Basic Hematology

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LEARNING OBJECTIVES

1. Define “CBC”
2. Define and explain red cell indices including derived values such as MCV, MCHC and MCH
3. Define and explain erythrocyte sedimentation rate
4. Describe the main cell types observed in peripheral blood.
5. Describe what is meant by the term “differential count”.
6. Describe the distribution & morphology of cells in certain common “Blood Pictures”
Clinical Diagnostic Laboratories offer:

1. “CBC”
2. “CBC plus Diff.” [CBC + Differential count]
3. Above ± “Reticulocyte count”

What do these terms mean, and how are they used?
CBC

1. White Cell count (leukocyte count)

2. Platelet count

3. Red cell count, hemoglobin concentration and red cell indices
What Specimen?

• Whole blood, uncentrifuged, anticoagulated with EDTA (Purple or lavender cap)
Cell Counts

• White cells, red cells (erythrocytes) and platelets are counted per unit volume of whole blood.

• Unit volume: per cubic millimeter (mm³) which is the same as µL

• WBC  4.0–10.0 x 10³/cu mm
• Platelets  150–450 x 10³/cu mm
• RBC  4.5–5.9 x 10⁶/cu mm
Cell Counts

- Unit volume: per liter (SI units).

- WBC  $4.0-10.0 \times 10^9/L$
- Platelets  $150-450 \times 10^9/L$
- RBC  $4.5-5.9 \times 10^{12}/L$
Hemoglobin & Red Cell Indices

1. Hemoglobin
2. Hematocrit
3. Red cell count
4. Mean cell (corpuscular) *volume* or MCV
5. Mean cell *hemoglobin concentration* or MCHC
6. Mean cell *hemoglobin content* or MCH
7. Red cell distribution width or RDW
1) **Hemoglobin concentration**
in grams/dL (grams per 100 mL)

Normal: 12 - 16 g/dL or 120 – 160 g/L
Hemoglobin concentration

- Hemoglobin is converted to CYANOMETHEMOGLOBIN which absorbs maximally at 540 nm

- Hb plus KCN plus potassium ferricyanide + NaHCO₃
- Heme iron (Fe²⁺) → Fe³⁺
- Methemoglobin binds with CN
Hemoglobin concentration

• Most current hematology analyzers have switched to a new, alternative method: *sodium lauryl sulfate* (SLS, SDS) binds to hemoglobin.

• Sulfated derivative absorbs at 535 nm
CYANMETHEMOGLOBIN

DEOXYHEMOGLOBIN

OXYHEMOGLOBIN

Absorbance

540 nm
Na Lauryl Sulfate (SLS)-Hemoglobin

Absorbance

540 nm

CYANMETHEMOGLOBIN

Professional Practice in Clinical Chemistry
2) Hematocrit (the percentage of blood that is represented by the packed red cells) determined by blood centrifugation are termed “spun hematocrits.”

Normal: 35 - 45%

Hematocrits determined by blood centrifugation are termed “spun hematocrits.”
3) **Red cell count.** *Number of red blood cells per microliter of blood (or per liter)*

Normal: 4 - 5.5 \( \times 10^6 \) /uL
4) **Mean Cell Volume** (MCV) = red cell volume in *femtoliters* or $10^{-15}$ liter

Normal: 78 - 100 *femtoliters*

Low MCV = Microcytic
High MCV = Macrocytic
5) **MCHC** (Mean cell hemoglobin concentration):

Hemoglobin concentration of the packed red cells (minus plasma)

- **Low MCHC:** Hypochromic
- **High MCHC:** Hyperchromic

Normal: 31-37 g/dL (of erythrocytes)
6) **Mean Cell Hemoglobin** (MCH) = red cell hemoglobin **content** in picograms or $10^{-12}$ grams

Normal: 26 - 32 pg per red cell
MCHC: reflects the concentration of Hb in the red cell (g/dL)

MCH: reflects the Hb content (in picograms) of each red cell

Both

Small vs Big

“Pale” vs “Deep Red”

