Evidence-Based Hematological Solutions

Beyond the Routine CBC

Objectives

• Describe novel hematology parameters and their derivation.
• Investigate the evidence for their clinical utility.
• Discuss how new information can be applied to patient care.
• Explore the operational benefits these parameters provide.
• Discuss implications for cost of care episode.
Escalating Healthcare Pressures

Hematological Challenges

- Infection/Inflammation Response
- Iron Deficiency Management
- Thrombopoiesis Management
Significance of Left Shift

70% of HAI Occur Outside the ICU

Pathogenesis of Sepsis

- Increased neutrophil migration and adhesion
- Increased coagulation
- Decreased fibrinolysis
- Increased inflammation
- Endothelial injury and loss of barrier integrity
- Microvascular injury results in altered microcirculatory perfusion

Clinical Challenges

- How soon can we identify a neutrophil response?
- What is a “left shift”?
- How do we ID infection when WBC and ANC are normal?
## Manual WBC Differential Imprecision

<table>
<thead>
<tr>
<th>#</th>
<th>N = 100</th>
<th>N = 200</th>
<th>N = 500</th>
<th>N = 1,000</th>
<th>N = 10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0 - 3.6</td>
<td>0.0 - 1.8</td>
<td>0.0 - 0.7</td>
<td>0.0 - 0.4</td>
<td>0.0 - 0.1</td>
</tr>
<tr>
<td>1</td>
<td>0.0 - 5.4</td>
<td>0.1 - 3.6</td>
<td>0.3 - 2.3</td>
<td>0.5 - 1.8</td>
<td>0.8 - 1.3</td>
</tr>
<tr>
<td>2</td>
<td>0.2 - 7.0</td>
<td>0.6 - 5.0</td>
<td>1.0 - 3.6</td>
<td>1.2 - 3.1</td>
<td>1.7 - 2.3</td>
</tr>
<tr>
<td>3</td>
<td>0.6 - 8.5</td>
<td>1.1 - 6.4</td>
<td>1.7 - 4.9</td>
<td>2.0 - 4.3</td>
<td>2.6 - 3.4</td>
</tr>
<tr>
<td>4</td>
<td>1.1 - 9.9</td>
<td>1.7 - 7.7</td>
<td>2.5 - 6.1</td>
<td>2.9 - 5.4</td>
<td>3.6 - 4.5</td>
</tr>
<tr>
<td>5</td>
<td>1.6 - 11.3</td>
<td>2.4 - 9.0</td>
<td>3.3 - 7.3</td>
<td>3.7 - 6.5</td>
<td>4.5 - 5.5</td>
</tr>
<tr>
<td>6</td>
<td>2.2 - 12.6</td>
<td>3.1 - 10.2</td>
<td>4.1 - 8.5</td>
<td>4.6 - 7.7</td>
<td>5.5 - 6.5</td>
</tr>
<tr>
<td>7</td>
<td>2.9 - 13.9</td>
<td>3.9 - 11.5</td>
<td>4.9 - 9.6</td>
<td>5.5 - 8.8</td>
<td>6.5 - 7.6</td>
</tr>
</tbody>
</table>

*The 400-cell diff may be acceptable for relatively high counts, it is not suitable for counts of less than 5%.*

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### Sooner or Later?

- **11%**
- **5.5%**
- **5%**
- **4.5%**
- **1.0%**

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Automated 6-Part Differential

