Fight the Flight
Simplifying Body Fluid Analysis

Heather Lai MLS(ASCP)
Clinical Instructor of Hematology/Coagulation/Urinalysis
UnityPoint Health St. Luke’s Hospital
Cedar Rapids, IA
Disclosure

• I am receiving an honorarium from Sysmex for today’s presentation
I’m just going to head to lunch now...
Objectives

• Discuss manual and automated counting techniques
• Recognize normal cells found in body fluids
• Recognize characteristics of malignant cells
• Review patient case studies
Manual Counting

• Time consuming
• Error prone
  – Dilutions
  – Area correction
  – More RBC than Nucleated Cells (NC) or vice versa
  – Crenated RBC vs NC
  – Tech Inexperienced
HEMOCYTOMETER FLUID COUNTS

Patient Name

Accession #

Fluid Type

NOTE: If the whole side is counted, multiply the results by 1.1. If you count any number of large squares other than those listed; use the following guide for the appropriate area correction multiplication factor:

<table>
<thead>
<tr>
<th># Large Squares Counted</th>
<th>Multiplication Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

If you count the 5 small center squares (marked R) multiply the result by 50.

Count:

Tech 1 _____ (initials)

TNC \( x \) ______ = ______ RBC \( x \) ______ = ______

Tech 2 _____ (initials)

TNC \( x \) ______ = ______ RBC \( x \) ______ = ______

Average: The results of the two sides should be within 20% of each other - if not, flood another counting chamber and repeat the count.

Average TNC ________ RBC ________

Differential

<table>
<thead>
<tr>
<th></th>
<th>Tech 1:</th>
<th>Tech 2:</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Granulocytes</td>
<td>% Granulocytes</td>
<td>% Granulocytes</td>
<td>% Granulocytes</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>% Lymphocytes</td>
<td>% Lymphocytes</td>
<td>% Lymphocytes</td>
</tr>
<tr>
<td>% Maco/Meso</td>
<td>% Maco/Meso</td>
<td>% Maco/Meso</td>
<td>% Maco/Meso</td>
</tr>
<tr>
<td>% Other</td>
<td>% Other</td>
<td>% Other</td>
<td>% Other</td>
</tr>
</tbody>
</table>

Describe any other morphology:

________________________

________________________

________________________
IT'S THE 21 CENTURY MAN
THERE'S GOTTA BE A BETTER WAY
Automation!!!

Faster, Increased Precision, Fewer Dilutions
Preparing Sample for Analysis

• DON’T MAKE YOUR ANALYZER ANGRY
  – Treat synovial fluids with hyaluronidase
    • An aliquot, NOT the original

  – Remove clots and snots
    • Report count as > or Approximate
TNC? AMR? CRR?
Let’s break it down...

• TNC - Total Nucleated Cell count
  – Includes lining cells (such as mesothelial) as well as WBC’s

• AMR - Analytical Measurement Range
  – Range of analyte values that a method can directly measure on a specimen without any dilution, concentration, or other pretreatment not part of the usual assay process

• CRR - Clinical Reportable Range
  – How low and high YOU can report results
Establishing AMR and CRR

• XN AMR
  – 2,000-5,000,000 RBC/uL
  – 3-10,000 TNC/uL

• UF-1000 AMR
  – 0-5,000 RBC/uL
  – 0-5,000 TNC/uL

• St. Luke’s CRR
  – 0-5,000,000 RBC/uL
  – 0-100,000 TNC/uL

• Why are they different?
  – If we run a body fluid on XN and RBC is 0, we know it is really <2,000; could report as <2000
  – If we run a body fluid on UF-1000 and TNC is >5,000, could report as >5,000
I have automation, why would I ever do a manual count?

- **Low sample volumes**
  - Depends on sample requirement of your testing system
    - UF-1000 sample volume is 800 u/L
    - XN sample volume is 88 u/L

- **Sample not approved/correlated for automated analysis**
  - Bronchial Lavages
  - Low RBC count w/ small sample volume

- **Flags on Automated counts**
  - Or if cytospin doesn’t match automated counts

- **When you want to be mean**
  - Students
  - New Techs
What do these counts even mean?

• RBC counts are really only important for CSF
  – Hemorrhage (SAH) vs traumatic tap
    • RBC > in tube 1 than tube 4

• **Type of cell present is more important than the count**
  – Can still do a diff on clotted sample
  – Can still possibly do a diff on QNS sample
If you thought cell counts were scary...