Hypo- vs Hyperchromic

MCV: reflects the cell volume in femtoliters
\[
\text{MEAN CELL femtoliters} = \frac{\text{Hematocrit} \times 10}{\text{RBC}}
\]
\[
\text{MEAN CELL pg per RBC} = \frac{\text{Hemoglobin} \times 10}{\text{RBC}}
\]
\[
\text{MEAN CELL HEMOGLOBIN g/dL of erythrocytes} = \frac{\text{Hemoglobin} \times 100}{\text{HCT}}
\]
All automated cell counters can generate a red cell volume - distribution histogram.
7) Red cell Distribution Width (RDW)

- The *coefficient of variation* of the red cell volume distribution histogram
<table>
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<tr>
<th>Formula</th>
<th>Units</th>
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<td><strong>MEAN CELL VOLUME (MCV)</strong></td>
<td>RBC</td>
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<td><strong>MEAN CELL HEMOGLOBIN CONC (MCHC)</strong></td>
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<tr>
<td><strong>Hematocrit</strong></td>
<td>RBC</td>
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<td>Directly measured</td>
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Reticulocytes

1. Immature red cells containing residual RNA
2. Indicate rate of new RBC production
The % reticulocytes assess the bone marrow response to anemia.

• In a hemolytic anemia → ↑ reticulocytes

• In bone marrow disease (impaired erythrocye production) → ↓ reticulocytes
Normal stain

Reticulocyte

Normal stain

Using special reticulocyte (supravital) stain
Reticulocytes
RETICULOCYTES

- The retic count multiplied by the patient's hct divided by the expected hct (45%) is the standard **reticulocyte correction formula**.

\[
\text{Patient HCT} \times 100 \quad \frac{\text{Standard HCT (45%)}}{\text{Patient HCT}}
\]
Erythrocyte sedimentation rate or ESR

- Anticoagulated whole blood (4 parts whole blood: 1 part Na Citrate) is placed in a narrow-bore glass tube. Erythrocytes are allowed to sediment at room temperature.

- ESR measures the distance, in millimeters, that erythrocytes fall in 1 hour.
Blood Column
Plasma
Sedimenting RBCs
Blood Column
Professional Practice in Clinical Chemistry
Erythrocyte sedimentation rate or ESR

- Rate of sedimentation is determined by plasma proteins. ESR increases with acute phase response
- This is an *indirect* determination of inflammation
- Used to follow rheumatoid arthritis, SLE, vasculitis and many inflammatory conditions
- VERY LOW SPECIFICITY
Erythrocyte sedimentation rate or ESR

- **Westergren Method**: 200 mm tube
- **Wintrobe Method**: 100 mm tube
- **Modern Methods**: Semi-automated systems that measure sedimentation by infra-red light. Measured over a shorter period than 1 hour & extrapolated to give Westergren ESR.
- Can interface with LIS
BLOOD SMEAR
The Blood Smear
Stained Blood Smear
A “Differential Count”

1. Stained peripheral blood smear is examined carefully using 40 x to 100 x objective – usually oil immersion lens
2. 100 white blood cells are counted
3. Cells are classified by morphology→

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<td>Promyelocytes</td>
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<tr>
<td>Blasts</td>
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Eosinophil, Lymphocyte & Neutrophil
Neutrophil and Lymphocyte
Neutrophils - The main phagocytic cells of peripheral blood.
Monocyte
Monocytes
Monocyte and Lymphocyte
Monocyte and Neutrophil
Eosinophil
**Basophils** - involved in IgE-mediated hypersensitivity response
Neutrophil maturation
Promyelocyte → Metamyelocyte → Band → Myelocyte → Mature segmented Cell
Neutrophil “Bands” - immature cells, classically with a “horseshoe-shaped” nucleus.
METAMYELOCYTES
Myelocytes - very immature neutrophils with a round or near-round nucleus
Lymphocytes - classic small variety;
Lymphocytes – Large granular ("LGL")
Atypical Lymphocytes
Blast
Common Blood “Pictures”
Reactive neutrophilia with “left shift”
Atypical Lymphocytes (reactive)
Acute Leukemia with larger blasts
Blast
Acute Leukemia with smaller blasts
Chronic Myelogenous Leukemia (CML)
Chronic Lymphocytic Leukemia (CLL)
END
Self-Assessment Question #1

Regarding the mean cell volume or MCV:

a) **This is now determined directly by electrical or flow cytometric methods.**

b) it is always calculated from the spun hematocrit.

c) it is determined by light microscopy.

d) it is determined by electron microscopy.

e) it is derived from the white cell count.

