The International System for Reporting Serous Fluid Cytopathology

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Conflict of Interest

- Dr. Kurtycz is one of the Editors of the International System for Reporting Serous Fluid Cytopathology.
- There are no financial or other conflicts
Educational Objectives

- Understand the rationale behind the International System for Reporting Serous Fluid Cytology diagnostic scheme and the quest for standardization.
- Describe a systematic approach to serous fluid interpretation.
- Apply the International System for Reporting Serous Fluid Cytology diagnostic scheme to serous fluid samples.

History: Bethesda - GYN

- The Bethesda GYN System was created in response to the US Federal government’s need for corrective action in the face of poor performance by a number of laboratories.
- There was a need for a simpler consistent diagnostic terminology based upon knowledge of HPV.
- Successive interobserver reproducibility studies (BIRST1 and BIRST 2) have shown improved performance all diagnostic categories in using the system since its inception.

History: Bethesda - Thyroid

- Like the Bethesda GYN, Bethesda Thyroid began at the NIH and attempted to regularize thyroid FNA.
- One of the major contribution was the attempt to provide a literature based risk of malignancy (ROM) for a given cytologic diagnosis.
- Another was an international effort to try and develop orthodoxy in an area of FNA.
History: Paris System - Urinary

Issues:
- No uniform reporting system, unlike Cervical Cytology
- Diagnostic accuracy for LGUC is low
- Cannot rely on WHO/ISUP Classes.
- Are papillary lesions that do not invade or metastasize really cancer?
- Diagnostic accuracy for HGUC is high
- Must understand that clinicians and patients were frustrated

Result:
- Focus on what urinary cytology does well
- Diagnose High Grade Urothelial Carcinoma

History: Milan System - Salivary

Arose Because of Reporting confusion in a difficult area:
- Diversity of Diagnostic cytology categories, vs.
- Descriptive reports (no categories), vs. Surgical pathology terminology
- No general correlation with Risk of Malignancy or clinical management
- Little use of genetic studies on salivary gland cell block material

History: Milan System - Salivary

Consensus on the need for a defined set of categories for salivary gland FNA:
- Need for clarity of communication: Milan answered the repeated calls for consistency of terminology and risk of malignancy for a given diagnosis
- Better exchange of data across institutions and to clinicians
Other Systems

- The International Academy of Cytology (IAC) sponsored the Yokohama System for Breast.
- The Papanicolaou Society
  Created a reporting system for Pancreatobiliary Cytology. This work is entering into its second edition.

All the systems seek definition of terms, standardization of terminology and restriction of atypical and suspicious categories.

Beginnings of TIS

Why This System?

- Consistency in diagnosis and terminology for a significant volume of cytologic practice
- Consistency in communication to clinicians and management
- Consistency in most relevant ancillary studies for patient samples
- Defining what is a true negative
- Putting bounds on atypia
- What is needed to make the diagnosis of mesothelioma on a cytologic sample?
- What are the implications of benign epithelial cells in peritoneal fluids
- Encourage direct comparison of results for studies
Initial Survey

- The Survey was truly international with 503 participants responding to 54 questions.
- Africa: 3.3%
- Asia: 21.6%
- Australia: 3%
- Central America: 0.9%
- China: 7.7%
- Europe: 26%
- Mexico: 2.4%
- Middle East: 2.1%
- South America: 10.9%
- USA: 24.2%
- Other: 3.2%
- Years in practice: 0-99 (mean: 15.4)
- Volume: 4-10,000 samples (mean: 1,899) with a large standard deviation > 2K

Survey Results

- 90% (385/429) add a general category (negative, atypical, suspicious or positive) to their reports.
- 78% (371/477) generate reports with a general category (negative, atypical, suspicious, malignant) rather than a surgical pathology type diagnosis.
- 32% (150/475) report adequacy on all samples, 33% on some samples and 36% do not report on adequacy at all.
- Those that only report adequacy on some specimens do so when samples are too degenerate, poorly stained or poorly cellular for interpretation.
Survey Results

- 76% (362/477) do not require a specific number of cells for adequacy and 59% (271/463) do not believe it is reasonable to do so.
- 61% (281/463) would make a diagnosis of mesothelioma if the cytology and immunocytochemistry are supportive. 97% (388/402) want to see a section on mesothelioma in the forthcoming Serous Fluid Monograph.
- 74% (295/401) believe that both Papanicolaou stains and modified Giemsa stains (e.g., Diff-Quik) should be standard.

