Salivary Gland Cytology: A Clinical Approach to Diagnosis and Management of Atypical and Suspicious Lesions

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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
Proposed Classification Scheme

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 Benefits of a Uniform Reporting System for Salivary Gland Cytopathology

- Improve communication between pathologists and clinicians
- Improve patient care
- Facilitate cytologic-histologic correlation
- Facilitate research into the epidemiology, molecular biology, pathology, and diagnosis of salivary gland diseases
- Facilitate sharing of data from different laboratories for collaborative studies
The Milan System for Reporting Salivary Gland Cytopathology

- Sponsored by the ASC and the IAC
- The goal is to produce a practical classification system that will be user-friendly and internationally accepted.
- The system will be evidence-based, and will provide a useful & uniform format for clinicians who treat salivary gland disease.
The Milan System for Reporting Salivary Gland Cytopathology

Core Group and over 40 Participants from 14 Countries

- Co-Chairs: Bill Faquin, MD, PhD & Diana Rossi, MD
- Zubair Baloch, MD, PhD
- Guliz Barkan, MD
- Maria Pia Foschini, MD
- Daniel Kurtycz, MD
- Marc Pusztaszeri, MD
- Philippe Vielh, MD
## The Milan System for Reporting Salivary Gland Cytopathology

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>ROM*</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-Diagnostic</strong></td>
<td>10-20%</td>
<td>Clinical and radiologic correlation/ repeat FNA</td>
</tr>
<tr>
<td><strong>Non-Neoplastic</strong></td>
<td>TBD (0-20%)</td>
<td>Clinical follow-up and radiologic correlation</td>
</tr>
<tr>
<td><strong>Atypia of Undetermined Significance (AUS)</strong></td>
<td>TBD</td>
<td>Repeat FNA or surgery</td>
</tr>
<tr>
<td><strong>Neoplasm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Benign</td>
<td>5-7%</td>
<td>Conservative surgery or clinical follow-up</td>
</tr>
<tr>
<td>ii. Uncertain Malignant Potential (SUMP)</td>
<td>20-40%</td>
<td>Conservative surgery</td>
</tr>
<tr>
<td><strong>Suspicious for Malignancy (Low grade vs High grade)</strong></td>
<td>70-80%</td>
<td>Surgery: Correlate LG vs HG</td>
</tr>
<tr>
<td><strong>Malignant (Low grade vs High grade)</strong></td>
<td>85-95%</td>
<td>Surgery: Correlate LG vs HG</td>
</tr>
</tbody>
</table>

*Based on literature review, criteria have not been validated; SUMP-Salivary gland neoplasm of uncertain malignant potential; TBD: Needs further literature review and data.
Insufficient quantitative and/or qualitative cellular material to make a cytologic diagnosis.

At present no set validated adequacy criteria has been established in the literature.

This diagnostic category should only be used when the entire material is processed and examined.
Non-Neoplastic

- Specimens lacking cytomorphologic evidence of a neoplastic process; consisting of benign acinar and/or ductal epithelium with or without inflammatory component, metaplastic and reactive changes (includes benign entities such as: acute and chronic and granulomatous sialadenitis, sialolithiasis, sialadenosis, etc…)
- Specimens showing evidence of reactive lymphoid tissue (flow cytometry is recommended based on clinical and morphologic suspicion).
- The ROM for this category is expected to be low (0-20%) if strict criteria are used for inclusion
- Clinico-radiological correlation is essential to ensure that the specimen is representative of the lesion.
Atypia of Undetermined Significance

- Cytologic features of greater dysmorphology than those assigned to the Non Neoplastic category but falling qualitative or quantitative short of those assigned to the Suspicious for Malignancy category, and an insufficient number of features for diagnosis of a Neoplasm (Neoplastic category).
- AUS favors a benign-non neoplastic process, but cannot entirely exclude a neoplasm (benign or malignant) after examination of all the cellular material. These samples are therefore indeterminate for a neoplasm.
- A majority of these samples will represent reactive atypia or poorly sampled neoplasms.
- A contributing factor to the uncertainty is often (but not always) specimens that are compromised due to scant cellularity and/or preparation artifacts (eg, air-drying, blood clot).
- The AUS diagnostic category is needed to accommodate certain FNA samples, but should be used rarely (<10 % of all salivary gland FNAs).
**i) Benign Neoplasm:**
Reserved for clear-cut benign neoplasms diagnosed based on established cytologic criteria
This category will include classic cases of PA, WT, lipoma, etc…

