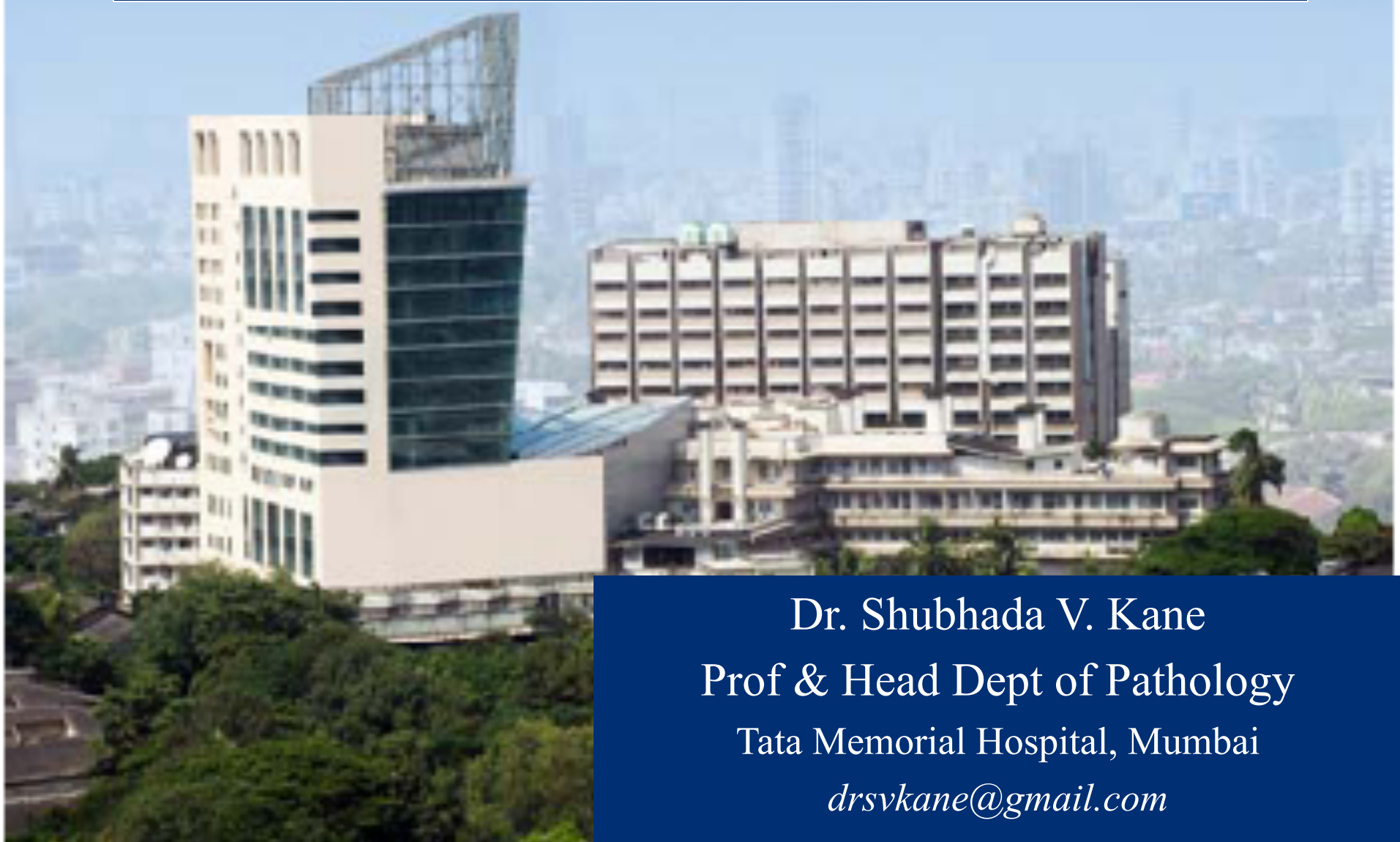
Role of cell block in Pleural Fluid Cytology



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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.