**IG% and IG#**

### DIFF Channel
- Reagent Reaction: STROMATOLYSER-4DL & 4DS
- Blast, I.G.
- Normal Cell
- Lyse
- Stain

### IG Precision

<table>
<thead>
<tr>
<th>Specimen No/Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
<th>Manufacturer Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 No. of IGs (≥ 10%)</td>
<td>0.38</td>
<td>0.03</td>
<td>6.63</td>
<td>SD &lt; 0.12; CV &lt; 25%</td>
</tr>
<tr>
<td>IGS (%)</td>
<td>6.13</td>
<td>0.37</td>
<td>6.01</td>
<td>SD &lt; 1.0; CV &lt; 25%</td>
</tr>
<tr>
<td>WBC count, {1L ≤ 10%}</td>
<td>6.36 (6.3)</td>
<td>0.09</td>
<td>1.48</td>
<td>CV &lt; 3%</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>53.0 (54)</td>
<td>1.19</td>
<td>2.22</td>
<td>CV &lt; 8%</td>
</tr>
<tr>
<td>3 No. of IGs (≥ 10%)</td>
<td>0.08</td>
<td>0.06</td>
<td>5.84</td>
<td>SD &lt; 0.12; CV &lt; 25%</td>
</tr>
<tr>
<td>IGS (%)</td>
<td>5.46</td>
<td>0.47</td>
<td>5.33</td>
<td>SD &lt; 1.0; CV &lt; 25%</td>
</tr>
<tr>
<td>WBC count, {1L ≤ 10%}</td>
<td>11.50 (11.5)</td>
<td>0.35</td>
<td>2.27</td>
<td>CV &lt; 3%</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>66.33 (66.3)</td>
<td>0.81</td>
<td>0.98</td>
<td>CV &lt; 8%</td>
</tr>
<tr>
<td>3 No. of IGs (≥ 10%)</td>
<td>0.35</td>
<td>0.03</td>
<td>8.53</td>
<td>SD &lt; 0.12; CV &lt; 25%</td>
</tr>
<tr>
<td>IGS (%)</td>
<td>2.39</td>
<td>0.20</td>
<td>8.24</td>
<td>SD &lt; 1.0; CV &lt; 25%</td>
</tr>
<tr>
<td>WBC count, {1L ≤ 10%}</td>
<td>44.44 (44.4)</td>
<td>0.15</td>
<td>0.24</td>
<td>CV &lt; 3%</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>72.96 (72.9)</td>
<td>0.49</td>
<td>0.67</td>
<td>CV &lt; 8%</td>
</tr>
</tbody>
</table>

CV: Coefficient of variation; IGs = immature granulocytes.

IG Correlation Studies


Figure 1. ROC curves comparing ability of IG (blue), ANC (green), and WBC count (red) to predict infection. The IG and ANC curves are superimposable.

IG: Better than WBC


Figure 1. ROC curves comparing ability of IG (blue), ANC (green), and WBC count (red) to predict infection. The IG and ANC curves are superimposable.
WBC vs. IG as Predictors of Sepsis

Prediction of infections or bacteremia might be improved by adding IG into an algorithm with other lab parameters to target a careful workup of a subset of patients.

Table 2
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Sepsis

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive (n = 91)</th>
<th>Culture Negative (n = 51)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD WBC count (× 10^9/L)</td>
<td>10,300 ± 2,300 (10.3 ± 2.3)</td>
<td>10,200-5,500 (10.2 ± 5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SD immature granulocyte percentage</td>
<td>2.0 ± 1.6</td>
<td>2.7 ± 2.5</td>
<td>.209</td>
</tr>
<tr>
<td>Proportion (percent)</td>
<td>39 (43%)</td>
<td>32 (24%)</td>
<td>.02</td>
</tr>
<tr>
<td>Proportion (count)</td>
<td>11 (12%)</td>
<td>1.2 (1.2%)</td>
<td>.94</td>
</tr>
</tbody>
</table>

IG: Elevated When Other Markers are Not

Diagnosis or Clinical Feature | Patients, n | Samples, n | Diagnosis or Clinical Feature | Patients, n | Samples, n
---|---|---|---|---|---
INFECTION AND PARASITIC DISEASES | | | | | |
Tuberculosis | 15 | 24 | Systemic Lupus Erythematosus | 6 | 9
Human Immunodeficiency Virus | 4 | 8 | Thrombotic Thrombocytopenic Purpura | 2 | 5
Pneumonia | 3 | 3 | | |
Cellulitis | 2 | 5 | | |
Malaria | 3 | 3 | | |
Hepatitis C | 2 | 2 | | |
POST-OPERATION/BLEEDING | 14 | 18 | OTHER | 13 | 14
Coronary Artery Bypass Grafting | 5 | 8 | Pancreatitis | 3 | 3
Gastrointestinal Bleeding | 3 | 4 | Fistula | 1 | 1
Arteriovenous Fistula | 1 | 1 | Crohn's Disease | 1 | 1
Laparotomy | 1 | 1 | Heart Failure | 1 | 1
Whipple Procedure | 1 | 1 | High Blood Pressure | 1 | 1
Kidney Transplantation | 1 | 1 | Carotid-cavernous Sinus Fistula | 1 | 1
Ischemic Bowel Disease | 1 | 1 | Sickle Cell Disease | 1 | 1
RENAL FAILURE | 10 | 19 | Protein S Deficiency | 1 | 1
End-stage Renal Failure | 8 | 15 | Sarcoidosis | 1 | 1
Chronic Renal Failure | 2 | 4 | Pyelonephritis | 1 | 1
TOTAL | 43 | 84 | Adrenoleukodystrophy | 1 | 2