Survey Results

- 78% prefer “suspicious for malignancy” over other terms
- 58% use both “suggestive of or suspicious for” and “consistent with” terminology
- 65% use definitive terminology, e.g., “Metastatic ovarian carcinoma”, but 86% use “consistent with” when additional information or studies are not available
- 83% agree with AUS as an atypical term
- 96% agree that malignant cells should be qualified as specifically as possible with ancillary studies

Survey Results: Ancillary

- 76% (374/532) regularly perform ancillary studies on Serous fluid samples.
- For ancillary tests 53% (345/649) use cell blocks, 15% (98/649) use Cytospin®, 13% use direct smears, 12% use ThinPrep®
- 73% (279/378) Perform ancillary testing on atypical and suspicious samples.
- 32% (158/501) indicate that they can perform genetic studies on serous fluid samples. 48% (115/234) could do FISH and 41% (96/240) Next Generation Sequencing (NGS). Approximately 56% (138/246) perform such testing in house.
- 76% (383/739) of respondents would accept a two-stage report while awaiting confirmatory studies. 64% (242/376) indicate that they currently practice with a two-stage report.
Proposed Diagnostic Categories

- Non-diagnostic (ND)
- Negative for malignancy (NFM)
- Atypia of undetermined significance (AUS)
- Suspicious for malignancy (SFM)
- Malignant (P) – primary (mesothelioma)
- Malignant (M) – secondary (indicate cell type and possible site of origin)

Risk of Malignancy

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>% Risk (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic (ND)</td>
<td>17% (± 8.9%)</td>
</tr>
<tr>
<td>Negative for Malignancy (NFM)</td>
<td>21% (± 0.3%)</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance (AUS)</td>
<td>66% (± 10.6%)</td>
</tr>
<tr>
<td>Suspicious for Malignancy (SFM)</td>
<td>82% (± 4.8%)</td>
</tr>
<tr>
<td>Malignant (MAL)</td>
<td>99% (± 0.1%)</td>
</tr>
</tbody>
</table>


A meta-analysis of 34,941 samples

Risk of Malignancy = SE + Standard Error
Monograph Structure

Monograph Production

Non-Diagnostic (ND)

Considerations:
- Is the sample non-representative (i.e., blood)?
- Is Volume Important?
- Is Cellular Content Important?
- Is Cellular Preservation Important?

Decisions:
- Clinicians give you what they have, volumes and cellular components vary. No data on valid number of mesothelial cells.
- Any abnormal cell makes a sample diagnostic.
- Only if the sample is totally blood or totally degenerate do we consider it non-diagnostic.
- Report what is given.
Adequacy

- In most diagnostic of our diagnostic systems authors have chosen a reasonable but admittedly arbitrary starting point and then asked the literature to validate or invalidate that point. (Eg, thyroid, urine, Salivary gland)
- A probabilistic approach to all samples that are suboptimal...
- A well-mixed 75 ml or greater sample is likely to have the correct information, unless it is a bloody tap
- A 10-30 ml sample may have the correct information, but is somewhat less likely to contain the answer than the sample with greater volume and the greater the chance for a false negative (impaired sensitivity).

Adequacy

- As in the hypothetical graph to the right... The greater the volume the more likely one is to have a representative sample of the disease process, and more likely to be able to make the right call
- When the volumes are low you have a somewhat decreased chance of seeing the cause of the effusion.
- With low volumes, I put in a statement “due to small sample size the identifying features of a disease process may not have been present in the sample”.

