Role of cell block in Pleural Fluid Cytology

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Limitations of fluid cytology

- Atypical cells -? Reactive mesothelial cells ? Adenoca cells
- Atypical cells -? Reactive mesothelial cells ? Mesothelioma
- Poorly differentiated malignancy :?Carcinoma? lymphoma ? sarcoma
- Poorly differentiated ca: Non-small cell ca or small cell ca
- Non- small cell ca : ? Adenoca ? Squamous cell ca
- Adenoca ? Primary origin
Limitations of Immunocytochemistry Vs Immunohistochemistry

- Positive Immunocytochemistry controls
- Optimal antibody concentrations – Customized for cytology specimens
- Inadequate immunocytochemistry panels
- Haemorrhagic necrotic samples or with acute inflammatory exudate- background wash
- Availability of same set of cells for multiple markers
Why cell block in Fluid cytology?

- The cell block is an ancillary technique used in cytology to increase the diagnostic accuracy in the analysis of effusions and aspirations.

- Also used for prognostic & THERAPEUTIC purpose. With the availability of molecular targeted therapy for many cancers, a large number of recent studies have used cytological material or CBs for molecular characterization.

Indications of cell block

• For classification of atypical cells detected in fluid smears
  – Carcinoma/mesothelioma cells vs reactive mesothelial cells
  – Lymphoma cells vs reactive lymphocytes
• For classification of poorly differentiated carcinoma cells
  – Adeno vs squamous vs small cell carcinoma
• To detect primary origin of tumor in c/o metastasis
• Treated primary malignancy, when another mass & pleural effusion to know whether new primary
• Poorly processed cytology smears: Hemorrhagic, thick smears hampers visibility

30% of pl fl at TMH
Preparation of Cell Block

• Centrifuge fluid sample with clot or suspended tissue fragments.
• Decant supernatant and prepare smears from sediment, if possible.
• Wrap the clotted material or tissue flakes with Whatmann’s tissue paper no. 1 in a cassette, fix with 10% neutral buffered formalin and process as per histological procedure.
• If the sediment is scanty, embed it in 4% molten agar or clot it with plasma-thrombin.
• Put the agar/plasma-thrombin clotted tissue in a tissue cassette, fix with 10% neutral buffered formalin & forward for histological processing.
• In case of a thick smear, the smear can be scraped with the help of a disposable scalpel blade; wrapped in molten agar and processed as cell block. (The stained thick smear should be destained with 1% acid alcohol and then scraped to retrieve the tissue material for cell block)
Cell block preparation methods

1. Cell concentration
2. Fixation of sediment
3. Cell hardening
4. Pellet embedding

Adequate residual sediment
- Direct sedimentation

Scanty residual sediment
- Agar embedding
- Thromboplastin + pooled plasma
CB Technique can be applied to

- Any cytology sample with adequate sediment
- All Fluids except CSF
- Respiratory / alimentary lavage or brush
- Sputum, urine,
- FNAC
- Commonly used samples: pleural fluid [rich in cellularity]
- Not applied when no sediment
Various Techniques of cell block preparation relation with vol of sediment

- Direct sedimentation • > 1ml sediment; cheapest
- Agar embedding • 0.5ml --1ml sediment ; cheaper
- Thromboplastin- plasma • < 0.5ml Sediment; expensive
- Scrape cell block • Thick , poorly spread FNAC smear, cheap but tedious
<table>
<thead>
<tr>
<th>Method</th>
<th>Merit</th>
<th>Demerit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Direct sedimentation</td>
<td>Simple, No extra cost</td>
<td>Large sediment required (&gt;1ml) DNA fragmentation and Denaturation, Sequence artefacts, Potential false positives Poor yield of RNA</td>
</tr>
<tr>
<td>2. Agar (HG)</td>
<td>Cheap, suitable for IHC &amp; molecular tests</td>
<td>Tedious, heat related artifacts, quantity of sediment required 0.5 to 1ml</td>
</tr>
<tr>
<td>3. Thromboplastin – Plasma[TP]</td>
<td>Simple &amp; easy, can be used when sediment is scant (&lt;0.5ml)</td>
<td>Relatively Expensive, reagent stored at 2°C to 8°C</td>
</tr>
<tr>
<td>4. Cytolyt prefixed thrombin clot (with prothrombin) CTC</td>
<td>Simple &amp; easy</td>
<td>Expensive, collection in cytolyt, cell shrinkage, molecular tests need validation</td>
</tr>
<tr>
<td>5. Scrape cell block</td>
<td>Cheap, no reagent required</td>
<td>Tedious, Skill required</td>
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</tbody>
</table>
# Automated cell block method: Cellient cell block: vacuum assisted filtration