IG: 
**Elevated When Other Markers are Not**

IG can elevate in infection even when WBC, ANC, and other markers are normal.

Briggs, C. et al. (2003). Laboratory Hematology; 117 - 123

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**Clinical Utility:**

**Automated Immature Granulocyte Count**

Briggs concludes, "IG count can highlight a potential acute infectious inflammatory response at a relatively early state ... when other blood count parameters are in the overall normal range and are generally nonspecific indicators." (Discussion)

Briggs, C. et al. (2003). Laboratory Hematology; 117 - 123
Summary: Automated IG Count

- More precise than 100-cell diff (Fernandes).
- Good correlation with flow (Fernandes).
- Better sensitivity and specificity than WBC alone in predicting infection in patients admitted through the ED with suspected bacteremia (Ansari-Lari).
- 92% PPV in patients with positive blood cultures and IG >3% (Ansari-Lari).
- IG can elevate in infection/inflammation even when the WBC and other markers are not elevated (Briggs).
- IG, a direct cellular measure of leukopoiesis, may aid the ability to detect infection if added to current protocols.

Anemia Management
Anemia Prevalence

Prevalence of Anemia
- 3.4 million people in US
- 2 billion people globally
  - 30% of world’s population
- Many of these anemias are due to iron deficiency (ID)
- In children, ID may adversely affect neurodevelopment and behavior and the effects may be irreversible

Patients at Highest Risk of Anemia
Traditional Lab Anemia Work-up

- Hgb less than 12g/dL
- MCV less than 80 fL
- Microcytic / Hypochromic RBC
- RDW-SD & RDW-CV Increased
  - (measures of anisocytosis)
- Reticulocytes Decreased

Finding Balance in Anemia Management

Challenge
- How do you balance dosage and timing of iron therapy?
- What is the best assessment to balance ESA and Iron therapy?
- What is the best assessment of iron stores?
## Serum Iron Studies

<table>
<thead>
<tr>
<th></th>
<th>Iron Deficiency Anemia</th>
<th>Anemia of Inflammatory Chronic Disease</th>
<th>Functional Iron Deficiency Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron:</td>
<td></td>
<td></td>
<td>Low or Normal or High</td>
</tr>
<tr>
<td>(Will be High with Iron therapy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIBC:</td>
<td>Green</td>
<td>Blue</td>
<td>Low or Normal or High</td>
</tr>
<tr>
<td>Transferrin Saturation:</td>
<td>Normal</td>
<td>Low in 20% of cases</td>
<td></td>
</tr>
<tr>
<td>(Changes with dietary protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin:</td>
<td>Normal</td>
<td>Normal or High</td>
<td>Normal or High</td>
</tr>
<tr>
<td>(Acute phase reactant)</td>
<td></td>
<td></td>
<td>High with persistent anemia</td>
</tr>
<tr>
<td>sTFR:</td>
<td>Green</td>
<td>Normal</td>
<td>SI High but may be unavailable in EMAO</td>
</tr>
<tr>
<td>Recommendation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>More useful to use sTFR / log Transferrin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Iron Metabolism

If we want to know about iron metabolism at the cellular level... Shouldn’t we directly measure changes to developing RBC?
Reticulocyte Method

- Reticulocytes
  - # and % of immature RBC’s
- Immature Reticulocyte Fraction
  - Newly released from the marrow, a direct cellular measurement of erythropoiesis
- Reticulocyte Hemoglobin
  - Direct cellular measure of iron availability
Anemia Assessment