I see cell clumps
A few things to help simplify

• Know how to make an adequate cytospin
  – How to recognize when it’s not good
• Be familiar with what cells are present in each fluid type
• Be familiar with characteristics of malignant cells
• Always scan on 10x first, perform diff on 50x
• Have 2 techs perform differential
• Have a good resource available for reference
  – Books
  – Pathology
Cytospin

• 20 fold concentration of cells
  – Cell count in fluids lower than peripheral blood
• Preserves cellular morphology
• Monolayer of cells
<table>
<thead>
<tr>
<th>Nucleated cell count</th>
<th># of Drops of Fluid</th>
<th>Add Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 500</td>
<td>5 drops</td>
<td>To bottom line if QNS fluid</td>
</tr>
<tr>
<td>501 – 1,000</td>
<td>3 drops</td>
<td>To bottom line</td>
</tr>
<tr>
<td>1,001 – 2,500</td>
<td>1 drop</td>
<td>To bottom line</td>
</tr>
<tr>
<td>2,501 – 10,000</td>
<td>1 drop</td>
<td>To top line</td>
</tr>
<tr>
<td>10,001 – 25,000</td>
<td>1 drop of 1:5 diluent (1 drop fl. + 4 drops NaCl)</td>
<td>To top line</td>
</tr>
<tr>
<td>25,001 – 50,000</td>
<td>1 drop of 1:10 diluent (1 drop fl. + 9 drops NaCl)</td>
<td>To top line</td>
</tr>
<tr>
<td>&gt;50,000</td>
<td>1 drop of 1:15 diluent (1 drop fl. + 14 drops NaCl)</td>
<td>To top line</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>#WBC/ cumm</th>
<th>#Drops 30% Albumin</th>
<th>Amount of CSF</th>
<th>Add Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>1</td>
<td>Fill to top line if enough CSF</td>
<td>To bottom line if QNS CSF</td>
</tr>
<tr>
<td>200 – 499</td>
<td>2</td>
<td>15 drops</td>
<td>To bottom line if QNS CSF</td>
</tr>
<tr>
<td>500 – 999</td>
<td>2</td>
<td>10 drops</td>
<td>To bottom line</td>
</tr>
<tr>
<td>1000 – 2499</td>
<td>3</td>
<td>4-5 drops</td>
<td>To top line</td>
</tr>
<tr>
<td>2500 – 4999</td>
<td>3</td>
<td>2 drops</td>
<td>To top line</td>
</tr>
<tr>
<td>5000 – 9999</td>
<td>4</td>
<td>1 drop</td>
<td>To top line</td>
</tr>
<tr>
<td>10000 – 24999</td>
<td>4</td>
<td>1 drop 1:5 dil (1 CSF + 5 NaCl)</td>
<td>To top line</td>
</tr>
<tr>
<td>25000 – 49999</td>
<td>5</td>
<td>1 drop 1:10 dil (1CSF + 9 NaCl)</td>
<td>To bottom line</td>
</tr>
<tr>
<td>≥ 50000</td>
<td>6</td>
<td>1 drop 1:15 dil (1 CSF + 14 NaCl)</td>
<td>To bottom line</td>
</tr>
</tbody>
</table>
Albumin or not?

- Textbook\(^1\) says to add albumin to CSF and serous fluids
  - If protein is too high cells shrink, making them difficult to identify
    - Remake slide without albumin
    - If no albumin used, use a little saline when preparing the cytospin
  - Lots of smudge cells = add albumin

Albumin or not?

Pleural Fluid

Albumin

No Albumin
Cell Types Present in Body Fluids

- All body fluids can have
  - WBC’s
    - Become comfortable with the characteristics of cell types in peripheral blood where they look less scary
      - Neutrophils can look hypersegmented or degenerated
      - Lymphs often appear more reactive with artifactually prominent nucleoli and cytoplasmic projections
      - Monocytes may have more abundant or vacuolated cytoplasm, or have phagocytosed material
  - RBC’s
  - “phages”
  - Malignant cells
    - Found most often in serous fluids
Cell Types Present in Body Fluids

- **Cerebrospinal fluid (CSF)**
  - Lining Cells-often present in clumps, more common in neonates
    - Ependymal
    - Choroid Plexus
    - Arachnoid

- Mesothelial cells are NOT present in CSF
  - Presence of large tissue cells are suspicious for malignancy
Cell Types Present in Body Fluids

• **Serous - Pleural, Pericardial, Peritoneal**
  
  – Mesothelial-lining cell
    
    • Individual or seen in clumps
      
      – No cytoplasmic molding
      
      – Flat clusters
    
    • Low NC (nuclear-cytoplasmic) ratio
    
    • Multinucleate
    
    • Cytoplasm can be light or dark blue (biphasic)
    
