Complete Blood Count (CBC) Basics

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Intro to the CBC

- Blood = plasma (albumin, clotting factors) + cells
- CBC = complete blood count, quantifies 3 types of blood cells
  - Red blood cells (RBCs)
  - White blood cells (WBCs)
  - Platelets
- Gives additional info, e.g.,
  - % of blood composed of RBCs – “hematocrit”
  - Different types of WBCs present – “differential”
Collection

- 3 to 10 ml of whole blood drawn into tube containing anticoagulant
  - EDTA
  - Heparin
  - Citrate
- Most common: purple-top tube with EDTA to chelate Ca2+
Cell count analysis

• Sample dilution and even distribution of cells essential to accurate counts

• Different solution for different cells
  • RBC counts need isotonic solution
  • WBC and platelet counts use RBC-lysis solution

• Performed by manual counts or automated analyzers
Manual counts

- Rarely used for absolute count
- May use hemocytometer for platelet or low WBC counts
  - Counting chamber with specific volume
  - Viewed with microscope
Automated analyzers

• Increased accuracy and speed
• Based on electrical impedance, light scattering, radiofrequency conductivity, or cytochemical reactions
• Electrical impedance
  • Change of voltage when cells pass through aperture
Automated analyzers

• Flow cytometry and light scatter
• Laser hits single stream of cells, light scatter interpreted into info on size, structure, granularity
CBC parameters

RBC
- Oxygen carrying cells
- Anucleate (unless immature)

WBC
- Immune function

Platelets
- Clotting
- Anucleate cell “fragments”

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Minimizing error

- All laboratory processes are subject to error
- Examples of sources of error in the CBC will be discussed under each cell type
- Automated analyzer computers have multiple programs to detect possible error
- If data meets possible error criteria, data is “flagged” for operator review prior to release
RBC parameters

• Quantitative
  • Hemoglobin (Hgb, g/dL)
  • Hematocrit (Hct, %)
  • RBC count (per µL)

• Qualitative (averages)
  • Mean corpuscular volume (MCV, fL)
  • Mean corpuscular hemoglobin (MCH, pg)
  • Mean corpuscular hemoglobin concentration (MCHC, g/dL)
  • Red cell distribution width (RDW, %)

• Sometimes – reticulocyte count
RBC parameters

Hemoglobin (Hgb, g/dL)
- Colored protein, measured by absorbance at 540nm

Hematocrit (Hct, %)
- Proportion of volume occupied by RBCs
- Manual – height of column after centrifugation
- Automated – RBC number/RBC volume

RBC count (cells per µL)
- Obtained via electrical impedance and/or light scatter
- RBCs and WBCs counted together by analyzer
  - RBC outnumber WBC ~500:1, negligible error
RBC parameters

• Mean corpuscular volume (MCV, fL)
  • Average volume of each RBC
  • RBC volumes/RBC count

• Mean corpuscular hemoglobin (MCH, pg)
  • Average Hgb in each RBC
  • Hgb/RBC count

• Mean corpuscular hemoglobin concentration (MCHC, g/dL)
  • Average Hgb concentration in each RBC
  • Hgb/Hct

• These three are calculated averages – may not accurately describe mixed population of cells
RBC parameters

- Red cell distribution width (RDW, %)
  - Range of RBC sizes

- Reticulocytes
  - Immature, anucleated cells containing RNA
  - Reflect bone marrow’s ability to make new RBCs
  - Manual – “supravital” stain of ppt RNA
  - Automated – fluorescent dye stains RNA
RBC parameters – clinical scenarios

- Decreased Hgb, Hct, or RBC count – **anemia**
  - 1° - iron deficiency anemia
  - 2° - acute blood loss

- Increased Hgb, Hct, or RBC count – **polycythemia**
  - 1° – bone marrow proliferative disease
  - 2° – compensation due to chronically low oxygen from smoking, sleep apnea, high altitude
## RBC parameters – examples of error

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Falsely increased</th>
<th>Falsely decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBC count</td>
<td>High WBC count</td>
<td>Hemolysis (in vitro), clotting</td>
</tr>
<tr>
<td>Hct</td>
<td>Giant platelets</td>
<td>Hemolysis (in vitro), clotting</td>
</tr>
<tr>
<td>MCV</td>
<td>Cell clumping</td>
<td>Giant platelets</td>
</tr>
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</table>

- **Giant platelet**
- **Hemolyzed**
- **Non-hemolyzed**
WBC parameters

- Quantitative cell count (cells per µL)
- Differential (cells per µL and % of total WBCs)
WBC parameters

• Quantitative cell count (cells per µL)
  • Dilution of blood in RBC lysis buffer, usually acid or detergent
  • Total count of nucleated cells obtained by electrical impedance or flow cytometry (automated) or hemocytometer (manual)
WBC parameters

- Differential (cells per µL and % of total WBCs)

- Immature neutrophil “band form”
- Neutrophil
- Basophil
- Eosinophil
- Lymphocyte
- Monocyte
WBC differential

- Automated
  - Individual cells analyzed by flow cytometry
- Light scatter
  - Forward (cell size)
  - Side (complexity, granularity)
- Cells identified based on expected profile
  - E.g., neutrophils – larger than lymphocytes with granular complexity, monocytes – fewer granules than neutrophils and therefore, less SSC

Image was originally published in ASH Image Bank. Maslak and Rose. White cell differential -1. ASH Image Bank. 2008; 00003658. © the American Society of Hematology.
WBC differential

- Abnormal cells that do not identify as WBCs are tagged for manual review
  - E.g., atypical lymphocytes, immature blasts

- Manual review
  - Drop of blood smeared on glass slide
  - Dyes
    - Basic (nuclei, basophilic)
    - Acidic (eosinophilic)
WBC parameters – clinical scenarios

- Decreased total WBC count – leukopenia
  - 1° - HIV, bone marrow disease, e.g., aplastic anemia
  - 2° - immunosuppressants

- Increased total WBC count – leukocytosis
  - 1° - acute leukemia
  - 2° - neutrophilia and lymphocytosis from infection

This image was originally published in ASH Image Bank. Maslak. Neutrophilia-1. ASH Image Bank. 2008; 00003785. © the American Society of Hematology.
WBC parameters – examples of error

• Total WBC count – falsely increased
  • Nucleated RBCs (counted as WBCs)
  • Antibodies that cause RBC clumping

• Differential
  • Automated –
    - Abnormal cells incorrectly identified
  • Manual –
    - Poor staining leading to cell recognition errors
    - Larger cells pushed to edge, missed in count
Platelet parameters

- Platelet count
  - Obtained by electrical impedance or light scatter (automated) or hemocytometer (manual)
Platelet parameters – clinical scenarios

- Decreased platelet count – **thrombocytopenia**
  - 1° - decreased bone marrow production
  - 2° - immune-mediated destruction/sequestration

- Increased platelet count – **thrombocytosis**
  - 1° - proliferative bone marrow disease
  - 2° - acute phase reactant, e.g., inflammation
Platelet parameters – examples of error

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<tr>
<td>Platelet count</td>
<td>Hemolysis (in vitro), microcytic (small) red cells</td>
<td>Giant platelets, platelet clumping</td>
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[Images of microcytes and giant platelet]
Summary

• CBC is an important screening and diagnostic tool
  • Majority of specimens run on automated analyzers which use impedance and flow cytometry for cell identification and enumeration
  • Manual review and count reserved for abnormal specimens or patients with clinical history
• Knowledge of error sources essential to accurate interpretation
References


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