Reticulocyte Hemoglobin

• RET-He / CHr
  – Measured at cellular level
  – Monitor acute changes in hemoglobin incorporation into the erythron
  – More sensitive than indirect chemical measurements
  – Detect non-responders to ESA (Functional Iron Deficiency)
Precision Analysis

Table 2. Precision analysis for Ret He and RBC He parameters

<table>
<thead>
<tr>
<th></th>
<th>Ret He SD (pg)</th>
<th>Ret He CV (%)</th>
<th>RBC He SD (pg)</th>
<th>RBC He CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run precision</td>
<td>0.32</td>
<td>1.49</td>
<td>0.27</td>
<td>1.23</td>
</tr>
<tr>
<td>SD of the run means</td>
<td>0.29</td>
<td>1.32</td>
<td>0.16</td>
<td>0.74</td>
</tr>
<tr>
<td>SD of the daily means</td>
<td>0.79</td>
<td>3.66</td>
<td>0.19</td>
<td>0.86</td>
</tr>
<tr>
<td>Total imprecision</td>
<td>0.85</td>
<td>3.92</td>
<td>0.29</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Reticulocyte Hemoglobin Comparison

Figure 1. Method Comparison Data, XE 2100 and ADVIA 2120
200 Clinical Samples

Figure 2. Method Comparison Data, XE 2100 and ADVIA 2120
125 Normal Adults

Screening Children for Iron Deficiency

"...Screening for anemia with a Hb determination neither identifies children with ID nor specifically identifies those with IDA."

Pediatrics. 2010;126;1040-1050. Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in and Infants and Young Children (0 - 3 Years of Age). Baker, R., Greer, F. and The Committee on Nutrition.
Recommendations for Screening

“A low CHr concentration has been shown to be the strongest predictor of ID in children.”

“For infants with Hb <11.0 mg/dL or with significant risk of ID or IDA, SF and CRP or CHr levels should also be measured to increase the sensitivity and specificity of the diagnosis.”

Pediatrics. 2010;126;1040-1050. Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in and Infants and Young Children (0 - 3 Years of Age). Baker, R., Greer, F. and The Committee on Nutrition.

RET-He in the Diagnosis of IDA

Diagnostic performance of RET-He is excellent compared to traditional parameters

Cutoff 27.2 pg
Sensitivity 93.3%
Specificity 83.2%

Iron Deficiency Anemia diagnostic criteria:
- Fe <40
- Tstt <20
- Ferritin <100
- Hgb <11

* For patients on maintenance hemodialysis

KDOQI Guidelines for Evaluation of Anemia

Initial Anemia Evaluation

- Cellular Assessment
  - Hgb < 12 g/dL
  - RBC indices
  - Absolute Retic
  - WBC & Diff
  - Platelet

- Iron Assessment
  - Serum ferritin
  - Serum TSAT or CHr

Iron Assessment Indices

- HD-CKD Target
  - Ferritin > 200 ng/ml and
  - Tsat > 20% or CHr > 29 pg/cell

Variations in Tests of Anemia and Iron Status

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb</td>
</tr>
<tr>
<td>Analytical</td>
<td>2.0</td>
</tr>
<tr>
<td>Biological</td>
<td>4.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>6.0</td>
</tr>
</tbody>
</table>

"Hb, Hct, and RET-H\(_c\), but not TSAT or Ferritin, are useful analytes to guide dose adjustment for ESA or IV iron."


Variations in Tests of Anemia and Iron Status

<table>
<thead>
<tr>
<th>Level of Closeness to True Mean</th>
<th>Analyte</th>
<th>Hb</th>
<th>Hct</th>
<th>Chi</th>
<th>TSAT</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>20%</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>5%</td>
<td></td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>226</td>
<td>40</td>
</tr>
</tbody>
</table>

The presence of inflammation and uremia makes this diagnosis particularly challenging for dialysis patients.

By directly measuring the RET-He, early stages of iron deficiency may be identified, at a time that other traditional biochemical parameters are non-informative.

