Cytology of Effusion Fluids

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Cytology of Effusion Fluids
Lecture Objectives

- Clinical importance of malignant effusions
- Handling of effusion specimens
- Features of benign and malignant effusions
- Pitfalls in effusion cytology
- Use of ancillary studies
- Accuracy of cytology of effusions
Anatomy

- Four main serosal body cavities
  - 2 pleural, 1 pericardial, 1 peritoneal
- Lined by single layer of mesothelium
- Normally contains very little fluid
- Effusion develops from imbalance of fluid formation and removal
Types of Effusion Fluids

- **Transudate**
  - Changes in hydrostatic or osmotic forces
  - Ultrafiltrate of plasma
  - S.G. $<1.015$, protein $< 3$ g/dL

- **Exudate**
  - Increased capillary permeability
  - Rich in protein and inflammatory cells
  - S.G. $>1.015$, protein $> 3$ g/dL

- **Chylous**
  - Rare cause of pleural effusion
  - Leakage from thoracic duct
Causes of Effusions

Transudates
- CHF, cirrhosis of liver, nephrotic syndrome, Meigs’ syndrome

Exudates
- Malignant neoplasms
- Infections, collagen vascular diseases, pulmonary infarction, post radiotherapy

Chylous
- Malignant neoplasms
- Trauma, infections
Sampling of Effusion Fluids

- Thoracentesis, paracentesis, pericardiocentesis
- Diagnostic, therapeutic or both
- Sample size, a few ml to litres
- For cytology, microbiology, chemistry, cell count, flow cytometry, other ancillary studies
Handling of Effusion Fluids

- Fixatives not necessary
- Cannot do DQ stain if fixed
- Effusion fluids act like culture medium; cells will remain viable and retain morphology for several days in fridge (4 °C)
- Process fluids promptly to minimize degenerative artifacts
Handling of Effusion Fluids

Gross examination
- Volume, colour, opacity, odour, blood
- Clues to underlying etiology

Slides
- Direct smears, Pap or DQ
- Liquid-based - SurePath, ThinPrep
- Cytospin

Cell block
- Clot and/or sediment preparation
Question #1

When do you prepare a cell block for an effusion specimen in your lab?

1. Routinely for every specimen
2. Only if cytology looks suspicious or positive
3. Only if stains are required
4. Never
Cytology Cell Block

- Various techniques
  - HistoGel, agar, gelatin, albumin, plasma / thrombin
- Allow special studies
  - IHC, ISH, molecular
Significance of Malignant Effusions

- In patients with known malignancy
  - Indicates advance stage, poorer prognosis
- Initial manifestation of malignancy
  - Identification of primary site
- Treatment implications
  - Palliative versus curative intent
  - Excludes surgery or radiation therapy
Malignant Effusions - Statistics

- Any malignancy may lead to an effusion
- Not all effusions in cancer patients are malignant

Malignant pleural effusion
- Men: lung, GI, pancreas
- Women: breast, lung, ovary

Malignant ascites
- Men: GI, Pancreas, Lung
- Women: Ovary, GI, pancreas
Approach to Effusion Cytology

- Get clinical history, imaging and other laboratory findings
- Presence of malignant cells?
- Determine primary site?
- Require ancillary studies?
- Require another sample or biopsy?
Benign Cells in Effusions

- Mesothelial cells
- Macrophages
- Blood & inflammatory cells
- Endometrial cells, tubal cells
- Liver, colon, lung cells
Benign Mesothelial cells

- Spectrum of features
- Monotonous, central nucleus
- Nucleoli inconspicuous to prominent
- Two-zone cytoplasm
- Fuzzy cell border
- Windows between cells
- Multinucleation, signet-ring like vacuoles
- Occasional papillary groups
Benign effusion
Reactive Mesothelial cells

- Avoid the term “atypical”
- Wide morphologic spectrum overlapping with malignant cells
  - High N/C ratio
  - Nuclear hyperchromasia & pleomorphism
  - Coarse chromatin clumps
  - Prominent macronucleoli
  - Irregular nuclear membrane
  - Numerous mitotic figures
Reactive Mesothelial Cells
Peritoneal Washing

- Sampled before operative procedure for suspected gynecological malignancy
- Unique cytological features
- Fluid is saline not plasma
- Beware of the specimen labeled as “peritoneal fluid”
Peritoneal Washing
Benign versus Malignant

- “Two-cell population”
- Mesothelial cells show continuum of morphology
- Malignant cells stand out as distinct second population
- IHC if uncertain
Mesothelial cell  Adenocarcinoma cell

Cytomorphological Clues

Benign
- More single cells
- Cell clusters
  - Knobby, flower-like
  - Flat shape
  - Windows
- Cilia
- Degenerative vacuoles
- Inflammatary cells

Malignant
- Large cell aggregates
  - Smooth outlines
  - Rounding up
  - Bizarre shapes
  - Distinct cell borders
- Secretory vacuoles
- Nuclear pleomorphism
- Lymphoma, melanoma may show single cells
Malignant Effusions
What is the primary site?

