Evidence-Based Hematological Solutions

Beyond the Routine CBC

Economic Considerations

Cost of Care

- Patient Safety
- Quality Measures
- Patient Satisfaction
- MS-DRG Coding
- Patient Flow
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.

Hematopoiesis

- Erythropoiesis (Retics, NRBCs)
- Red Cell Hemoglobinization (Ret-He)
- Leukopoiesis (IG)
- Thrombopoiesis (IPF)
- Stem Cells (HPC)

Immature cell count data can be used in conjunction with mature cell counts to assess pathophysiological mechanisms.
Hematological Challenges

- Infection/Inflammation Response
- Iron Deficiency Management
- Thrombocytopenia Management

Significance of Left Shift
Infection Surveillance
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%.*

WDF Channel  Scattergram - Normal Pattern

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</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Glu (x 10^3/μL)</td>
<td>0.36</td>
<td>0.03</td>
<td>6.63</td>
<td>SD &lt; 0.12, CV &lt; 25%</td>
</tr>
<tr>
<td>Leu (x 10^3/μL)</td>
<td>6.13</td>
<td>0.03</td>
<td>6.61</td>
<td>SD &lt; 1.0, CV &lt; 25%</td>
</tr>
<tr>
<td>WBC count, (x 10^3/μL)</td>
<td>6.350 (8.3)</td>
<td>0.00</td>
<td>1.48</td>
<td>CV &lt; 3%</td>
</tr>
<tr>
<td>Neutrophil, %</td>
<td>53.8 (5.0)</td>
<td>1.19</td>
<td>2.22</td>
<td>CV &lt; 8%</td>
</tr>
</tbody>
</table>

3
| No. of Glu (x 10^3/μL) | 0.06 | 0.06 | 5.94   | SD < 0.12, CV < 25%         |
| Leu (x 10^3/μL)       | 5.46 | 0.47 | 9.55   | SD < 1.0, CV < 25%          |
| WBC count, (x 10^3/μL)| 11.520 (11.5) | 0.25 | 2.17   | CV < 3%                     |
| Neutrophil, %         | 63.22 (8.2)  | 0.61 | 0.98   | CV < 8%                     |

| 3                     |      |     |        |                             |
| No. of Glu (x 10^3/μL) | 0.35 | 0.03 | 8.53   | SD < 0.12, CV < 25%         |
| Leu (x 10^3/μL)       | 2.35 | 0.03 | 8.24   | SD < 1.0, CV < 25%          |
| WBC count, (x 10^3/μL)| 14.440 (18.4) | 0.16 | 1.04   | CV < 3%                     |
| Neutrophil, %         | 72.96 (8.7)  | 0.49 | 0.67   | CV < 8%                     |

CV: coefficient of variation; Eo: eosin; gran: 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

Table 38
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Sepsis:

<table>
<thead>
<tr>
<th>Culture Positive (n = 51)</th>
<th>Culture Negative (n = 51)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD WBC count (All ≤ 10%)</td>
<td>10,300 ± 7,000 (10.3 ± 7.3)</td>
<td>10,200 ± 5,500 (10.2 ± 5.5)</td>
</tr>
<tr>
<td>Mean ± SD immature granulocyte percentage</td>
<td>2.0 ± 3.6</td>
<td>2.7 ± 3.6</td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>38 (41%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Proportion &gt;3%</td>
<td>11 (13%)</td>
<td>10 (22%)</td>
</tr>
</tbody>
</table>

NS, not significant.

“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.”


IG: Elevated When Other Markers are Not

Table 39

<table>
<thead>
<tr>
<th>Diagnosis or Clinical Feature</th>
<th>Patients, n</th>
<th>Samples, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFECTIOUS AND PARASITIC DISEASES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Urinary Tract infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Malaria</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>POST-OPERATION/BLEEDING</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Coronary Artery Bypass Grafting</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Gastrointestinal Bleeding</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Aortic Valve Replacement</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glaucoma Excision</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Whipple Procedure</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Kidney Transplantation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ischemic Bowel Disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RENAL FAILURE</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>End-stage Renal Failure</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Chronic Renal Failure</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis or Clinical Feature</th>
<th>Patients, n</th>
<th>Samples, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISEASES OF THE MUSCULOSKELETAL SYSTEM AND CONNECTIVE TISSUE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Thrombotic Thrombocytopenic Purpura</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>OTHER</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fistula</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Crohn’s Disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carotid-cavernous Sinus Fistula</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Protein S Deficiency</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Peptic Ulcer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>43</td>
<td>54</td>
</tr>
</tbody>
</table>

