What’s In and What’s Out?  
New CBC Parameters for Clinical Diagnosis  

Part 1: Beyond Bands:  
The Immature Granulocyte Count  

Becky Socha, MS, MLS, (ASCP)CM BB CM
Disclosures

- I am receiving an Honorarium from Sysmex.
- The views expressed in the presentation are those of the author and their healthcare facility. Results of case studies are not predictive of other cases and results may vary.
Objectives

- Describe the historical transition in hematology from the manual differential to the automated differential
- State the definitions of bands, immatures granulocytes (IG), and absolute neutrophil count
- Describe clinical benefits of the automated immature granulocyte count over the manual band count
- Identify applications of the IG% in diagnosis and monitoring disease
Early analyzers

Model A Coulter Counter, 1956.
https://www.beckman.com/resources/discover/fundamentals/history-of-flow-cytometry/the-coulter-principle

TOA CC-1001 automated hematology analyzer, the first blood cell counter in Japan, 1963

CBC and differential are historically the highest volume tests in the clinical laboratory
History of automated cell counters

- Early cell counters -1950’s, 60’s, 70’s
  - Only performed counts and indicies
  - 1968; fully automated hematology analyzer, Coulter Counter Model S, seven-parameter CBC
  - Impedance counting
  - Platelet counts added 1977; Coulter Model S Plus (12 parameters)
  - 1978; fully automatic blood cell counter featuring a sampler, Sysmex CC-720.

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## Manual Differentials

### 100 Cell Manual Differential

| Enumerates percentage of each cell type | Detects presence of abnormal cells |

### Manual differential traditionally performed on all samples

| Labor intensive, slow, expensive | Subjective; Imprecise, Inaccurate | Absolute counts need to be calculated |
### History of the Automated Differential; How to count different types of WBCs?

<table>
<thead>
<tr>
<th>Perkin-Elmer Cellscan; 1966</th>
<th>1970’s</th>
<th>100-200 cells counted in 1-2 minutes</th>
</tr>
</thead>
</table>
| • Neutrophils, lymphocytes, monocytes counted  
  • But….Results took overnight! | • Introduction of several instruments using different technologies  
  • Variations of cell scanning techniques, recognition logic and automation  
  • 3 part diffs | • Accuracy about same as manual differential  
  • No microscopic evaluation of red cell and platelet morphology  
  • Manual diffs considered superior by many labs and doctors |

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History - Automated Differentials

The 80’s
- 10 and 12 parameter CBCs
- Automated retics
- The 5 part Differential
- 10,000 cells counted

Sysmex; 1988
- First automated hematology system that combined cell analysis with slide making

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Sysmex HS- series

First fully automated system handling blood cell counts, white blood cell differentiation, reticulocyte measurement and preparation of smears. Sysmex America 2018

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1990’s

The Next Generation; Flow Cytometry

- Flow cell technologies based on impedance or light scatter
- Sysmex first to use fluorescent flow cytometry to reliably detect abnormal samples and reduce false positive results.
- New parameters added
- Digital cell images and automated manual differentials

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Today's Analyzers - We’ve come a long way!

Technologies include impedance, hydrodynamic focusing, flow cytometry

Today's hematology analyzers report 26-30 parameters

Automated differentials can count over 30,000 cells

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Beyond Flags: Where are we now?

- An automated 5 part differential will give instrument flags if abnormalities seen
  - ?IG
  - Cannot count immature cells
- What to do?
  - Perform ‘scan’
  - Perform manual differential, if indicated
- What about counting cells instead of just flagging?
  - Immature granulocytes
  - Nucleated RBCs

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Advanced Clinical Parameters

- New test availability
- An extension of the traditional CBC and automated differential
- Direct cellular measures of hematopoiesis
- New tools for patient care
- Can provide a wealth of knowledge to physicians in making diagnoses
  - Ret-He
  - IPF
  - IG#, IG%
Traditionally used as a clinical indicator of infection, inflammation, sepsis.

Bands can be subjective.

Remarkable variability with CV up to 198% reported by studies.

Therefore, is the band count truly useful?
Bands are subjective

- This is a band
- And this is a band
Is This a band?