Adequacy

- I never tell my clinicians that the sample is insufficient. It just causes distress, stimulates unnecessary phone calls, and if the patient reads the chart... leads to lots of unnecessary questions.
- For small samples I do indicate that the sample size is small, or that the material is scant.
- It helps keep the peace.
Adequacy

- We do know that every with additional sample and additional amount of sample, findings come closer to reflecting the true biology of a patient (Sister, LAST Project).
- The more you get the more you know... but past a certain point the incremental increase in knowledge is small. For us in serous fluids, it may be around 75 ml.
- As another example, in lung FNAs, you do not get much more information after 4 passes. (Xia)

Non-Diagnostic

a. An abscess is diagnostic. It gives information about a condition and the sample is suitable for culture
b. A hemolyzed sample or degenerate sample does not provide much information.
c. Degenerate Geimsa
d. Papanicolaou prep with air drying

Negative for Malignancy (NFM)

Negative:
- There are clearly no neoplastic elements
- Only expected cell elements in variable numbers these may include: mesothelial cells, macrophages, and inflammatory cells.
Negative for Malignancy (NFM)

This chapter has many examples of normal and abnormal cell types, especially describing forms of mesothelial cells.

Microvilli on Mesotheial cells

Normal microvilli keep mesothelial cells apart, but cells are connected to one another by desmosomes.

There may be infectious, immunologic or other non-neoplastic elements. Those are not deemed atypical. Atypia is should be reserved for concerns about malignancy.
Immunocytochemical studies, clinical and radiographic correlations required.

**Effusions**

**Adequate**

Expected cellular findings (mesothelial cells, some inflammatory cells)

**Inadequate**

Unexpected cellular and non-cellular findings

In regard to volume and distribution:

**Dx. Negative for malignancy (NFM)**

- Mostly mesothelial cells arranged singly and/or in small clusters. No cellular atypia. Some histiocytes, lymphocytes, neutrophils

**Increased volume and/or cell distribution:**

- Predominantly mesothelial cells (single and/or numerous clusters)
  - **Dx. NFM**
  - **Dx. Mesothelioma**

- Predominantly histiocytes
  - **Dx. NFM**

- Predominantly lymphocytes
  - **Dx. NFM**
  - **Dx. Lymphoma**

- Predominantly or increased eosinophils

- Predominantly neutrophils

**Second (malignant) cell population**

- Single cells
  - **Dx. Melanoma, lymphoma, breast (lobular) ca, sarcoma**

- Small clusters
  - **Dx. AdenoCa, breast, lung, small cell carcinoma**

- Large clusters
  - **Dx. AdenoCa, ovarian, pancreatic**

- Psammoma bodies
- Collagen balls
- Asbestos bodies
- LE cells
- Necrosis, spindle and giant cells
- Detached ciliary tufts

**Dx. NFM**

- Infectious organisms

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**Figure 1. Algorithmic Approach to Serous Effusion. NFM - Negative for malignancy – E. Wojcik**

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**Atypia of Undetermined Significance (AUS)**

- Some people do not use “Atypia” at all
- There may be times due to the quality of cellular elements that you actually need to use the term.
- It may be used as part of a two step process where the sample is deemed atypical awaiting ancillary tests including possible cytochemical, immunologic or genetic analyses (such as TTF-1, Bap1 inactivation, p16 loss, calretinin, etc.)
- Sometimes a sample cannot be resolved and it must be used as a final diagnosis, such situations should be avoided if possible, and certainly explained.