DIAGNOSIS BY ORGAN SITE

“TEXTBOOK” KNOWLEDGE

CYTOMORPHOLOGICAL & ANCILLARY FEATURES

CYTOLOGY PRACTICE
Malignant Effusions
What is the primary site?

**History**

**Cytomorphological patterns**
- Average nuclear size
  - S, M, L, G
- Mucin-secreting activity
- Degree of cell cohesion
  - Proliferation spheres, papillary structures, cohesive clusters, solitary cells
- Specific unique features
  - Melanin, psammoma bodies

**Ancillary studies**
Ancillary Studies

- **Histochemistry**
  - Mucin

- **Immunocytochemistry**
  - Typing of tumour – epithelial, mesothelial, lymphoid, melanocytic, germ cell, etc
  - Determine primary site for adenocarcinoma
  - Confirm presence of few tumour cells

- **Flow cytometry**
  - Lymphoma / leukemia

- **Molecular**
  - Breast, lung, gastric
A Few Cytomorphological Patterns

- **Proliferation spheres**
  - Breast ca, small cell ca lung

- **Isolated tumour cells**
  - Gastric ca, lobular breast ca, melanoma, lymphoma

- **Signet ring cells**
  - Gastric ca

- **Papillary groups containing psammoma bodies**
  - Ovarian ca, thyroid ca
Proliferation Spheres
Proliferation Spheres

- Unique to effusion cytology
- Proliferation of tumour cells in fluid medium (in vivo)
- More common in chronic malignant effusions
  - Breast ca, small cell ca, ovarian ca
- Spheres may fuse to mimic papillary structures
- May also be associated with reactive mesothelial proliferations
Psammoma Bodies
Psammoma Bodies

**Malignant**
- Ovary – serous carcinoma
- Thyroid – papillary carcinoma
- Lung – some BAC
- Mesothelioma – papillary epithelial type

**Benign**
- Pelvic inflammatory disease
- Non-specific finding in women in ascitic fluids and pelvic washings
Signet-ring Cells
Signet-ring Cells

- **Malignant**
  - Gastric adenocarcinoma
  - Colonic adenocarcinoma

- **Benign**
  - Degenerative vacuoles in mesothelial cells and macrophages
Single Tumour Cells
Single Tumour Cells

- **Malignant**
  - Gastric adenocarcinoma (diffuse type)
  - Breast lobular carcinoma
  - Lymphoma
  - Melanoma

- **DDx - Benign**
  - Reactive lymphoid population
Immunohistochemistry

- Formalin-fixed cell block preferred
- Try to follow large tumour cell clusters on consecutive levels
- Cell blocks containing mixed populations of single benign and malignant cells most difficult to interpret
- Stain panels guided by clinical suspicion
Cell Block Stain Patterns

Isolated Tumour Cells

 Mostly benign

 Mixed benign / malignant

 Mostly malignant
Immunohistochemistry

- **Mesothelial markers**
  - Calretinin, WT1, CK7, LMWK, CK5/6
  - EMA membrane pattern (mesothelioma)

- **Adenocarcinoma markers**
  - CK7, CK20, LMWK, BerEP4, m-CEA
  - EMA cytoplasmic pattern
  - Organ specific
    - GI tract – CDX2
    - Lung – TTF-1, Napsin A
    - Breast – ER/PR
    - Ovarian – ER/PR, WT1, CA-125, PAX-8
    - Prostate – PSA, PSAP
    - Renal cell – RCC, CD10, vimentin
Immunohistochemistry

- Squamous cell
  - HMWK, p63
- Melanoma
  - HMB-45, Melan A, S100, Sox-10
- Lymphoma / Leukemia
  - CD45, B-cell panel, T-cell panel, etc
  - CD30, HHV8, EBV
- Macrophage
  - CD68
Flow cytometry

- Effusions ideally suitable for flow cytometry
- Suspected cases of lymphoma / leukemia
- Coordinate with hematology lab
- May not be available in community labs
- Rapid transport to flow lab for best results
Case 1

History of pancreatic carcinoma. Ascites.
Case 1 - Macrophages

- CD68
- Calretinin
- BerEP4-, mCEA-, EMA-, CK20-, TTF1-, CDX2-
Case 2
Pericardial effusion. Hx of pleural mesothelioma 1 year ago.
Mesothelioma

- Clinical suspicion
- Pleuritic chest pain, recurrent unilateral bloody pleural effusion
- Viscous fluid (hyaluronic acid)
- Distinction from benign mesothelial cells
  - Bigger clusters, bigger cells
  - Rarely may have only single cells, psammoma bodies
Comparison