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Definitions

Definition #1: As soon as the nucleus is threadlike and constricted at any given place, it can be called a segmented neutrophil. Before that it is called a band neutrophil (The rule of filament)

The ratio of band neutrophils to segmented neutrophils is normally around 1 to 4 (the rule of filament)

Definition #2: As soon as the diameter of the nucleus at any given place is less than 1/3 that of its widest point, it is a segmented neutrophil (The rule of one third).

The ratio of band neutrophils to segmented neutrophils is normally around 1 to 12-15 (the rule of one third)
A Matter of Statistics- Is the Band Count Truly Useful as a Clinical Indicator of Sepsis?

<table>
<thead>
<tr>
<th></th>
<th>Band %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tech 1</td>
<td>5%</td>
</tr>
<tr>
<td>Tech 2</td>
<td>16%</td>
</tr>
<tr>
<td>Tech 3</td>
<td>8%</td>
</tr>
</tbody>
</table>

- >10% is an indicator of sepsis
- 5% and 8% do not indicate sepsis
- 16% exceeds cutoff by 1.5x
- Variation can occur depending on tech training, definition of bands used and # cells counted
- Different normal values result depending on which definition is used

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Counting More Cells gives a More Accurate Manual Differential

<table>
<thead>
<tr>
<th>TRUTH</th>
<th>N=100</th>
<th>N=200</th>
<th>N=1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0-7</td>
<td>1.0-5.5</td>
<td>2.0-4.1</td>
</tr>
<tr>
<td>5</td>
<td>1-10</td>
<td>2-8</td>
<td>3.7-6.4</td>
</tr>
<tr>
<td>10</td>
<td>5-16</td>
<td>6-14.5</td>
<td>8.2-11.9</td>
</tr>
</tbody>
</table>

95% confidence intervals (%) for differential counts on smears from one sample of Blood (Rumke)

Rumke’s research showed that there is a statistical chance that a 100-cell differential from a blood smear with a "true" 10% band count might be reported as anything between 5 and 16%.


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Beyond Bands: What is an immature granulocyte count?

- Mature Neutrophils - segmented neutrophils and bands
  - The Mature Neutrophil Count is the Absolute Neutrophil Count (ANC)
  - Neuts and bands are separated from IG’s
- Sysmex’s Advanced Clinical parameter - IG%, IG#
  - IG - Metamyelocytes, Myelocytes, Promyelocytes
  - Indicators of leukopoiesis
  - Cells just coming out of the bone marrow
  - Earliest indicator of inflammation and infection
- Older analyzers could not identify or count immature granulocytes
  - manual differential was the only way to identify cells less mature than neutrophils.
  - Coulter can also provide IG%
Let’s talk about band% vs IG%!

**Band%**
- Manual differential performed
- Bands counted, 100 cells, subjective
- Bands manually calculated into ANC count
- There is a danger in making a clinical decision based on apparent changes in band counts that are not real but only reflect subjectivity of band count

**Immature Granulocytes %**
- Sysmex automated differential: thousands of cells counted
- 6 part Diff- auto diff separates bands and immature granulocytes
  - Bands flagged when bands are seen
- Band cells considered mature cells
  - Bands included in neutrophil count
  - Can fight infection
  - Included in Instrument ANC count
- Statistically superior automated differential
Measuring IG on Sysmex

- IG% and absolute are part of the automated diff and can be reported with CBC
- WDF channel
  - Classifies WBCs
  - Flow cytometry using a semiconductor laser
  - X axis - intensity of the side scatter light
    - Cell interior
    - Size of the nucleus
  - Y-axis - intensity of side fluorescent light
    - Degree of blood cell staining
WHAT ARE SOME OF THE CLINICAL USES OF THE IMMATURE GRANULOCYTE COUNT?

Case Studies

The views expressed in the case studies are those of the presenter. Results of case studies are not predictive of other cases and results may vary.
Case Study

- 60 year old woman was admitted to the ER with high fever and chills
- CBC with auto diff ordered, performed on Sysmex XN
- WBC $9.2 \times 10^3/\mu L$
- Total and percent neutrophils are high
- Physician suspects sepsis despite normal WBC
- CRP and blood cultures ordered
  - CRP results are within normal limits
  - Streptococcus pneumoniae was isolated from the blood cultures at 48 hours
- Patient admitted to the hospital
## Case Study 1