**ii) Salivary Gland Neoplasm of Uncertain Malignant Potential:**
Reserved for FNA specimens which are diagnostic of a neoplasm; however, a diagnosis of a specific entity cannot be made. This diagnosis should be used for cases where a malignant neoplasm cannot be excluded. A majority of these cases will include cellular benign neoplasms, neoplasms with atypical features, and low grade carcinomas (eg, basaloid tumors, oncocytic tumors, atypical PAs, etc…).
• This category is for aspirates of neoplasms which show features that are highly suggestive of carcinoma but are not unequivocal for carcinoma (ROM: 70-80%).

• An attempt should be made to sub-categorize these FNA specimens as suspicious for low grade vs high grade carcinoma.

• A majority (but not all) of specimens in this category will be high grade carcinomas.
• This category is for aspirates which are diagnostic of malignancy.

• An attempt should be made to sub-classify these aspirates into specific types and grades of carcinoma: eg, low grade (low grade muco-epidermoid carcinoma) vs high grade (salivary duct carcinoma).

• Other malignancies such as lymphomas, sarcomas and metastases are also included in this category and should be specifically designated.
SALIVARY workshop

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LIQUID-BASED CYTOLOGY (LBC)

Collect all the aspirated material in the same vial

Rinse the needle and the syringe in a hemolytic and preservative solution (skip the smearing step)

Obtain a slide made up of an uniform layer of the cells present in the vial
RINSE IN THE HEMOLYTIC SOLUTION

THE NEEDLE IS LEFT IN THE SOLUTION

THE THIN PREP 2000™ DEVICE

COMPARISON BETWEEN LBC AND CONV.
All the material is processed and the procedure is standardized

The cells are immediately preserved and the erythrocytes with the fibrin are lysated

The cells are layered on a single slide

Store a variable amount of cells for being used for further investigations (up to six months after the FNAB)
LIQUID-BASED CYTOLOGY (LBC): LIMITS

It is not cost-effective in the short term

The technical work is higher than conventional

Some morphological features are different in conventional smears compared to LBC

The on-site assessment of the material adequacy is not possible
Conventional FNAB and LBC

1) Background (less tissue fragments in LBC)

2) Stromal material: denser, in fragments and small droplets in LBC

3) Cells: slightly smaller and uniform cytoplasms, better nuclear details in LBC
WHAT’S ABOUT LBC?

The Utility of Liquid-Based Cytology in Salivary Gland Fine-Needle Aspirates: Experience of an Academic Institution

Table 2. Tips for circumventing artifacts in TP-processed aspirates

<table>
<thead>
<tr>
<th>Problem</th>
<th>Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distinguishing true inflammation from peripheral blood elements</td>
<td>Peripheral blood elements are of mixed cell type containing both neutrophils and lymphocytes</td>
</tr>
<tr>
<td>Reduced lymphocytes</td>
<td>Look for lymphocytes entrapped within the clusters or the fibrin clots</td>
</tr>
<tr>
<td></td>
<td>Look at the periphery of the preparation where they tend to concentrate</td>
</tr>
<tr>
<td>Reduced tumor diathesis</td>
<td>Look for nuclear debris and old blood entrapped within fibrin clots</td>
</tr>
<tr>
<td>Markedly decreased mucin</td>
<td>Look for intracellular mucin</td>
</tr>
<tr>
<td></td>
<td>Look for mucin entrapped within cell clusters or fibrin clots</td>
</tr>
<tr>
<td>Altered matrix</td>
<td>Better identified within larger groups</td>
</tr>
<tr>
<td></td>
<td>Frequently loosely detached in the background</td>
</tr>
<tr>
<td>Variable cellularity across the preparation</td>
<td>Scan the slide at low magnification since the center tends to have less clusters and cells than the periphery</td>
</tr>
<tr>
<td>Ring effect</td>
<td>This results from blocked filter holes</td>
</tr>
<tr>
<td></td>
<td>Specimen may need reprocessing to eliminate obscuring blood and repeat when relevant</td>
</tr>
<tr>
<td>Cells appear flat or not well preserved</td>
<td>Avoid evaluating cells that are at the periphery, which are usually subjected to pressure by the ring</td>
</tr>
</tbody>
</table>