- REF : **Cytopathology Vol 25 [6] pages 356–371, 2014**

Indications of cell block

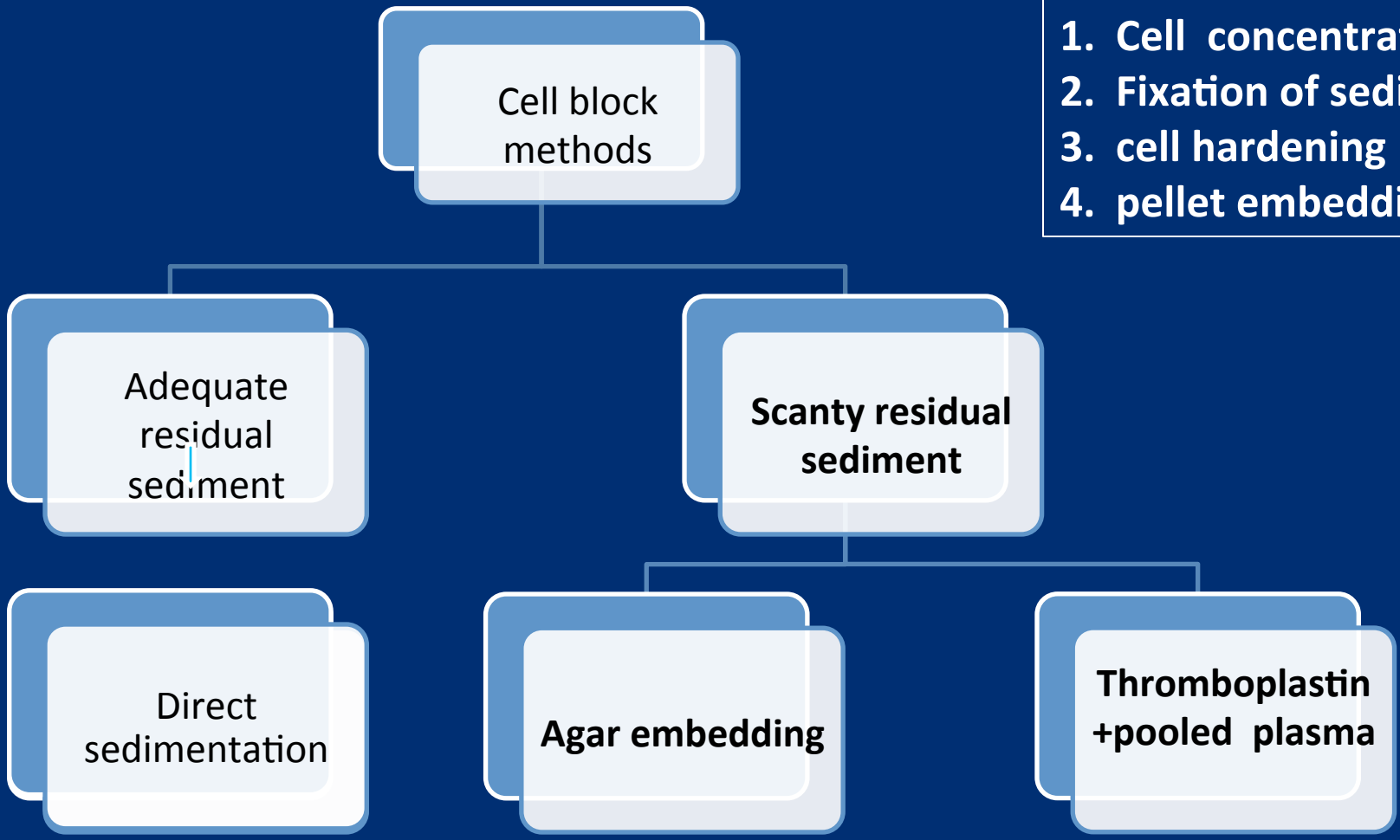
- For classification of atypical cells detected in fluid smears
  - Carcinoma/mesothelioma cells vs reactive mesothelial cells
  - Lymphoma cells vs reactive lymphocytes
- For classification of poorly differentiated carcinoma cells
  - Adeno vs squamous vs small cell carcinoma
- To detect primary origin 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