<table>
<thead>
<tr>
<th></th>
<th>Day 1 on admission</th>
<th>+6 hours</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG %</td>
<td>4.2%</td>
<td>5.9%</td>
<td>2.4%</td>
<td>1.7%</td>
<td>0.8%</td>
</tr>
<tr>
<td>IG absolute#</td>
<td>.38</td>
<td>.54</td>
<td>.22</td>
<td>.16</td>
<td>.07</td>
</tr>
<tr>
<td>(2 diffs performed on each slide)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bands % Range</td>
<td>5%- 19%</td>
<td>9%-26%</td>
<td>4%-12%</td>
<td>3%-8%</td>
<td>1%-3%</td>
</tr>
<tr>
<td>metas</td>
<td>1-2%</td>
<td>1-2%</td>
<td>1%</td>
<td>0%</td>
<td>0-1%</td>
</tr>
<tr>
<td>Myelos</td>
<td>3%</td>
<td>4%</td>
<td>1-2%</td>
<td>1-2%</td>
<td>0%</td>
</tr>
<tr>
<td>Pros</td>
<td>0%</td>
<td>0-1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

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Case Study 1

- Band counts were high or low depending on who performed the diff
  - Demonstrates the inherent subjectivity and imprecision of band counts and manual differential counts (using only 100 cells)
- Immature granulocyte count is automated, not subjective
  - Presence of these immature granulocytes was seen from slide correlation
Case Study 1

- IG% of >1% is indicative of a true left shift and >3% may predict positive blood cultures
  - 1st 2 CBCs, on admission and 6 hours later, IG > 3%
  - Positive blood cultures
  - Admission to Day 3, >1%
- The band count could not have been used to predict this because of subjectivity
- If the IG is elevated on admission, ED may determine if an infection is community acquired vs. nosocomial

Advantages of IG

- IG available with CBC, no additional testing, fast, no need to wait for manual differential
- Study concluded IG% may be a better predictor of sepsis than the WBC and band count¹
- IG% and absolute counts can be increased when other markers of infection are inconclusive²
- With the availability of a 6 part diff, labs have encouraged physicians to order and use automated differential

High IG% Study findings

- Study of patients with high IG% and normal ANC
- Mean IG% 3.9% (range 2.0-12.0%) (positive)
- Mean WBC $5.21 \times 10^3/\mu L$ (range 2.01-7.49 $\times 10^3/\mu L$)
- IG% compared to qualitative values for CRP (84%), ESR (95%) and CD64 (80%) despite normal WBC
- Conclusion: automated IG count can be used as part of the CBC to highlight a potential inflammatory or infectious process even in the absence of leukocytosis or neutrophilia.

Determining Infection: The Absolute Neutrophil Count

- WBC count x % neutrophils in the differential count
- Absolute neutrophil count can also be used as an indicator of infection and inflammation.
- ANC is also very important in chemotherapy
  - Risk of infection is much higher when the ANC is < 500
  - ANC from instrument is measured and can be reported.
  - No need to wait for manual diff for ANC

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Determining Infection: Immature to total neutrophil ratio = ITR

- ITR is used by neonatologists to determine infection.
- Calculation based on a 100 cell manual differential.
- In this formula, the immature neutrophils are bands, metas, myelos, and pros.
- An ITR <0.2% is a negative predictive value for sepsis.

$$\frac{\text{Immature neutrophils}}{\text{Total neutrophils}} = \frac{\text{Bands + myelo + meta + pros}}{\text{Total neutrophils}} = \text{ITR}$$
## Case Study 2

<table>
<thead>
<tr>
<th></th>
<th>Band range %</th>
<th>ITR range</th>
<th>IG%</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby A</td>
<td>15-26%*</td>
<td>0.29-0.44</td>
<td>5.4%</td>
<td>Fever, with sepsis</td>
</tr>
<tr>
<td>Baby B</td>
<td>5-17%*</td>
<td>0.06-0.22</td>
<td>0.3%</td>
<td>Fever, no infection</td>
</tr>
</tbody>
</table>

*2 diffs counted
Case Study 2

- I2 babies less than 30 days old, wide variety of bands counted by 2 techs
- In the ITR, bands are counted as immature cells.
- Despite the imprecision of the band count, Baby A has >10% bands counted by both techs and the ITR range is >0.2% in both calculations
- In Baby B, band count from one differential was 5% and the second tech counted 17% bands.
  - Since the bands are used to calculate the ITR, one calculation gives an ITR of 0.06%, a negative predictor of infection
  - second count gives an ITR of 0.22%, above the 0.2% threshold
Case Study 2