    • Galagan book-illustrations and images
Cells Present in Body Fluids

- **Synovial - joint fluid**
  - Synovial lining cells
    - Resemble mesothelial cells, but have denser cytoplasm
  - Crystals
    - Monosodium Urate - gout
    - Calcium Pyrophosphate - pseudogout
    - Cholesterol - chronic arthritis (RA)
  - Malignant cells are extremely rare
Cells Present in Body Fluids

• **Bronchial Lavage/Brushing**
  – Bronchial lining cells-row of cilia at one end
  • Considered “contaminant”
    – We report as # seen per 100, not including them in the differential
Characteristics of Malignant Cells

**Benign**
- Individual/flat clusters
- Separation window
- Low N:C ratio
- Uniformity
  - Size and shape of nucleus
  - Loose, homogeneous nuclear chromatin
  - Small/regular nucleoli

**Malignant**
- Ball like clusters
- Nuclear molding
- High N:C ratio (varies)
- Non Uniformity
  - Nuclear shape/size variation
    - Irregular/jagged/folded
  - Unevenly distributed chromatin
  - Nucleoli
    - Prominent, frequently multiple, irregular membrane
Characteristics of Malignant Cells

• None of the features can be used alone

• What not to use to differentiate benign and malignant cells
  – Mitotic activity-reactive mesos also undergo mitosis
  – Cytoplasmic vacuoles-can also represent early degradation
Characteristics of Malignant Cells

The most common nonhematopoietic malignancies seen in body fluids are small-cell carcinoma and adenocarcinoma

**Small cell carcinoma**
- High N:C ratio
- Blast like chromatin
- Absent or non-prominent nucleoli
- Frequent nuclear molding
- Paranuclear blue bodies

**Adenocarcinoma**
- Overall larger cell size
- Moderate to abundant cytoplasm (Low N:C ratio)
- Nuclear chromatin partially clumped and heterogeneous
- Prominent irregular nucleoli
How do I know I’m not missing something

• Start out all diffs by scanning on 10x
  – Look for malignant cells
  – Find representative area to count

• Perform count on 50x

• Always have 2 techs diff body fluids
  – Most don’t have abnormal cells, so it’s easy to assume that they won’t or techs can be in a hurry and miss these cells
Patient 1

• 60yr Female
• History of lobular carcinoma of the breast
• CSF
  – TNC-31
  – RBC-1
  – Diff= 1% Neuts, 6% Lymphs, 13% Macrophages, 80% other
Mesothelial cells are NOT present in CSF.
Patient 2

- 75 yr Female
- Presents with pleural effusion
- Pleural fluid
  - TNC-4300
  - RBC-2900
  - Diff= 18% Neut, 53% Lymph, 26% Mono/Macro/Meso, 3% Other
Pleural 10x

Why only 3% "others"?
Non-small cell carcinoma- cytologic and immunohistochemical features favor adenocarcinoma (large cells with moderate cytoplasm)
Patient 3

• 69 yr Female
• History of ovarian cancer
• Presents with ascites and left sided pleural effusion on evening shift
• Peritoneal fluid
  – TNC-2172
  – RBC-2848
  – Diff=31% Neuts, 30% Lymphs, 39% Macrophages
• Pleural fluid
  – TNC-476
  – RBC-25,825
  – Diff left for days
PLEURAL
10X
I FOUND ONE NORMAL FIELD!!
Adenocarcinoma admixed with inflammatory cells and reactive lymphocytes
My inner nerd is showing....
Patient 3 con’t

- Patient report had to be amended, had this been the only fluid sent, it would have probably been missed
Patient 4

- 67 yr Male
- History of renal transplant
- Pleural Fluid
  - TNC-3331
  - RBC-179,000
  - First tech counted primarily macrophages
SECOND TECH COUNTED 87% "OTHER"

Diffuse Large B-cell Lymphoma
Patient 5

• 93yr Female
• Presents with bilateral pleural effusion
• Pleural fluid
  – TNC-531
  – RBC-2346
  – Diff= 5% Neut, 15% Lymph, 80% Macro/Meso
Patient had pneumonia of both lower lobes. Mesothelial cells look reactive, but very uniform throughout the sample.
Why are these cells ok and the others weren’t?

OK

Not OK

Flat vs Ball like clusters
Separation window vs Nuclear Molding

Low N:C ratio vs High N:C ratio

Loose homogenous chromatin vs Unevenly distributed chromatin

Uniform nuclear size/shape vs Varied nuclear size

Uniform nuclear size/shape vs Varied nuclear size

No visible nucleoli vs Multiple prominent, irregularly shaped nucleoli
Being unfamiliar or unsure of what a cell is doesn’t make you a bad tech. Pretending it’s not there does.
Summary

• Don’t make it harder than it needs to be
• Know what’s normal
• Practice what you aren’t comfortable with
• Utilize rules that prevent errors
• Use your resources
Questions?