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**Atypia of Undetermined Significance (AUS)**

- Just a few cells, making a cluster and you are not quite sure
Atypia of Undetermined Significance (AUS)

- It is difficult to find any medical test that can always separate a "diseased" population from a "normal" population.
- There is always overlap in the values. This valid in clinical chemistry, hematology and pathology. In morphology, there are patterns that do not allow an observer to resolve tumor versus non-tumor.
- The performance of the test will also change depending on where the observer makes the decision point on the scale.
- Move to the right, the less tendency for false positives and the higher the diagnostic specificity, but with lower sensitivity.
- Move to the left, the higher the false positives, lowered specificity and higher sensitivity.
- TES proposes a right shift the decision, and decrease the numbers of atypical diagnoses.

The domain of atypia resides somewhere between benign reactive change and suspicious for malignancy (SFM).
- AUS should have a higher ROM than benign changes but less than SFM.
- Degeneration or preparation artifacts can impair morphology.
- Experience and good quality control can help. Laboratory practices that correlate the histology and the cytology may fine tune criteria, leading to more secure diagnoses and the avoidance of indeterminate categories.
Suspicious For Malignancy (SFM)

- There are few studies that concentrate on SFM. The Farahani and Baloch study found a rate of 2.3% in 34941 cases.
- Where the pattern is more worrisome, but you cannot bring yourself to make the call.
- The worrisome sample is either quantitatively or qualitatively deficient but a bit more than AUS.
- May be part of the two stage process, with a preliminary SFM while awaiting ancillary tests.
- One should not overuse SFM.
- If you say suspicious, clinicians will either interpret it as positive or be frustrated with the unclear answer.

Adenocarcinoma?

Lobular carcinoma?
In TIS there are two malignant chapters.
- Malignant (P) for Primary malignancies
- Malignant (M) for secondary or Metastatic Malignant disease
- Usually the latter is the easy diagnosis save for well differentiated lesions

In the past the ability for cytology to make the diagnosis of mesothelioma was questioned, but that is not the case in the modern era.
- With clinical and radiologic correlation
- With appropriate ancillary tests.
- If there is a mesothelial proliferation with inconclusive ancillary testing and little clinical suspicion of mesothelioma, AUS may be an appropriate response
- If there is clinical/radiologic suspicion and ancillary tests are conclusive, then a malignant diagnosis is warranted.

Definitive primary malignancy can be determined with:
- Cellular samples that often exhibit spheres, berry like morules or individual cells
- Mesothelial origin by morphology and immunohistochemistry markers (eg, WT-1, Calretinin, CK5/6, D2-40)
- Malignant features of overt nuclear abnormalities including karyomegaly, anisonucleosis, macronucleoli, bii or multinucleation
- Loss of Bap-1 by IC, deletion of p16/CDKN2A by FISH or loss by IC
- Positive clinical and radiologic correlation
Mesothelioma
Loss of BAP-1 in mesothelial cells