- Absolute neutrophil count (ANC) = number of neutrophils available to fight infection
  - Calculated using bands and neutrophils
  - Bands included with mature cells
- ITR
  - Bands included with immature cells
- This can be confusing information!
- Using the automated differential from the Sysmex XN, the IG% gives us a more clear and precise measure of left shift
- Using criteria for infection, the IG% of Baby A is clearly above 3% and in Baby B is well below 1%
How do band counts compare to the IG?

- Should bands be eliminated as an indicator of infection?
- Study concluded that IG% and ANC were more reliable in predicting infection than WBC¹
- Suggestions have been made that the left shift should be redefined with IG% rather than bands
- Bands may be too subjective to be the best indicator of infection in newborns, as they lead to an imprecision in the ITR
- The IG count can highlight potential acute infection or inflammation at its earliest stages, even when other parameters are still within normal ranges²

Clinical uses of the IG count in Cancer Management

IG can be used for early detection of tumor related myeloid proliferation

Tumor cells enhance the growth of myeloid cells and lead to leukocytosis

| Inhibit differentiation of myeloid cells | Accumulation of immature forms | Increased immature cells may indicate tumor formation | Increased immature cells may also induce development of the patient’s anti-tumor immune suppression |

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Auto Diff in Cancer Management

- **Indirect usage:**
  - 6 part differential separates IG from neutrophils and bands (used to calculate the ANC)
  - Instrument ANC (IANC) is reported with CBC and can be used by oncologists to manage patient care
  - ANC is important because risk of infection is higher when below 500 (on chemotherapy)
  - Physicians may treat or delay treatment based on ANC
- **If slide still needs review, IANC available before man diff**
- **Faster results**
  - Reduces patient wait time
  - Time to start or delay therapy
Clinical uses of the IG Count in Cancer Management

- After chemo, WBC counts drop
- G-CSF given for support during treatment
- Shortens time patient is neutropenic and at risk for infection
- Increased IG count may indicate G-CSF is working
- IG reflects stimulated white cells released from the bone marrow before maturation
- Increased IG can be attributed to G-CSF (Neulasta)
Benefits

Clinical Uses
- Early indication of infection
- May detect community acquired vs nosocomial infection
- Management of cancer/chemotherapy

Cost and Time Savings
- Sysmex automated differential: thousands of cells counted
- Statistically superior automated differential
- Reduced turnaround time - IG reported with the automated differential
- Instrument ANC reported with CBC
- nRBCs reported with CBC
- No additional blood draws necessary
- Valuable when used in conjunction with other indicators for the diagnosis of infection and inflammation
Thank you!
Questions?
What’s In and What’s Out?  
New CBC Parameters for Clinical Diagnosis  
Part 2: What’s ‘In’ for Thrombocytopenia diagnosis?  
Advanced Platelet Parameters: The Immature Platelet Fraction (IPF%) and the Immature Platelet Count (IPF#)  

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Objectives

- Define and list symptoms of thrombocytopenia
- Describe the concept of reticulated platelets and Immature Platelet Fraction
- Identify applications of the IPF in diagnosis of and distinguishing the cause of thrombocytopenia
- Analyze CBC results with reported advanced clinical parameters and correlate with diagnoses
Advanced Clinical Parameters

- New test availability
- An extension of the traditional CBC and automated differential
- Direct cellular measures of hematopoiesis
- New tools for patient care
- Can provide a wealth of knowledge to physicians in making diagnoses
  - Ret-He
  - IPF#, IPF%
  - IG#, IG%
Platelet Counts

- Platelets: first line of defense in controlling bleeding
- Thrombocytopenia can lead to:
  - Easy bruising
  - Tiny leaks from capillaries into the skin and mucous membranes, causing petechiae
  - Bleeding
- With thrombocytopenia, platelet counts can be less reliable than with normal counts
  - Physicians rely on precision with very low platelet counts
  - Need to make informed decisions about when to transfuse
Traditional Platelet counting methods

Optical platelet counts
- Platelets measured by size
- Large platelets can be missed
- Can lead to falsely decreased counts

