Interpreting Laboratory Data:
Infectious Diseases – Immune Response

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Objectives

1. Identify the five major components of a WBC
2. Describe the significance of a rise in WBC as it relates to infectious diseases
3. Understand the significance of elevated bands, eosinophils, monocytes, basophils, and lymphocytes in a CBC
4. List other parameters (non-hematological) which may indicate the presence of an infection
5. Identify laboratory parameters which need to be monitored during antibiotic therapy
Opening Case
The case of Mr. T.
Case

- You are on your hospital rotation and you encounter Mr. T. He is lying in the ICU s/p GSW to the chest secondary to an A-Team Mission that went bad. Mr. T. was shot in his abdomen 2 days ago but did not present to the ER until today. He has lost a significant amount of blood over the last 2 days. The wound site is swollen, red, and has a greenish-yellow colored discharge. You review his chart and find the following information:

<table>
<thead>
<tr>
<th>CBC</th>
<th>Temp</th>
<th>AST/ALT</th>
<th>BP</th>
<th>Pulse</th>
<th>SCR</th>
<th>BUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.8</td>
<td>103.4</td>
<td>140/191</td>
<td>92/54</td>
<td>110 bpm</td>
<td>1.72mg/dl</td>
<td>35.91</td>
</tr>
<tr>
<td>21</td>
<td>28.0</td>
<td>42/12/0.5/1.0/25/3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CBC Shorthand

CBC:

WBC → Hgb → platelets → MCV/MCH/MCHC

Hct → segs/bands/lymphs/monos/basos/eos
Components of a CBC
Focus on WBC
<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Phagocytosis</td>
<td>3-5 % (bands)</td>
</tr>
<tr>
<td>Banded Neutrophils (bands)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segmented neutrophils (segs/PMN)</td>
<td></td>
<td>50-60 % (segs/PMN)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>acidophils</td>
<td>0-3 %</td>
</tr>
<tr>
<td>Basophils</td>
<td>histamine release</td>
<td>0-1 %</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Phagocytosis</td>
<td>3-7 %</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Antibodies</td>
<td>25-35 %</td>
</tr>
</tbody>
</table>
## WBC Components and Function

<table>
<thead>
<tr>
<th>Component</th>
<th>Increase in this component can be indicative of an infection caused by</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutrophils (PMN)</strong></td>
<td>Elevated in bacterial infections</td>
</tr>
<tr>
<td>Banded Neutrophils (bands)</td>
<td>Elevated from high dose or prolonged steroid use</td>
</tr>
<tr>
<td>Segmented neutrophils (segs)</td>
<td>Low in patients on chemotherapy</td>
</tr>
<tr>
<td></td>
<td>Low in patients on immunosuppressive therapy</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>Elevated in patients with a history of allergies</td>
</tr>
<tr>
<td></td>
<td>Elevated in parasitic infections</td>
</tr>
<tr>
<td><strong>Basophils</strong></td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Monocytes</strong></td>
<td>Elevation indicative of auto-immune disease</td>
</tr>
<tr>
<td></td>
<td>Elevation may be due to chronic bacterial infection</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td>Low in cancer (leukemia)</td>
</tr>
<tr>
<td></td>
<td>Elevated in most viral and bacterial infections</td>
</tr>
</tbody>
</table>
Leukocytosis secondary to neutrophilia especially increase in bands due to infection.

3% of NE in circulation (PMN/seg)
7% of NE in organs (PMN/seg)
90% of NE in bone marrow (bands)

7-10% of NE in circulation (Segs/PMN and bands)
6% of NE in organs (segs/PMN)
84-87% of NE in bone marrow (bands)
Absolute Neutrophil Count (ANC)

- ANC is the total amount of neutrophils in the blood
- Calculation:  
  \[ \text{ANC} = (\% \text{ of Bands} + \% \text{ of Segs}) \times \text{WBC} \]

- Example 1:  
  - WBC = 9,500  Segs \% = 32\%  Bands\% = 2\%  
  - \[ \text{ANC} = (32\%+2\%) \times 9500 = 0.34 \times 9500 = 3230 \]

- Example 2:  
  - WBC = 3,100  Segs 24\%  Bands 3\%  
  - \[ \text{ANC} = (24\%+2\%) \times 3100 = 0.26 \times 3100 = 806 \]
ANC continued

- ANC is one of the main determinants of the presence of immunosuppression in a patient
- Often seen in patients with AIDS, Hepatitis C therapy with interferon, chemotherapy patients, and patients with opportunistic infections secondary to HIV/AIDS
A patient with a WBC of 8600, Band % of 6%, and ANC of 3440 would expect to have a Seg% of_______