Malignant (M)
Metastatic disease from ocular melanoma

Malignant (M)
Metastatic disease from Mullerian serous carcinoma

49

50

51
Table 8.2. Testing predictive mutations, other alterations in malignant effusions (common examples), and principal molecular techniques with reference ranges.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumor type</th>
<th>Targeted treatment</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>NSCLC</td>
<td>EGFR TKIs</td>
<td>FDA approved</td>
</tr>
<tr>
<td>BRAF</td>
<td>NSCLC, melanoma</td>
<td>BRAF inhibitors</td>
<td>FDA approved</td>
</tr>
<tr>
<td>KRAS</td>
<td>NSCLC</td>
<td>AMG 510</td>
<td>Emerging</td>
</tr>
<tr>
<td>HER2/ERBB2</td>
<td>NSCLC</td>
<td>Anti-HER2</td>
<td>Emerging</td>
</tr>
<tr>
<td>METex14</td>
<td>NSCLC</td>
<td>MET Inhibitors</td>
<td>Emerging</td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Ovarian, breast &amp; prostate cancer</td>
<td>PARP inhibitors</td>
<td>FDA approved</td>
</tr>
<tr>
<td>STK11/LKB1</td>
<td>NSCLC</td>
<td>Immune checkpoint inhibitors</td>
<td>Emerging</td>
</tr>
<tr>
<td>MSI</td>
<td>all tumor types</td>
<td>Immune checkpoint inhibitors</td>
<td>FDA approved</td>
</tr>
<tr>
<td>TMB</td>
<td>Different tumor types</td>
<td>Immune checkpoint inhibitors</td>
<td>Emerging</td>
</tr>
<tr>
<td>EGFR (p.T790M, p.C797S)</td>
<td>NSCLC later generation EGFR TKIs</td>
<td>FDA approved</td>
<td></td>
</tr>
<tr>
<td>ALK</td>
<td>NSCLC</td>
<td>ALK TKIs</td>
<td>Emerging</td>
</tr>
<tr>
<td>ROS1</td>
<td>NSCLC</td>
<td>ROS1 TKIs</td>
<td>Emerging</td>
</tr>
<tr>
<td>NTRK</td>
<td>NSCLC</td>
<td>NTRK TKIs</td>
<td>Emerging</td>
</tr>
<tr>
<td>STK11/LKB1</td>
<td>NSCLC</td>
<td>Immune checkpoint inhibitors</td>
<td>Emerging</td>
</tr>
</tbody>
</table>

Reference range LOD:
- all the mutations in the analyzed gene regions: 10%-20%
- only hotspot mutations: 1%-5%
- only hotspot mutations: 0.1%-1%
- all the mutations in the analyzed gene regions: 0.01%-5%

Abbreviations: ALK: anaplastic lymphoma kinase; BRAF: V-Raf Murine Sarcoma Viral Oncogene Homolog B1; BRCA1/2: Breast Cancer Type 1/2 Susceptibility genes.

Ancillary Techniques

A) PD-L1 in malignant effusion of NSCLC. Cell block section. Membranous PD-L1 staining in all tumor cells and weakly positive macrophages in the background.
B) Diffuse staining of tumor cells, smear
C) PD-L1 negative tumor cells and an adjacent macrophage serving as a positive internal control
D) Heterogenous PD-L1 staining.

FISH analysis using UroVysion® multi-probe FISH assay (increased copy numbers of chromosomes 3 (red), 7 (green) and 17 (aqua), and homozygous loss of 9p21 signals (gold). Note some benign cells with retained 9p21 signals.
Diagnostic categories & clinical management

- Diagnoses can be made on direct smears, Cyto/Spins, liquid preps, cell blocks and clot sections.
- Non-diagnostic: Repeat sample
- Negative for Malignancy: Clinical follow up based on suspicion. Specific diagnosis (e.g., serositis or features of auto-immune disorders for further investigation)
- Atypia of Undetermined Significance: Ancillary testing to exclude malignancy
- Suspicious for Malignancy: Ancillary testing to confirm malignancy
- Malignant – Primary (mesotheloma): Correlate with clinical findings and biopsy
- Malignant – Secondary (metastasis/symphoma): Ascertain the primary and prognostic/predective markers

Questions

A 58 year old man with no prior clinical history is presented with shortness of breath. A chest x-ray showed a lung mass. The pleural effusion is most likely positive for:

A. TTF-1 and napsin-A
B. WT-1 and calcitonin
C. PAX-8 and CD-10
D. SOX-10 and HMB-45
E. NKX3.1 and PSA

Questions

Answer: A. TTF-1 and napsin-A

- Malignant pleural effusion often the initial clinical presentation in lung adenocarcinomas.
- Metastatic lung adenocarcinoma to the body cavities can be confirmed by TTF-1 and napsin-A.
- Advanced lung adenocarcinomas are subjects for targeted therapies. WT-1 and calcitonin highlight mesothelial cells.
- PAX-8 and CD-10 are useful to diagnose renal cell carcinoma.
- SOX-10 and HMB-45 are used for diagnosis of melanoma.
- NKX3.1 and PSA are markers for prostatic adenocarcinoma.

