Hemoglobinopathies
Blood indices & PBS

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Hemoglobinopathy

- Structural hemoglobinopathy –
  Structure and synthesis of α-, β-, γ- and δ-globins produce abnormal and often dysfunctional Hb variants

- Thalassemia -
  cause decreased synthesis of normal Hb variants
Over 600 abnormal structural Hb variants have been reported.

95% differ from normal HbA by replacement of a single amino acid.

Although some structural mutations are benign.

Many (50% of β-variants and 20% of α-variants) alter Hb solubility, stability, or oxygen affinity in ways that adversely affect Hb function.

Thalassemia syndromes are caused either by deletions of entire genes or by mutations that affect the production or processing of normal globin mRNAs.
Hemoglobin

- Majority of hemoglobin variants - not associated with any clinical signs or symptoms

- Clinically significant variants – classification
  1. Sickling syndromes
  2. Unstable hemoglobin
  3. Structural variants with thalassaemic phenotypes
  4. Hb with abnormal $O_2$ affinity
  5. The M hemoglobins
Hemoglobin

- Common hemoglobin variants (HbS, HbE, HbC, HbD-Punjab) represent more than 90% of the abnormalities observed.

- It is needed to accurately and rapidly identify these variants using a minimal number of tests.
• estimated that 1.5% of the world’s population carries β thalassemia, ie, at least 80 to 90 million people with an estimated 60,000 new carriers born each year.

• The Southeast Asian region (which includes India, Thailand, and Indonesia) accounts for about 50% of the world’s carriers. The prevalence of β-thalassemia trait (BTT) is about 3.3% in India (1% to 13%)

• 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal
• β-Thalassemia has a high prevalence in some communities, such as Sindhi, Luvana, Tribes, and Rajputs.

• Gujarat is 10% to 15% in these communities, whereas the incidence in the general population is 2% to 3%.
The screening for BTT can be done by –

• Naked eye single-tube red cell osmotic fragility (NESTROF) test
• Cell counter–based formulas
• HbA2 by microcolumn chromatography
• Elution following cellulose acetate electrophoresis
• High-performance liquid chromatography (HPLC)
Peripheral blood smear
Microscopic Observations

Blood smear
Raw data from Cell analyzer

Order of signal generation

Cell size in fl

Cells

Noise
Organized data from Cell analyzer

Signals in ascending order

Cell size in fl

Cells

Noise
Frequency distribution curve of signals from Cell analyzer
RBC histogram

Main RBC population

RBC doublets

256 channel high resolution RBC histogram display cell population data between 24 and 350 fl
**Red cell Distribution width (RDW)**

The RBC distribution width gives a measure of anisocytosis.

- **RDW-CV**: 11 - 16 %
- **RDW-SD**: 37 - 46 fl

\[
\text{RDW-CV} \, (\%) = 100 \times \frac{\sigma}{\mu}
\]
Parameters provided by automated hematology analyzers

- Hemoglobin (Hb)
- Hematocrit (HCT)
- RBC count (RBC #)
- Platelet count (PLT #)
- WBC count (WBC #)
- WBC diff. (WBC %)
- MCH (Hb/RBC #)
- MCV (HCT/RBC #)
- MCHC (Hb/HCT)
- RDW (RBC volume)
- MPV (Plt TV/PLT histograms (RBC, WBC & PLT))
RBC Normal Histogram

RBC histogram starts at 50fl and ends at 150fl
Microcytic
RBC - Anisochromemia
Microcytosis

RBC graph has shifted to the left and is starting from close to the Y-axis. This indicates that maximum number of cells are smaller in size (MCV is low).
Microcytosis

Interpretation

WBC: Normal
RBC: Microcytic RBC. Histogram shifted to the left and starts at the Y-axis
PLT: Histogram shows interference due to microcytic RBC's. The ascend in the platelet curve signifies this interference. Plt curve fitting is not present and hence the interference due to RBC's is not eliminated. The platelet count is cannot be reported.