ANC = Total Neutrophil % x WBC
     = (Band % + Seg %) x WBC
3440 = (0.06 + X) x 8600

Rearrange Equation:

X = 3440 – (8600x 0.06)

------------------------- = 0.34 = 34% Segs
     8600
Other Hematological Markers for Infections

- **Non-Specific**
  1. C-Reactive Protein (CRP)
  2. Erythrocyte Sedimentation Rate (ESR)
  3. Procalcitonin (PCT)

- **Specific**
  1. IgM Antibody
  2. IgG Antibody
  3. Viral load/count
Clinical s/sx of infection

Localized Infection

- Four hallmark signs of inflammation:
  - Redness, heat, swelling, and pain
- Erythema
- Skin lesions/abcess
- Purulent discharge
- Increased secretions (sputum, polyuria, excessive tearing, unusual discharge)
- Diarrhea
- Headache, stiff neck, or unusual rigidity in a localized area
Localized Infection (staph)
Localized Infection
Staph vs Stept
Localized infection (pseudomonas)
Localized infection (Fungal)
Systemic s/sx of Infection

- Fever
  - Temperature of > 100.4 degrees Celsius
  - Two consecutive temperatures > 101.0 degrees Celsius
  - In Febrile neutropenia
    - > 101.0 degrees Celsius
    - > 100.4 degrees Celsius for > 1 hour
- Malaise
- Chills/rigors
- Tachycardia/hypotension
- Mental status changes/confusion
Radiographic Evidence of Infection

- Chest X-ray/MRI/CT:
  - Presence of consolidation
  - Presence of infiltrates
  - Presence of effusions
  - Presence of nodules
- Bone X-ray/MRI/CT/Bone Scan
- Head CT/MRI
  - Presence of lesions
- Radioactive Labeled WBC (Gallium Scan)
Radiographic Evidence of Infection
Radiographic Evidence of Infection
Radiographic Evidence of Infection

http://www.youtube.com/watch?v=LMWC85PN4WE
Radiographic Evidence of Infection
Culture & Sensitivity

1. **Gram Stain**
   
   Used to distinguish Gram positive from Gram negative bacteria and identify the shape of a bacteria

2. **Culturing Bacteria**
   
   the process of growing a small number of bacteria into a larger number to perform further testing

3. **Sensitivity Testing**
   
   the process where the cultured bacteria are tested against several antibiotics to determine which antibiotic kills the bacteria most effectively
Culture & Sensitivity

4. **Mean Bactericidal Concentration Testing**
   Used in specific clinical scenarios to detect “tolerance”

5. **Synergy Testing**
   A specific test to determine the advantage of combining more than one antibiotic to kill a bacteria compared to using only one agent

6. **D-Test**
   Used to determine if erythromycin induced clindamycin resistance is present in a MRSA colony
1. Gram Stain

- First discovered by Hans Christian Gram in 1884
- Used to distinguish Gram positive from Gram negative bacteria prior to culturing the bacteria
- Utilizes Crystal Violet, iodine, and Safranin to stain bacteria
- Gram positive bacteria stain violet/purple
- Gram negative bacteria stain pink
Gram Stain

(a) Application of crystal violet
(b) Application of iodine
(c) Alcohol wash
(d) Application of safranin

http://www.sirinet.net/~gjohnso/monorans.html
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive cocci in clusters</td>
<td>Staphlococcus sp</td>
</tr>
<tr>
<td>Gram positive cocci in pairs</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>Gram positive cocci in chains</td>
<td>Alpha/beta hemolytic streptococcus peptostreptococcus</td>
</tr>
<tr>
<td>Gram positive cocci in pairs and chains</td>
<td>Enterococcus sp</td>
</tr>
<tr>
<td>Gram negative coccobacilli</td>
<td>Haemophilus sp, Moraxella catarrhalis</td>
</tr>
<tr>
<td>Gram variable bacilli</td>
<td>Gardenella vaginallis</td>
</tr>
</tbody>
</table>
1. General Culture Information

- Bacteria are divided into three groups:
  - Gram positive, Gram negative, and Mycobacterium
- Culture refers to the process of growing a small number of bacteria obtained from the pt into a larger number of bacteria
- Purpose is to increase the number of bacteria to allow for identification and subsequent sensitivity testing to antibiotics
1. General Culture Information

- Multiple types of media are used to grow various bacteria and fungi
- Usually take 1-5 days to grow aerobic bacteria
- May take up to 14-21 days to grow anaerobic bacteria and some fungi
General Culture Information
General Sensitivity Information