## Merits
- Good cellular yield
- Uniformly distributed cells
- Improved cellular architecture & nuclear features
- Consistent results
- Reduced procedural time
- No cross-contamination
- Minimal cell loss
- High quality of DNA & RNA

## Demerits
- Expensive machines and consumables
- Requires trained staff for cutting thin blocks
- Limited studies with ancillary Techniques available
- Possible false negatives for hormone receptors
Histology  Immunocytochemistry  Cytogenetic and molecular testing

Alcohol: methanol  Formalin Poor discrimination of nuclear and cytological details Higher frequency of positivity for hormone receptors and other nuclear antigens, such as Ki67, PCNA and p53  DNA fragmentation and denaturation Sequence artefacts Potential false positives Poor yield of RNA in PreservCyt and CytoLyt used in LBC and CellientTM CB53,54 Ethanol in SurePath LBC Good cytological preservation, but cell shrinkage and increased nuclear-cytoplasmic holes Inhibition of S-100 and hormone receptors ISH for HPV can be performed  Superior nucleic acid quality
<table>
<thead>
<tr>
<th>Effusion with papillary clusters of balls</th>
<th>Effusion with singly scattered cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Breast carcinoma</td>
<td>• Lung adenocarcinoma</td>
</tr>
<tr>
<td>• Ovarian malignancy</td>
<td>• Stomach adenocarcinoma</td>
</tr>
<tr>
<td>• Lung adenocarcinoma</td>
<td>• Breast adenocarcinoma</td>
</tr>
<tr>
<td>• Prostate adenocarcinoma</td>
<td>• Renal cell carcinoma</td>
</tr>
<tr>
<td>• Colorectal ca</td>
<td>• Malignant melanoma</td>
</tr>
<tr>
<td>• Malignant mesothelioma</td>
<td>• Malignant mesothelioma</td>
</tr>
<tr>
<td>• Florid reactive mesothelial hyperplasia</td>
<td>• Reactive mesothelial proliferation</td>
</tr>
</tbody>
</table>
Limitations of cell block

- Good cellular yield needed in the cytology specimen to make a cell block.
- Poor discrimination of nuclear and cytological details
- Cell morphology is better preserved in properly processed conventional fluid smear than cell block.
- HE sec of CB VS pap smear of fluid cytology, morphologic interpretation may not enhance by CB
- Yet, CB scores over CS----- WHY?
- Availability of cellular material for IHC & molecular tests
Causes of poor cell yield in CB

- Improper centrifugation [2 tubes/15ml/3000 RPM] for 10 min
- Improper pipetting out the sediment
- Improper proportion of T & P added
- Pleural fl with anticoagulant added
- T & P not mixed well with sediment
- Mixture not allowed to stand for 10 min

No short cuts
Our experience with T-P CB

- Simple procedure, easy to process & cut
- Recovers minute cellular material
- Ready to use reagent with long shelf life
- Preserves the cell antigens for IHC
- No cross reactivity with other ag
- Relatively cost-effective [INR-20]
Comparison between CB & CC

1. Cellular material more in CB due to concentration

2. Benign Dx more common in CS

3. Pick of malignancy more in CB

4. Atypical Dx resolved on CB into benign or malignant with IHC

5. CB increases the diagnostic yield & malignancy Dx by 10-20%

6. Archival storage available, routine histology controls
Case No 01- CN7927

- 60 male,
- Presented with breathlessness on exertion since 3 months, dry cough, low grade fever, anorexia since 2 months
- X-ray chest: right pleural effusion
- Pleural tapping done twice: 800ml and 500ml fluid
- Fluid cytology: reactive mesothelial cells, negative for malignancy
Case 1: Final diagnosis

- Cell block: malignant mesothelioma
- CT scan done afterwards: nodular pleural thickening.
- Cell block diagnosis confirmed
- Pleural fluid cytology: False negative
Case No 02 - CJ3045- Ca breast

- 71 female, operated case of IDC-II right breast, (lumpectomy) In 2012
- In 2014, presented with right pleural effusion.
- Tapping done 4 times, cytology reported as suspicious for malignancy twice.