Findings

Microcytic Hypochromic Anaemia
Red Cell indices

Cell counter–based formulas:

1 - Shine and Lal Index: \( \text{MCV} \times \text{MCV} \times \frac{\text{MCH}}{100} \)
2 - Mentzer Index: \( \frac{\text{MCV}}{\text{RBC count}} \)
3 - Srivastava Index: \( \frac{\text{MCH}}{\text{RBC count}} \)
4 - England and Fraser Index: \( \text{MCV} - (5 \times \text{Hemoglobin}) - \text{RBC} \)
5 - Ricerca Index: \( \frac{\text{RDW}}{\text{RBC count}} \)
6 - Green Index: \( \text{MCV} \times \text{MCV} \times \frac{\text{RDW}}{(\text{Hemoglobin} \times 100)} \).
Indices

• Threshold values of the indices used to discriminate between iron deficiency anaemia (IDA) and the b-thalassaemia trait (b-TT)

<table>
<thead>
<tr>
<th>Indices</th>
<th>IDA</th>
<th>b-TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell (RBC) count</td>
<td>&lt; 5</td>
<td>&gt; 5</td>
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<tr>
<td>RBC distribution width</td>
<td>&gt; 14</td>
<td>&lt; 14</td>
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<tr>
<td>Mentzer index (MI) = MCV/RBC</td>
<td>&gt; 13</td>
<td>&lt; 13</td>
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<tr>
<td>Shine and Lal (S&amp;L) index = MCV2 × MCH × 0.01</td>
<td>&gt; 1530</td>
<td>&lt; 1530</td>
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<tr>
<td>England and Fraser (E&amp;F) index = MCV – RBC – 5Hb – 3.4</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Srivastava index (S) = MCH/RBC</td>
<td>&gt; 3.8</td>
<td>&lt; 3.8</td>
</tr>
<tr>
<td>Green and King (G&amp;K) index = MCV2 × RDW/100Hb</td>
<td>&gt; 65</td>
<td>&lt; 65</td>
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<tr>
<td>RBC distribution width index (RDWI) = MCV × RDW/RBC</td>
<td>&gt; 220</td>
<td>&lt; 220</td>
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<tr>
<td>Ricerca (R) index = RDW/RBC</td>
<td>&gt; 4.4</td>
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Predictive values of evaluated indices for patients with mild-to-moderate iron deficiency anaemia (IDA; Hb 8.5 – 11 g/dl) and patients with the b-thalassaemia trait (b-TT)

<table>
<thead>
<tr>
<th>Indices</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Youden's index</th>
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<td><strong>Green and King (G&amp;K) index</strong></td>
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</table>

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• Shine and Lal Index
• MCV (<71.3 fl)
• MCH (<22.5 pg)
• Srivastava Index
• Mentzer Index
• RBC count ( >5.28 )
• England and Fraser Index
• Green Index
• Ricerca Index
• RDW (12.4 %)

New Indices

- New parameters –
  
  - Percentage of microcytes ( % Mi )
  
  - Percentage of hypochromic cells ( % Hy )
Thalassaemia

DF = (MCV)^2 x RDW
    HGB x 100
    = 56.8

DF < 65 = b-thal
> 65 = IDA

RBC high, MCV low, Hgb low. Discriminant function (DF) is less than 65
This is a histogram of a patient with β Thalassaemia Major.

The red cell histogram is shifted to the left, intercepting the Y-axis, suggesting the presence of many very small red cells.

This is also suggested by the platelet curve, which does not come to baseline at 20 fL; there is a non-fitted curve and incomplete computation for the platelet count.

The white cell histogram is interesting because we see a very large peak to the left of the lymphocyte population which suggests that the white cell count might include small particles that are not white cells (characteristic of nucleated red cells).
Thalassaemia
Anisocytosis

RBC graph shows a broad base with graph shifted to the right. The broad base indicates anisocytosis (RDW high). The graph also indicates macrocytosis (MCV high).
Hemoglobin

- Common hemoglobin variants (HbS, Hb E, HbC, HbD-Punjab) represent more than 90% of the abnormalities observed.

- It is needed to accurately and rapidly identify these variants using a minimal number of tests.
PBS
• As evidence on spurious data generated by these instruments increases, blood smear examination is regaining its importance as a vital tool in haematology reporting.
Thank you