Impedance platelet counts
- At low end, other cellular elements can be counted as platelets
  - RBC fragments
  - Schistocytes
  - Microcytic RBCs
- Can lead to falsely increased count
Platelet counts on Sysmex

- For normal samples, Impedance counting used
- If abnormal scattergram or a low platelet count, PLT-F is reflexed
- PLT-F is more reliable, counting time 6x
- Uses dye specific for platelet organelles - Oxazine
- Eliminates interferences seen with other methods
- Fluorescent dye labels the RNA
  - Forward scatter is used to determine size; fluorescence is used to measure RNA content
  - Gating set based on cell volume and RNA content

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What can we use to help determine etiology of thrombocytopenia?

Etiology helps guide treatment!
Diagnosis: Based on Etiology of thrombocytopenia

Thrombocytopenia
- Decreased platelet production
- Increased destruction

Determining etiology
- Physical with attention to bleeding symptoms and organ enlargements
- Medical history
  - Family history - genetic platelet disorders
  - Recent viruses
  - Drug therapies
- Bone marrow aspirate and biopsy
Mean Platelet Volume (MPV)

- Analogous to RDW for Red Cells
- Used as an indicator of the maturity of platelets
  - Young platelets largest
  - Size decreases with maturity
- Normal range 9-12 fl
- MPV higher when more platelets are being released from the bone marrow
- MPV lower if fewer are being newly released
- Indirect marker of platelet production
- As with impedance platelet count, MPV can be unreliable because any RBC fragments or particles may interfere
Reticulated Platelets

- Concept of reticulated platelets (retPLT), first researched late 1960’s
  - Immature, functional platelets in the peripheral blood.
  - retPLT are to mature platelets as reticulocytes are to mature RBCs
- Youngest platelets
  - Within 24 hours of being released from the bone marrow
  - Large, with increased amounts of RNA
- Can provide an estimate of the rate of thrombopoiesis
- Originally stained with new methylene blue and counted manually
  - Similar to manual reticulocyte count
  - Tedious, Imprecise

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Automated retPLT- 2000’s

- Flow cytometry used for measuring retPLT
- Reticulated platelets stained with Thiazole Orange, passed through flow cytometer
- Normal ranges wide: 1-15%
- No standardization
- Variations in
  - Dye concentration
  - Timing
  - Gate settings
- Time consuming, Labor intensive, Costly

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What’s new for Platelets?

- Newer flow cytometry methods
  - Fluorescent dye-platelet specific
  - 2 dimensional gating
- RetPLT Available on 2 Hematology analyzers
- Abbott CELL-DYN
- Sysmex XN
  - When PLT-F is reflexed, IPF% and IPF# are also reported.
  - RetPLT expressed as absolute Immature Platelet count, IPF# and IPF%
Immature Platelet Fraction

- Routine CBC reagents and controls used
- Same K2 EDTA CBC tube
- Automated
- Simple to perform
- Fast
- Tight normal ranges
- Standardized

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Clinical Utility of the IPF?

- The IPF helps physicians to differentiate pathogenesis
  - Increased IPF indicates more newly formed immature platelets circulating
    - Suggests thrombocytopenia caused by platelet destruction or consumption
  - Decreased IPF
    - Thrombocytopenia caused by deficient platelet production, bone marrow failure
  - Must know etiology to determine treatment
Plt-F and IPF

Low PLT +
Normal/Low IPF
(Consistent with production disorder)

Normal

Low PLT+ High IPF
(Consistent with destruction disorders)

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Large platelets, High IPF
Immature Platelet Fraction

- Immature platelets are functioning platelets
  - Decisions can often be made without costly, time consuming flow cytometry
  - Without an invasive bone marrow biopsy
  - Without waiting for the results of such biopsy
  - May improve blood management.
Case Studies

The views expressed in the case studies are those of the presenter. Results of case studies are not predictive of other cases and results may vary.
Case Study 1

A 4 year old child was brought to the pediatrician by her mother with a complaint of sudden onset of severe bruising on her legs. She could not recall any falls or bumps that would have caused the bruising. On exam, the physician also noted mucosal bleeding in the oral cavity. Questioning revealed that the patient had experienced flu like symptoms several weeks earlier.

