FNA Diagnosis of Head and Neck Tumors

- Immunocytochemistry
  - LBP
  - Smears
  - Cell block
- FISH
- RT-PCR
- Next Generation Sequencing
Anchored Multiplex PCR (AMP)

- ~190 target amplicons across 39 genes and 50+ rearrangements

High-quality sequence:
- Staggered start sites
- >100X target coverage
- Molecular indexing
- Bi-template coverage
- ~2% analytical sensitivity

Fast turn-around (~2 weeks)

Cost-effective (<$500)

Small tissue amounts (5-10 ng)
NORMAL SALIVARY GLAND
Immunohistochemical Markers

- SOX10
- Ker 7, 19, CAM 5.2, EMA
- Ker 5/6, p63
- Ker 5/6, S-100, p63, calponin, SM actin
- Ker 7, CAM 5.2, DOG1
# Increasing Availability of Molecular Markers For Salivary Gland Tumors

- **Mammary analogue secretory carcinoma:**
  - ETV6-NTRK3; t(12:15)

- **Pleomorphic adenoma & Ca ex PA:**
  - PLAG1; t(3;8)
  - HMGA2 rearrangement

- **Clear cell carcinoma:**
  - EWSR1-ATF1; t(12:22)

- **Mucoepidermoid carcinoma:**
  - MECT1/MAML2; t(11:19)

- **Cribriform Adenocarcinoma:**
  - PRKD rearrangement

- **Adenoid cystic carcinoma:**
  - MYB-NFIB; t(6:9)

- **Basal cell adenoma:**
  - CTNNB1 mutations
IHC and Molecular Analysis Applied to Selected Salivary Gland Tumors
Pleomorphic Adenoma
Diagnostic Problems Arise from Variants of PA

- Cellular PA with sparse matrix
- Focal adenoid cystic–like areas
- Cytologic atypia
- Metaplasia
  - Squamous
  - Mucinous
  - Sebaceous
  - Oncocytic
Pleomorphic Adenoma with Mucinous Metaplasia:
Can be difficult to distinguish from MEC
Pleomorphic Adenoma

**Immunohistochemistry:**

- **Markers of both ductal and myoepithelial cells:**
  - Keratin 7, CEA, EMA, SOX10
- **Myoepithelial markers:**
  - Smooth muscle actin
  - Calponin
  - S-100
  - Keratin 5/6
  - P63
  - GFAP
Pleomorphic Adenoma

Cytogenetics:

- **PLAG1 rearrangements (50-60%)**
  - Present in PAs in different tissues
  - Nuclear localization
  - Functions to activate transcription (Zn finger)

- **HMGA2 rearrangements (10%)**

http://www.uniprot.org/uniprot/Q6DJT9
PLAG-1 Immunoreactivity:
Overexpressed in 94% of PA

Does not distinguish benign from malignant

Contributed by Dr. J. Krane, BWH
Carcinoma Ex Pleomorphic Adenoma:
Most cases have PLAG1 or HMGA2 Rearrangements

Salivary duct carcinoma ex PA
Adenoid Cystic Carcinoma
Adenoid Cystic Carcinoma: Classic Cribriform Pattern
Adenoid Cystic Carcinoma

**Immunohistochemistry:**
- Positive for keratin 7, CEA, EMA
- Positive for myoepithelial markers:
  - Smooth muscle actin
  - Calponin
  - S-100
  - Keratin 5/6
  - P63
- SOX10+
- CD117 (KIT) +
- MYB +
Adenoid Cystic Carcinoma

**CD117 Immunohistochemistry:**

- Over 90% are strongly positive for CD117 (KIT)
- Protein overexpression but no mutation identified
- Useful for all variants including solid forms
- Unusual IHC pattern
Immunoreactivity for CD117 (KIT) in AdCC:

Tubular Type

Luminal Cells +

Solid Type
MYB immunostaining is a useful ancillary test for distinguishing adenoid cystic carcinoma from pleomorphic adenoma in FNA specimens
Adenoid Cystic Carcinoma:
Recent Advance – MYB Translocation

Cytogenetics:

• t(6:9) MYB oncogene-NFIB transcription factor
• In salivary gland, this finding by FISH is specific for AdCC

FISH contributed by Dr. Joaquin García, Mayo Clinic
Basal Cell Adenoma/Adenocarcinoma
Basal Cell Adenocarcinoma – Cytologically indistinguishable from adenoma
Basal Cell Adenoma & Adenocarcinoma

**Immunohistochemistry:**
- Positive for keratin 7, CEA, EMA
- Positive for myoepithelial markers
- **Nuclear beta-catenin +** (esp. common in adenoma)
Basal Cell Adenoma & Adenocarcinoma

- **CTNNB1 mutation** – 3p21
- **Beta-Catenin overexpression**
  - Present at cell junctions
  - Part of WNT signaling pathway
Nuclear Beta-Catenin in Basal Cell Adenoma
Mammary Analogue Secretory Carcinoma
MASC

Immunohistochemistry:

• Positive for:
  - S-100
  - Mammaglobin
  - Keratin 7, 8, 19
  - EMA
  - GCDFP-15
  - MUC1, MUC4

• Negative for:
  - Myoepithelial markers (p63 etc)
  - Androgen receptor
  - DOG1 +/-
MASC: Immunohistochemical Studies

- GCDFP-15+
- S-100+
- Mammoglobin+
Mammary Analogue Secretory Carcinoma:

Cytogenetics: For difficult cases, test for rearrangement

- ETV6-NTRK3 rearrangement:
  - T(12:15)(p13;q25)
  - MASC is only known salivary gland primary
- Detected on histology or cytology using:
  - FISH
  - RT-PCR
  - Next-Gen Sequencing

FISH Contributed by Dr. Joaquin Garcia, Mayo Clinic
Acinic Cell Carcinoma
Acinic Cell Carcinoma
DOG1 and Acinic Cell Carcinoma

- Discovered on GIST
- Calcium-activated chloride channel protein
- Strong diffuse staining seen in most acinic cell carcinomas
SOX-10 and Acinic Cell Carcinoma

- SRY-related HMG-box 10 (SOX10) protein
- Transcription factor: neural crest formation, and maintenance of Schwann cells and melanocytes.
- Acinar cells and intercalated ducts
- Diffuse strong nuclear staining seen in most acinic cell carcinomas
## SOX10 and Salivary Gland Tumors

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MUCOEPIDERMOID CARCINOMA
Low Grade Mucoepidermoid Carcinoma:
Hypocellular with few groups of bland epidermoid cells;
Common cause of FN FNA
Mucoepidermoid Carcinoma: IHC is non-specific

**Immunohistochemistry:**

- **Positive for:**
  - Keratin 5,6,7,8,19
  - EMA
  - CEA
  - p63

- **Negative for:**
  - SM actin
  - Calponin
  - S-100
  - SOX10
Mucoepidermoid Carcinoma: Can be useful for difficult cases

Cytogenetics:
- t(11:19) translocation
- MECT1/MAML2
- FISH or NGS
- More common in low grade
  - 60% overall
  - LG 74%, IG 63%, HG 32%
  - Useful in small biopsies

FISH contributed by Dr. Joaquin Garcia, Mayo Clinic
SUMMARY

• Salivary gland cytology presents many diagnostic challenges

• The Milan System for Reporting Salivary Gland Cytopathology will help to produce a more uniform diagnostic structure
  – Improved communication between treating clinician and pathologist
  – Improved patient care

• Availability of IHC and molecular markers can greatly improve the accuracy of salivary gland FNA!
The Milan System for Reporting Salivary Gland Cytopathology

We look forward to implementing the Milan system – Thank You!