- Follows a culture and is the process where the isolated bacteria are tested against specific antibiotics
  - Reason for this testing is to determine which antibiotics are able to kill the bacteria most effectively
- Based on two parameters:
  - MIC (most important)
  - MBC (limited clinical utility)
General Sensitivity Information

- The lower the MIC value for a specific Abx-bacteria combination, the more sensitive the bacteria is to the Abx.
- The closer the MIC and the MBC values, the more bacteriocidal (ability to kill bacteria) the abx, and more rapid bacterial elimination occurs.
- MIC can not be translated across abx entities or different bacteria.
- MIC breakpoints differentiate whether the bacteria is sensitive, intermediate, and resistant to the given abx.
- MIC for a certain bacteria-Abx combination can change and are updated annually the CLSI (previously known as NCCLS).
Laboratory Interpretations for Urinary Tract Infections/ Urinalysis
Urine Content

• Urine is a healthy person is sterile (bacteria free)

• **Urine in healthy people contains:**
  • a pH of
  • a specific gravity (density) of
  • a yellow color
  • Minerals such as Na, Ca, Sulfates, K, water (95%), HCO3…ect

• **Urine in healthy people should not contain:**
  • Bacteria
  • Odor
  • Turbidity
  • Nitrites
  • Casts, proteins, RBC, and WBC
Urine Analysis

• In regards to urinary tract infections, the following as important indicators on a urine analysis:
  • Presence of WBC
  • Abnormal specific gravity (usually higher than normal)
  • Abnormal pH (usually a higher pH than normal)
  • Presence of nitrites
  • Presence bacteria
  • Presence of leukocyte esterase
  • Presence of casts in the urine

• Remember a urine analysis does not necessarily help identify the organism but helps determine if bacteria are present
Urine Analysis

- **Specific Gravity**
  - General range: 0.002 - 0.035
  - Hydrated/non-impaired range: 0.01 – 0.02
  - UTI range:

- **pH**
  - Normal urine pH is ~6.0
  - UTI cause urine pH to increase (more alkaline) from 6-8
    - Due to the production of urase by some bacteria (increased ammonia)
Urine Analysis

• **Leukocyte Esterase:**
  • An enzyme present in WBC and thus a result of increased WBC in the nephrons and renal tubules
  • Evidence of immune system response to UTI

• **Casts:**
  • A product of WBC and RBC breakdown in the renal tubules which result in cast shaped objects indicative of WBC and/or RBC presence

• **Nitrites:**
  • Certain bacteria in the urine are able to convert nitrates to nitrites. Presence of nitrites is indicative of the presence of these bacteria
## Test Your Knowledge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Increase/Present</th>
<th>Decrease/Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>✅</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>✅</td>
<td></td>
</tr>
<tr>
<td>Leukocyte Esterase</td>
<td>✅</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>✅</td>
<td>✅</td>
</tr>
<tr>
<td>Casts</td>
<td>✅</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>✅</td>
<td></td>
</tr>
</tbody>
</table>
TEST YOUR KNOWLEDGE

• Which of the following are not indicators of a urinary tract infection in a urine analysis:
  1. Increase in pH of the urine
  2. Increase in specific gravity of urine
  3. Absence of nitrite in the urine
  4. Presence of Leukocyte esterase in the urine
  5. Presence of casts in the urine
When a urine analysis is indicative of a possible infection causing a urine culture to be performed.
Urine Culture

- High likelihood of contamination from skin bacterial flora and flora in the GU tract
- Higher incidence of contamination in female vs male patients
- Ideally should be obtained from first void of the day and not after a recent void/bowel movement
- Mid-stream sample recommended in both males and females
- Most precise method is “straight catheterization” where a catheter is placed into the bladder to obtain urine within the bladder
- Know the method used to collect the sample
Urine Culture and Sensitivity

Specimen: Urine (mid-stream collection)
Report: Final
Organism Identified: >100,000 CFU E. Coli

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>XX</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>XX</td>
<td>Resistant</td>
</tr>
<tr>
<td>Imipenem</td>
<td>XX</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>XX</td>
<td>Sensitive</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>XX</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>
Blood Cultures
Blood Cultures

- Differ from other cultures as the infected site is the Blood, hence blood is collected to identify the bacteria
- High incidence of contamination from the needle puncturing the skin
- Must be obtained from more than one site to decrease the chances of contamination from the skin
- Consists of 4 bottles of blood (~10ml each)
  - 2 bottles for aerobic testing
  - 2 bottles for anaerobic testing
  - Each pair of one aerobic and anaerobic bottles = 1 SET
Blood Cultures