Microscopic Examination
20 ml. pale colored fluid received at room temperature
Smears show mesoepithelial cells, lymphocytes, polymorphs & RBCs. A few cells with enlarged hyperchromatic eccentric and occasional nucleoli noted.

MPRESSION
LT PLEURAL FLUID : Atypical cells suspicious adenocarcinoma
Case 2 : Final diagnosis

• PI fluid negative for malignant cells

• PI fluid cytology = FP

• Reactive mesothelial cells interpreted as adenocarcinoma cells : common diagnostic pitfall
Case No 03-- CN10927

- 77 male, retired police officer, Chronic smoker
- Presented with lower thoracic & upper abdominal pain, weight loss and anorexia since 1 year.
- Investigated outside
- CECT: Endoluminal mass in right main bronchus, right pleural effusion, and mild pleural thickening.
- USG abdomen : Multiple liver metastasis also present
- Clinical Dx : Stage IV CA lung
case 3 : Final Diagnosis

- Small cell ca of lung with pleural & liver metastasis

- Implications: tissue block asked for molecular testing in view of adenoca; TEST withheld

- THERAPEUTIC IMPLICATIONS
Case no 4 [CK 34175]

- 40 yr female,
- C/O Breathlessness, productive cough and hoarseness of voice since 4 months.
- CT Thorax s/o left lung lower lobe mass infiltrating mediastinum, with pleural thickening and effusion on same side.
- CT guided Bx: inconclusive
- Bronchoscopic Bx and pleural fluid tapping done
Case 4

- PI fluid cytology: positive
- CB SEC with IHC: NEGATIVE
- FINAL DX: POSITIVE
- PITFALL Selection of AB
- Ck 7 came positive in which calret negative
- CB: false negative
CASE 5

- 38 male,
- Presented with breathlessness, weight loss and anorexia
- Chest X-ray- right pleural effusion
- No other information available
- Pleural tapping done and cent for cytology and cell block preparation.
- Patient’s condition worsened and he expired on the day of tapping.
Case 5: Final diagnosis

- Metastatic amelanotic melanoma with UPM
- DD of plasmacytoid cells
- Adenoca
- Melanoma
- Neuroendocrine ca
- Osteogenic sarcoma
Ca breast

• 45/ F operated for ca breast in April 2013
• DX: micropapillary carcinoma
• ER +, PR−, Cerb B2 −
• On FU developed pleural effusion
• No SC/ axillary nodes
• Pleural fluid cytology: positive
• Cell block prepared
Advantages of cell block

• Preserves & concentrates cellular material
• Maintains tissue architecture
• Concentrates cells in a limited area permitting for an easier, more detailed, less time consuming analysis
• **Multiple sections of the same SET of cellular material available for special stains and immuno-stains**
• For molecular techniques like BRAF, Alk gene rearrangement & cerbB2 amplification
• Can be safely stored for long period
• Avoids biopsy or other invasive & expensive diagnostic tests
• Simple, cost-effective technique which Facilitates DX
### Concordance of CB with pleural bx

<table>
<thead>
<tr>
<th>CB</th>
<th>CB IHC</th>
<th>Pl. Bx</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive /ND</td>
<td>TP</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>FP</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>FN</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>TN</td>
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<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>P</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>FN</td>
</tr>
</tbody>
</table>
## Comparative Analysis