Utility of RET-He for ID
Pathway to Identify and Evaluate Anemia

Premedication:
- Complete Blood Count with Differential
- Hemoglobin Abnormal
  - Male >150
  - Female >120

Evaluation for Anemia of Chronic Disease
- Yes
  - Reticulocyte Count Abnormal
    - Yes
      - Rule Out Blood Loss
      - Rule Out Nephropathy
    - No
      - Creatinine ≤ 1.5 mg/dL
    - Yes
      - Ferritin ≤ 150 ng/mL
      - Transferrin Saturation ≤ 15%

If iron deficiency confirmed:
- Iron Supplementation
  - Consider Genitourinary Evaluation

Early Identification for Appropriate Intervention

The course of 2 clinical parameters during preoperative epoetin treatment... There is a clear difference between responders and non-responders. The haemoglobinisation level of reticulocytes is an early detector of functional iron deficiency due to epoetin injections.
Outcomes with Faster Identification of Non-responders

RET-He identified non-responders more quickly, allowing faster hemoglobin recovery in severe anemia, greater efficiency in OR scheduling, better transfusion management, and faster post surgical recovery of blood loss.

Decrease in Blood Transfusions

Fig. 3. Blood transfusion orthopaedic recovery plan 2001 - 2008.
Summary: *Reticulocyte Hemoglobin*

- More comprehensive workup of patients with suspected IDA
- Direct cellular measurement for faster indication of patient response
- Less variation than acute phase reactants
- May improve care of patients on ESA / IV Iron therapy when used with other information
- Manage cost of care for severe iron deficiency and iron deficiency anemia

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**Thrombopoiesis Management**
Clinical Challenge in Thrombocytopenia

- What is the cause of the thrombocytopenia?
- Is this a disorder of decreased production?
- Is platelet destruction increased?
- Might this patient have HIT?
- Should we withhold ARV?
- Is patient’s bone marrow recovering adequately without intervention?
- Will we need to consider platelet transfusions?

Platelet Transfusions

- 10 million platelet units are given to 2.2 million recipients each year
- 1:2,000 platelet units may be contaminated with bacteria
  - 1:20,000 recipients die from transfusion-transmitted sepsis
  - 2nd highest rate of transfusion-related death
- Alloimmunization
- Product Availability
What about PLT Transfusions?

Comprehensive Platelet Count

Immature Platelet Fraction (Reticulated Platelets)

- Platelets newly released from the bone marrow
- % of total platelets that are immature
- Indicates thrombopoiesis
  - ↓ Plts ↓ IPF = ↓ Production
  - ↓ Plts ↑ IPF = ↑ Destruction
Immature Platelet Fraction

- Fluorescent dye binds to platelet granules and RNA
- Immature platelets fluoresce more than mature platelets
- IPF is a direct cellular measurement of thrombopoiesis.
- Reference Range is 1.1 – 6.1%

Immature Platelet Fraction Accuracy

Differentiate Physiological Mechanisms

Low PLT + Low IPF
Consistent with production disorder

Normal

Low PLT + High IPF
Consistent with destruction mechanism (ITP, TTP, DIC, autoimmune)

Immature Platelet Fraction for Differential Diagnosis

- In thrombocytopenia, does IPF help differentiate between consumptive and aplastic causes?
- In thrombocytopenia, does regular monitoring of IPF provide valuable information for treatment decisions?
Immature Platelet Fraction for Transfusion Management

Fig. 2 “IPF should allow a more controlled prophylactic platelet transfusion policy to be implemented at specified threshold count, particularly when platelet recovery is imminent.”

Clinical Utility: Transfusion Management

“Predicting platelet recovery would permit more reasoned use of prophylactic platelet transfusion and provide the potential to reduce the use of platelet concentrates, minimizing possible transfusion-transmitted infections.”
Immature Platelet Fraction to Assess Bone Marrow Recovery

How well can IPF predict platelet recovery following peripheral blood HPC transplantation?

Clinical Utility: Bone Marrow Recovery

“Following HPC transplantation, IPF recovered significantly earlier than platelet count, ANC, and IRF.”

“A persistently low IPF in this setting would suggest failure of thrombopoietic recovery.”
Summary: Immature Platelet Fraction

- Provides a direct cellular measurement of thrombopoietic activity
- May assist in determining cause/differential diagnosis of thrombocytopenia when used with patient information and platelet count
- May provide more information for prophylactic platelet transfusion management, rather than using platelet trigger alone.

Beyond the Routine CBC

Thank You