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

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

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)
Summary: Automated IG Count

- More precise than 400-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 and Iron Deficiency

- 3.4 million people in US, 2 billion people globally (1/3 of population)
- Iron deficiency (ID) is the most common cause of anemia (> 600 million people)
- Leading causes:
  - Gastrointestinal blood loss
  - Dietary iron deficiency (poor nutrition, malabsorption)
  - Infectious disease, Cancer treatment
  - Increased iron demand
- Rapid diagnosis and treatment can prevent blood transfusion

National Anemia Action Council, 2006

Challenges in Anemia Management

Which tests provide the best assessment of iron deficiency anemia?

- Assessing iron stores before treatment?
- Assessing efficacy of EPO and Iron therapy?
Diagnosis of Iron Deficiency

Biochemical parameters
- Transferrin, transferrin saturation (Tfsat)
- Ferritin
- Serum iron
- Circulating transferrin receptor (STfR)

Hematological parameters
- Hb, MCV, RDW
- Erythrocyte zinc protoporphyrin (ZPP)
- Reticulocyte Hb content (CHr / Ret-He)

Conditions Affecting Serum Iron, Transferrin, and Ferritin

<table>
<thead>
<tr>
<th>Test</th>
<th>Elevated Test Results</th>
<th>Decreased Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron</td>
<td>Sample late in the day</td>
<td>Infection</td>
</tr>
<tr>
<td></td>
<td>Meal iron intake</td>
<td>Inflammation</td>
</tr>
<tr>
<td></td>
<td>Supplement iron intake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemolysis</td>
<td></td>
</tr>
<tr>
<td>Serum Transferrin</td>
<td>Oral Contraceptives</td>
<td>Inflammation/infection</td>
</tr>
<tr>
<td>Serum Ferritin</td>
<td>Inflammation/infection, Hyperthyroidism, Aging Liver disease (HCV) Malignancy Alcohol consumption Oral contraceptives</td>
<td>Vitamin C deficiency Hypothyroidism Exercise</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?

RET Channel Scattergram on Normal Pattern
Reticulocyte Parameters

- **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

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)
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
120 Normal Adults


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><strong>TOTAL</strong></td>
<td>6.0</td>
</tr>
</tbody>
</table>

"Hb, Hct, and RET-Hec, 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 3. Number of Sampling Days Needed for the Observed Result to be within an Expected Level of Closeness to the True Mean with 99% Probability

<table>
<thead>
<tr>
<th>Level of Closeness to True Mean</th>
<th>Analyte</th>
<th>Hb</th>
<th>Hct</th>
<th>CHr</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>15</td>
<td>3</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>57</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>

Utility of RET-He for ID

“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.”
Hematological and Biochemical Markers of Iron Deficiency
Non-iron-deficient and Iron-deficient Infants at Initial screening


Screening Children for Iron Deficiency

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iron Deficiency Without Anemia</th>
<th>Iron Deficiency Anemia</th>
<th>Iron Overload</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF*</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Transferrin Saturation</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>TFR1</td>
<td>Normal</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>CHr</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Hb</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mean Corpuscular Volume</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

* Confounded by the presence of inflammation.

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.”

Pathway to Identify and Evaluate Anemia*

*in elective surgical patients

Anesth Analg. 2005;101:1858-1861. Goodnough, L. T. et al. Fig. 1
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
Thrombocytopenia Management

- 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?

Clinical Challenge in Thrombocytopenia
# 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

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## 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

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![Immunomorphology of platelet distribution](image-url)
**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%

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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.”

Zucker, M. Laboratory Hematology, 12:125 - 130
Platelet function in AML/MDS and ITP patients

- Patients with AML/MDS had smaller PLTs, lower IPF and lower expression of GPIIb-IIIa
- AML/MDS patients have lower in vivo PLT activation than ITP patients

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