<table>
<thead>
<tr>
<th>Pleural fluid cytology</th>
<th>Cell Block</th>
<th>CB-IHC</th>
<th>Pleural Bx</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Ad Ca Breast</td>
<td>Mesothelial cells</td>
<td>Mesoth. Markers positive</td>
<td>Negative</td>
<td>FP</td>
</tr>
<tr>
<td>Met Ad Ca lung</td>
<td>Mesothelial cells</td>
<td>Mesoth. Markers pos lung markers neg</td>
<td>Not done Rpt cyto neg</td>
<td>FP</td>
</tr>
<tr>
<td>Met Ad Ca lung</td>
<td>Mesothelial cells</td>
<td>Mesoth. Markers +ve, lung ad ca markers = TTF 1 - ve</td>
<td>Not done</td>
<td>TP (Lung Bx mucinous Ca, CK7 +ve)</td>
</tr>
<tr>
<td>Met SCLC</td>
<td>A cluster of atypical cells</td>
<td>Mesoth. Markers +ve, lung ad ca markers –ve</td>
<td>Not done</td>
<td>TP markers for SCLC not done-Reviewed Markers +ve</td>
</tr>
<tr>
<td>Mesothelial cells negative</td>
<td>Positive</td>
<td>Lung markers +ve</td>
<td>Not done</td>
<td>FN</td>
</tr>
<tr>
<td>PI Fluid Cyo</td>
<td>CB</td>
<td>PI. Bx</td>
<td>Interpretation</td>
<td></td>
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</tr>
<tr>
<td>Positive</td>
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<td>negative</td>
<td>FP</td>
<td></td>
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<td>Positive</td>
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<td>Negative /ND</td>
<td>FP</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>FFP(TP)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
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<td>Negative /ND</td>
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<tr>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>FFN[TN]</td>
<td></td>
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</table>
False positive fluid cytology

- Papillary mesothelial hyperplasia -> papillary adenocarcinoma [WT1, calretinin, D240]

- Dissociated large mesothelial cells -> poorly differentiated adenocarcinoma or mesothelioma

- Lymphocyte rich effusion vs low grade lymphoma
Causes for FP

Fluid Cytology positive CB negative

1. Mesothelial cells are over called as carcinoma in CC

2. CB preparation suboptimal (Ca cells not included)

3. Wrong IHC asked eg. CK7, Calret, (both positive) specific markers not asked

4. Specific markers asked but are negative eg. TTF1 & Napsin A can be negative in------% cells specially mucinous type of Ad Ca Lung
How to avoid FP on fluid cyto

1. Optimal preparation of fluid to get clear picture

2. Meticulous examination of smear. One cell or two cell type criteria for malignancy

3. Correlate with histology of primary (cytology & Bx)

4. Rule out other causes of pleural effusion Infarction infection, injury

5. Keep Dx open when in doubt or ask for repeat cyto with cell block preparation
False negative

- Well differentiated papillary adenocarcinoma -> papillary mesothelial hyperplasia
- Poorly differentiated GI adenocarcinoma OR MELANOMA missed as reactive mesothelial cells
- Small cell carcinoma missed for lymphocytes or reserve cells
- Low grade NHL missed for lymphocytes
### Met. Ad Ca in PI Fluid Cytology

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Immunomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Lung</td>
<td>• TTF1, Napsin, CK 7, CEA</td>
</tr>
<tr>
<td>• Breast</td>
<td>• GATA3, ER, PR, Cerb B₂,</td>
</tr>
<tr>
<td>• Ovary</td>
<td>• WT₁, PAX8, CK7</td>
</tr>
<tr>
<td>• GI T</td>
<td>• CK7, CK20, CEA, CD X₂</td>
</tr>
<tr>
<td>• UPM</td>
<td>• CK7, CK20, WT1, TTF1, CDX₂</td>
</tr>
<tr>
<td>• SCLC (Lung,</td>
<td>• AE/Æ₃, synapto, CD56, Ckit</td>
</tr>
<tr>
<td>Oesophagus)</td>
<td></td>
</tr>
</tbody>
</table>
• CB preparation is easy & cost effective
• Conventional smear is diagnostic in > 80% cases of fluids
• Specific indications should be followed for CB
• Molecular testing & targeted therapy have changed the scenario of diagnostic testing
• Cell block has definite advantages over CS. However one should know the limitations.