Jason M. Ranick  Jay Wasman  Claire W. Michael
<table>
<thead>
<tr>
<th>Technique</th>
<th>Resulting artifacts</th>
</tr>
</thead>
</table>
| CytoLyt and PreservCyt® fixation (methanol-based with mucolytic and hemolytic agents) | - Only fixed preparations with Pap stain  
- No air-dried preparations available  
- Nucleoli are more conspicuous  
- Cells appear smaller  
- Cytoplasm has denser quality  
- Less bare nuclei  
- Hemolyzed blood leaving behind few mixed inflammatory cells  
- Alter mucus and other extracellular matrix  
- Clear background |
| Cell dispersion (gentle agitation)             | - Breakdown of large fragments into smaller and less complex groups  
- Increase number of single cells in background (pseudo-dyscohesion) |
| Cell collection (filtration)                  | - Loss of small cells such as lymphocytes and myoepithelial cells  
- Loss of tumor diathesis  
- Large fragments may not get through the filter  
- Filter may be blocked by excessive blood or inflammation, resulting in ring effect  
- Controlled cell density with minimal over lapping |
| Cell transfer (positive air pressure)          | - Cells and clusters are artificially flattened  
- More flattening at the periphery (ring effect)  
- Cells are denser at the periphery than at the center of the preparation  
- Small cells are identified mostly at the periphery of the preparation |
LBC of salivary glands

Red blood cells, lymphocytes, inflammatory cells may be filtered and minimally represented.

Larger clusters of cells broken into smaller or single.

Reduced complexity of cellular groups.

Cells may appear relatively smaller and more spherical.

Nucleoli are more conspicuous.

Chromatin detail is not always crisp.

Extracellular matrix is altered in quality and quantity.

Rarick JM et al, Acta Cytol 2014; 58: 552-62
Comparison of ThinPrep and Conventional Smears in Salivary Gland Fine-Needle Aspiration Biopsies

Morphologic Parameters for ThinPrep and Conventional Smears in Salivary Gland FNABs

<table>
<thead>
<tr>
<th>Morphologic parameter</th>
<th>TP n = 98</th>
<th>CS n = 98</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity ≥2+</td>
<td>56 (57)</td>
<td>74 (76)</td>
<td>.010</td>
</tr>
<tr>
<td>Fragmentation ≥2+</td>
<td>48 (49)</td>
<td>22 (22)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Single cells ≥2+</td>
<td>34 (35)</td>
<td>37 (38)</td>
<td>NS</td>
</tr>
<tr>
<td>Crush artifact</td>
<td>2 (2)</td>
<td>12 (12)</td>
<td>.006</td>
</tr>
<tr>
<td>Air drying artifact</td>
<td>0 (0)</td>
<td>13 (13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Obscuring blood</td>
<td>1 (1)</td>
<td>26 (27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Better nuclear detail</td>
<td>24 (24)</td>
<td>12 (12)</td>
<td>.027</td>
</tr>
<tr>
<td>Larger cell size</td>
<td>1 (1)</td>
<td>12 (12)</td>
<td>.002</td>
</tr>
<tr>
<td>Better cytoplasmic preservation</td>
<td>10 (10)</td>
<td>12 (12)</td>
<td>NS</td>
</tr>
</tbody>
</table>

TP indicates ThinPrep; CS, conventional smear; NS, not statistically significant (P > .05).
Liquid-Based Cytology in Fine-Needle Aspiration Biopsies of the Thyroid Gland

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WHICH EXAMINATION CAN BE CARRIED OUT ON THE CELLULAR MATERIAL?

Immunocytochemistry

molecular biology