A 78 year old male presented with pleural effusion. What molecular test can be requested by the oncologist?

A. Her2-Neu  
B. ALK  
C. BRAF  
D. EGFR  
E. C-kit

Answer: C. BRAF

- Melanoma often a diagnostic challenge
- Malignant melanoma often presents with single cells resembling mesothelial cells
- Cell clusters are uncommon.
- Nuclei are large and the nucleoli are prominent.
- Cytoplasm is moderate to abundant and may contain brown melanin pigment.
- 40% to 60% of malignant melanomas harbor BRAF V600E mutation.
- Her2-NEU is tested on breast carcinoma. ALK and EGFR are tested on lung adenocarcinoma. C-kit is tested on gastrointestinal stromal tumors.


Abdominal fluid of a 72 year old male is shown here. The possible primary site is:

A. Colon  
B. Lung  
C. Stomach  
D. Mesothelium  
E. Bladder
Questions

Answer: C. Stomach
- A significant form of gastric carcinoma is characterized by isolated signet ring in body cavity effusions.
- The malignant cells often contain a large intracytoplasmic vacuole and an eccentric nucleus.
- Lobular breast carcinoma is also present in signet ring cells in effusions.
- Adenocarcinomas of other primary sites such as lung and pancreas occasionally present as signet ring cells in body effusions.

Questions

A 78 year old man presented with pleural effusion. The most likely diagnosis is:
A. Metastatic lung adenocarcinoma
B. Metastatic squamous cell carcinoma
C. Reactive mesothelial cells
D. Mesothelioma
E. Metastatic pancreatic adenocarcinoma

Questions

Answer: D. Mesothelioma
- Epithelioid and mixed types of mesothelioma are likely exfoliated malignant cells to serous body cavities.
- The smear are cellular comprised of single cells and large clusters.
- The cells contain abundant dense cytoplasm with a pale rim and large nuclei with prominent nucleoli.
- Deletions of 1p, 3p, 9p, 6q, 22q and p16 are commonly detected by fluorescence in situ hybridization and as mentioned loss of BAP-1 by immunohistochemistry.
Chapters

- Ch. 1 Introduction
- Ch. 2 Non-Diagnostic (ND)
- Ch. 3 Negative (NFM)
- Ch. 4 Atypia (AUS)
- Ch. 5 Suspicious for Malignancy (SUS)
- Ch. 6 Malignant Primary (mesothelioma)
- Ch. 7 Malignant Secondary (metastasis)
- Ch. 8 Ancillary techniques
- Ch. 9 Peritoneal Washings
- Ch. 10 Cytopreparatory Techniques
- Ch. 11 Quality Management
- Ch. 12 Pathophysiology of Body Cavities

Peritoneal Washings

- Special considerations relative to peritoneal samples are presented
- Cytologic considerations for benign non-mesothelial epithelial cells, e.g., ciliated epithelial cells, endometriosis
- Considerations for borderline and malignant tumors.

Cytopreparation

- Donna Russell from the University Rochester and Dr. Depali Jain add their cytopreparatory expertise
- Direct smears, liquid based preparations, molecular preps,
- Multiple cell blocks techniques are described with relevant references.
Quality Management

- This chapter considers all the activities contributing to quality analysis of serous fluids from the pre-analytic submission to the reporting of results to clinical colleagues.
- Policies, procedures, proficiency testing and accreditation are considered.

Pathophysiology

- TIS adds an excellent chapter by Dr. Stefan Pambuccian on the anatomy, embryology, physiology and general pathology of body cavities.
- The situations that generate transudates and exudates are discussed including Starling forces and aspects of inflammation.
- There is also an illuminating discourse on mechanisms of malignant cell involvement of serous membranes and fluids.

Final Thoughts

- The system needs to be used and then challenged.
- All the diagnostic systems purport to be living systems capable of evolution.
- As new information arises the systems must be revisited and revised every few years.
- The authors and editors invite your comments and thoughts for improvement.