SET 1
2 Bottles:
1 Aerobic
1 Anaerobic

SET 2
2 Bottles:
1 Aerobic
1 Anaerobic
Blood Cultures

- Usually reported as 2 separate reports
  - 1 report per blood culture set (1 report per 2 bottles from same site of blood draw)

- Results will usually read:
  - No growth in X hours
  - No growth in X days (Max 5 days)
  - S. aureus isolated from aerobic bottle in Set in X hours

- Results should be communicated to the healthcare team as:
  - No growth in both sets in X days
  - 2 out of 4 bottles positive for S. aureus in 2 aerobic bottles
  - 1 out of 4 bottles positive for S. epidermis in aerobic bottle
Blood Cultures

- Likelihood of actual bacteremia (blood infection):
  - 4 of 4 bottles positive for bacteria X
  - 3 of 4 bottles positive for bacteria X
  - 1 bottle positive from each set (same type bottle same bacteria)
  - 1 Set positive and 1 set negative (same bacteria)
  - 1 Set positive and 1 set negative (different bacteria)
  - 1 out of 4 bottles positive for a bacteria that is not a skin colonizer
  - 1 out of 4 bottles positive for a bacteria which is a known skin colonizer (e.g. Coagulase Negative Staphylococcus/CoNS)
Test Your Knowledge

- You watch as an ER nurse takes a blood culture. You watch the nurse as she takes an alcohol pad to clean the patient’s left and right arm site with the pad and then uses the same alcohol pad to wipe off the tops of the blood culture bottles prior to collecting the blood.

- What is the problem here?
Meningitis

- Often diagnosed based on clinical s/sx
- When suspicion is high, a lumbar puncture is often utilized to rule out/in infection in the CNS
- Invasive procedure = risk of introduction of infection or new organisms
- Risk of adverse effects secondary to procedure
## Meningitis

<table>
<thead>
<tr>
<th>Parameter for CSF Infection</th>
<th>Normal</th>
<th>Bacterial Meningitis</th>
<th>Viral Meningitis</th>
<th>Fungal Meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening Pressure</td>
<td>&lt;180 mm H₂O</td>
<td>&gt;195</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>WBC</td>
<td>0-5/mm³</td>
<td>400-20,000</td>
<td>5-2000</td>
<td>20-2000</td>
</tr>
<tr>
<td>WBC differential</td>
<td>Normal</td>
<td>&gt;80% PMNs</td>
<td>&gt;50% lymphs</td>
<td>&gt;50% lymph PMN variable</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt; 50 mg/dL</td>
<td>&gt;100</td>
<td>30-150</td>
<td>40-150</td>
</tr>
<tr>
<td>Glucose</td>
<td>45-100 mg/dL</td>
<td>&lt;45</td>
<td>45-70</td>
<td>30-70</td>
</tr>
<tr>
<td>Gram Stain (% positive)</td>
<td>N/A</td>
<td>60-90</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
# Test Your understanding

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</table>
Other Infections

- Tuberculosis (M. tuberculosis) pneumonia
- Legionella pneumonia
- Syphilis
- C. difficile infection
Viral infections

HIV Infection
HIV Viral Count and CD4+
HIV Infection

- Viral infection which involves invasion of CD4+ cells by HIV virus
- Effects all CD4+ cells including memory cells leading to cell death
- As HIV virus replicates, CD4+ cell number decrease
- When CD4+ reaches to < 200 cells/ml = AIDS
Diagnosis of HIV

- Several rapid ELISA tests are available on the market
  - Works by detecting antibodies to HIV
  - High sensitivity to HIV-1 but low specificity
  - Low incidence of false negatives
  - High incidence of false positives

- Western Blot:
  - Works but detecting certain proteins within HIV
  - Low sensitivity with High specificity
  - Used as a confirmatory test following ELISA
  - Not used for diagnosis of HIV without ELISA
HIV Testing

ELISA

W-B
HIV Infection

- In HIV patients who are treatment naive, HIV viral load is often detectable (>50 copies/ml) while CD4+ count will be below normal.

- Goal of therapy is to have an undetectable HIV viral load (<50 copies/ml) and a CD4+ > 200.

- The lower the CD4+ drops prior to initiation of therapy, the decreased capability of CD4+ to rise after treatment initiation.
  - In other words, the later treatment is started, the potential to recover CD4+ number back to normal becomes more difficult.

- The lower the CD4+, the higher the risk of secondary (opportunistic infections).
QUESTIONS?