Explanation: The red cell MCV is measured directly on cell counters; this is true whatever method is employed to enumerate cells. The counter is able to plot a red cell volume histogram, and the mean is determined. MCV can be calculated from the spun hematocrit as in option b. This was the original method for determining MCV but it is not commonly used anymore.
Self-Assessment Question #2

The Erythrocyte Sedimentation Rate (ESR) is:

a) the rate of sedimentation of fibrinogen in a 5 ml glass tube.

b) the rate of sedimentation of white cells in a standard narrow-bore glass tube.

c) the rate of clotting in the presence of erythrocytes

d) a test of platelet function

e) the rate of sedimentation of erythrocytes in a standard narrow-bore glass tube

Explanation: The ESR is the rate of sedimentation of red cells in a whole blood specimen enclosed in a narrow-bore glass tube. ESR is increased in acute inflammation.
Self-Assessment Question #3

Acute leukemia is characterized by presence of ____ in the stained peripheral blood smear:

a) blasts

b) neutrophils

c) lymphocytes

d) platelet clumps

e) basophils

Explanation: The hallmark of acute leukemia is the increased presence of blasts in the bone marrow and in peripheral blood. Blasts are immature progenitor cells with characteristic morphology and cell surface markers.
APPENDIX
Derivation of RBC Indices
\[ \text{MCV} = \text{HCT/RBC} \]

- \(0.45 \, \text{L/L divided by} \ 5.0 \times 10^{12}/\text{L}\)
  
  \[= 0.09 \times 10^{-12} \, \text{L}\]

- \(= 90 \times 10^{-15} \, \text{L or} \ 100 \, \text{fL (femtoliter)}\)

- A femtoliter (fL) is = "cubic micron (\(\mu\text{M}^3\))"
\[ \text{MCHC} = \frac{\text{Hb}}{\text{HCT}} \]

- 150 g/L divided by 0.45 L/L
- = 333 g/L
- = 33.3 g/dL
MCH = Hb/RBC

- 150 g/L divided by 5.0 x 10^{12}/L
  - = 30 x 10^{-12}g
  - = 30 pg
AUTOMATED CELL Analysis
Impedance

Light Scatter

CBC, Automated Differential

Conductivity

Differential Responses to Chemical Treatment
1) IMPEDANCE
Electrically conductive diluent

A low-voltage direct current is established
A cell pulled through the aperture interrupts the current.
Impedance

- Current interruption = “Impedance”
- Impedance can be recorded as voltage pulses
- The number of pulses = Cell Number
- Pulse height = Cell Volume [femtoliters]
2) CONDUCTIVITY
Conductivity is measured by a high voltage radio-frequency current

Conductivity is determined by cell granularity, nuclear density, nuclear/cytoplasm ratio
3) LIGHT SCATTER (Flow Cytometry)
LASER

SIDE SCATTER: Internal complexity

FORWARD SCATTER: Cell Volume

CELL
4) Differential Responses to Chemical treatment
Differential Responses to Chemical treatment

- Lysis of RBCs → Allows counting of white cells
- Selective *shrinking* and/or *lysis* of certain white cell populations
- Selective *stripping* of cytoplasm → Allows analysis of cell nuclei
- Selective staining of cells e.g., *myeloperoxidase*
New Generation Cell Counters

- High sensitivity & specificity for blasts
- Ability to distinguish lymphoblasts from normal lymphocytes
- Automated enumeration of nucleated RBCs with correction of WBC count
- Reticulocyte counting, unaffected by red cell shape and capable of providing *immature reticulocyte fraction* and/or CHr
- High sensitivity & specificity for *immature granulocytes*
- Some will enumerate immature granulocytes
- Automated slidemaker/stainer
- Can be connected to automated front-end processing