- Benign mesothelial cells
- "Reactive" mesothelial cells
- Mesothelioma
Case 3
65 M. Pleural effusion. 400 ml cloudy red fluid.
Case 3 – Lung adenocarcinoma

CK7

Calretinin

TTF-1

Napsin A

EMA+, CK20-
Immunohistochemistry – Napsin A

- Aspartic proteinase
- Positive in 80-90% of lung adenocarcinoma
- More sensitive and specific than TTF-1
- Negative in small cell carcinoma, squamous cell carcinoma and metastatic adenocarcinoma to lung
- Granular cytoplasmic staining
Case 4
78 M. Pleural thickening and effusion. 500 ml red fluid.
Case 4 – Small cell lung carcinoma

CB

Synapto

CD56

TTF-1
Case 5

42 F. Breast carcinoma. New pleural effusion.
Case – Breast carcinoma

CK7+, PR+, calretinin-
Case 6
88 F. Breast carcinoma. New pleural effusion.
Case 6 – Lobular breast carcinoma
Case 7

72 F. Hx breast and colon carcinoma. Ascites 1L cloudy brown.
Case 7 – Colon adenocarcinoma

CB

CK7

CDX2

CK20

ER-
Case 8
42 M. Hx gastric carcinoma. Ascites.
Case 8 – Signet-ring carcinoma
Case 9

Case 9 – Ovarian carcinoma

CK7

Calretinin

ER

PAX-8

CA125+, WT1+, CK20-, CDX2-, TTF1-
Immunohistochemistry – PAX-8

- Transcription factor
- Positive in
  - Mullerian tract (ovary, endometrium)
  - Thyroid, parathyroid
  - Kidney, thymus
  - Some neuroendocrine tumours
- Nuclear staining
- Stains B-cells (cross reacts with PAX-5)
Case 10

53 M. History renal cell carcinoma. Pericardial effusion.
Case 10 – Renal cell carcinoma

CD10+, calretinin-, TTF1-, CDX2-, PSA-
Case 11
64 F. History melanoma. Pleural effusion.
Case 11 - Melanoma

Panker-, CK7-, CK20-, WT1-, TTF1-
Case 12

34 M. History HIV. KS. Small pleural effusion.
Case 12

- **CD3**
- **CD30**
- **HHV8**
- **EBV-ISH**

- **CD45+**
- **CD138+**
- **MUM1+**
- **CD20-**
- **CD5-**
- **ALK1-**
Case 12 – Primary Effusion Lymphoma

- DLBCL with primary effusion involvement
- Mostly in HIV patients
- Most have HHV8+, some EBV+
- Typical CD45+ CD20- CD30+ CD138+
  HHV8+ EBV+ CD3+/-
- Poor prognosis
Case 13

- 48 F mediastinal mass, bilateral lung nodules
- Biopsy showed “squamous cell carcinoma”
- New pericardial effusion
- Sample: 50 ml bloody fluid
Case 13 – Diagnosis?

1. Lymphoma
2. Adenocarcinoma
3. Squamous cell carcinoma
4. Germ cell tumour
5. Do immunohistochemistry
Case 14

- 62 F 15 cm cystic/solid ovarian mass
- Ascites, bilateral pleural effusions
- CT chest – enlarged mediastinal nodes
- 700 ml cloudy yellow pleural fluid drained
Case 14 – Diagnosis?

1. Ovarian adenocarcinoma
2. Lung adenocarcinoma
3. Carcinoma unknown primary
4. Neuroendocrine carcinoma
5. Do more immunohistochemistry
Pitfalls in Effusion Cytology

- **Suboptimal specimen preservation or handling** may result in degenerative changes
  - Nuclear hyperchromasia, cytoplasmic vacuolization

- **The many faces of reactive mesothelial cells**
  - Features overlap with adenocarcinoma cells

- **Unexpected patterns or unusual entities**
  - Reactive lymphoid population, single population of pure tumour cells, 3-dimensional benign cell groups, psammoma bodies, megakaryocytes
Effusions - Accuracy
Motherby H et al. Diagn Cytopathol 1999;20:350-7

- 300 pleural, 300 ascitic fluids
- Sensitivity 50%, specificity 97%
- PPV 95.7%, NPV 86.4%
- FP 0.5%, FN 31.5%
- Of FN
  - 30% due to screening error
  - 70% due to sampling error

“… diagnostic accuracy of effusion cytology is still unsatisfactory and should be improved.”
Many effusions fluids can be reported without ancillary studies

Ancillary studies are helpful in determining primary site and confirming small populations of tumour cells

Avoid using “atypical” category if possible

Be conservative
   – Truly malignant effusions rapidly recur
   – Next sample may be more diagnostic

Try not be biased by clinical history