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

Merits & demerits of Cell block methods

Method	Merit	Demerit
1.Direct sedimentation	Simple, No extra cost	Large sediment required(>1ml) DNA fragmentation and Denaturation , Sequence artefacts, Potential false positives Poor yield of RNA
2. Agar (HG)	Cheap , suitable for IHC & molecular tests	Tedious, heat related artifacts , quantity of sediment required 0.5 to 1ml
3.Thromboplastin – Plasma[TP]	Simple & easy , can be used when sediment is scant(<0.5ml)	Relatively Expensive , reagent stored at 2^{o} to 8^{o}
4.Cytolyt prefixed thrombin clot (with prothrombin) CTC	Simple & easy	Expensive , collection in cytolyt ,cell shrinkage , molecular tests need validation
5.Scrape cell block	Cheap, no reagent required	Tedious ,Skill required

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 andmolecular testing

Alcohol: methanol Formalin56 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

Effusion with papillary clusters of balls

- Breast carcinoma
- Ovarian malignancy
- Lung adenocarcinoma
- Prostate adenocarcinoma
- Colorectal ca
- Malignant mesothelioma
- Florid reactive mesothelial hyperplasia

Effusion with singly scattered cells

- Lung adenocarcinoma
- Stomach adenocarcinoma
- Breast adenocarcinoma
- Renal cell carcinoma
- Malignant melanoma
- Malignant mesothelioma
- Reactive mesothelial proliferation

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 C S
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
- Pl 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, lymhocytes, 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

- **Pl fluid negative for malignant cells**
- Pl fluid cytology = FP
- Reactive mesothelial cells interpreted as adenoca 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

- Pl 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 April2013
- 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 diagnstic tests
- Simple, cost-effective technique which Facilitates DX

Concordance of CB with pleural bx

CB	CB IHC	Pl. Bx	Remark
Positive	Positive	Positive /ND	TP
Positive	Positive	Negative	FP
Negative	Negative	Positive	FN
Negative	Negative	Negative	TN
Negative	Positive	Positive	P
Positive	Negative	Positive	FN

Comparative Analysis

Pleural fluid cytology	Cell Block	CB-IHC	Pleual Bx	Remarks
Met Ad Ca Breast	Mesothelial cells	Mesoth. Markers positive	Negative	FP
Met Ad Ca lung	Mesothelial cells	Mesoth. Markers pos lung markers neg	Not done Rpt cyto neg	FP
Met Ad Ca lung	Mesothelial cells	Mesoth. Markers +ve, lung ad ca markers = TTF 1 - ve	Not done	TP Lung Bx mucinous Ca, CK7 +ve
Met SCLC	A cluster of atypical cells	Mesoth. Markers +ve, lung ad ca markers –ve	Not done	TP markers for SCLC not done- Reviewed Markers +ve
Mesothelial cells negative	Positive	Lung markers +ve	Not done	FN

Statistical Analysis Basis

Pl Fluid Cyo	CB	Pl. Bx	Interpretation
Positive	Positive	negative	FP
Positive	Negative	Negative /ND	FP
Positive	Negative	Positive	FFP(TP)
Negative	Negative	Negative /ND	TN
Negative	Positive	Positive /ND	FN
Negative	Positive	Negative	FFN[TN]

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 mucinouus 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 Pl Fluid Cytology

Primary Site	Immunomarkers
• Lung	• T TF1, Napsin, CK 7,CEA
• Breast	• GATA3, ER, PR, Cerb B_2 ,
• Ovary	• WT_1 , PAX8 ,CK7
• GI T	• CK7, CK20, CEA, CD X_2
• UPM	• CK7, CK20, WT1,TTF1 CDx_2
• SCLC (Lung, Oesophagus)	• AE/AE_3, synapto,CD56, Ckit

Take Home Message

- 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.