CBC performed

- WBC, RBC, Hgb, Hct, indicies normal
- Platelet count 36 x 10^3/μL
Case Study 1

- **Results**
  - Normal CBC
  - Decreased PLT
  - Bleeding symptoms
  - Normal physical exam (except for bleeding)
  - No family history of bleeding disorder
- **Additional results from Sysmex XN**
  - Low platelet count reflexed Plt -F and IPF
  - IPF 16% (normal IPF% 1.0-7.0%)
- Increased IPF indicates an increase in platelet production
- Suggests thrombocytopenia may be due to excessive destruction of platelets.
Case Study 1

- **Diagnosis:** Immune (Idiopathic) Thrombocytopenia - ITP
- An IPF reported with a CBC is fast, inexpensive, and be extremely beneficial in aiding a timely diagnosis
- As platelet count recovered, IPF% returned to normal range
- ITP can be monitored with CBC
  - IPF can be used not only to help diagnose but also as an indicator of remission
  - May improve blood management
- One month later, Plt 164 x 10^3/μL
- IPF% 4.0%

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ITP- Discussion

- ITP is an autoimmune bleeding disorder in which the immune system makes anti-platelet antibodies which destroy platelets.

- ITP is typically an acute disease in children
  - Thrombocytopenia
  - After viral illness or live vaccination
  - Antibodies bind to platelets and cause destruction
  - Self-limiting, resolves in several weeks to a few months

- Chronic form more commonly seen in adults
  - Diagnosis of exclusion
  - Does not typically follow viral illness
  - Requires treatment
Case Study 2

- Newborn, full term, male child, developed scattered petechiae and purpuric hemorrhages several hours after birth. The baby was moved to the NICU and a CBC was drawn.
- WBC, RBC, Hgb, Hct and indicies were normal
- Plt count $48 \times 10^3 / \mu L$
- Thrombocytopenia reflexed IPF on XN
  - IPF 17%, indicative of increased platelet production
  - Baby exhibited no symptoms of sepsis
- Mother is gravida 1, para 1 with normal CBC, no symptoms of ITP
- No history of drugs known to be associated with drug induced thrombocytopenia
Thrombocytopenia is not an uncommon finding in neonates

3 primary causes

- Neonatal sepsis can present with a high IPF, is typically found in very ill or premature babies, degree of thrombocytopenia is not as severe as with NAIT
- Neonatal thrombocytopenia due to placental insufficiency would exhibit a decreased IPF due to a deficiency in platelet production
- Neonatal alloimmune thrombocytopenia, (NAIT) caused by platelet destruction, IPF% is high.

IPF% and IPF# can help differentiate the causes of neonatal thrombocytopenia

Can help steer treatment and save infants from unnecessary invasive procedures
Case Study 2

- Diagnosis: NAIT
  - Similar in pathogenesis to hemolytic disease of the fetus and newborn (HDFN)
  - Incompatibility in human platelet antigens between mother and baby.
  - Can affect first born
- Mother is HPA-1b and the father and baby are HPA-1a.
- The mother forms anti-HPA-1a which crosses the placenta and destroys the fetus’ platelets.
- Thrombocytopenia caused by platelet destruction, with high IPF%
- Condition is self-limiting; resolves in 1-4 weeks

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Other Clinical uses of IPF

- IPF may also be a reliable indicator of bone marrow recovery.
- Traditionally, neutrophil counts have been used as an indicator of recovery after a bone marrow transplant.
  - IPF can be used as an indicator of imminent platelet recovery.
  - Post-transplant, the IPF% increases before the platelet count.
- In a study done with stem cell transplant patients, it was shown that the absolute neutrophil count took an average of 13 days to recover, compared to 9 days for the IPF.
- The IPF was shown to recover before the Immature reticulocyte count, platelet count and absolute neutrophil count, giving physicians earlier indication that the transplant was successful.

Other Clinical uses of IPF

- Investigation of secondary thrombocytopenias due to chronic liver disease, HIV, and Hepatitis C
- IPF useful in evaluation of hereditary platelet thrombocytopenias
- The IPF% and IPF# can be compared before and after platelet transfusion
  - after platelet transfusion, the IPF% will decrease due to the newly increased platelet count
  - IPF# remains the same.
  - This validates that the IPF is a reflection of continual platelet production by the bone marrow.\(^4\)

Conclusions

➢ IPF% and IPF# are expanded CBC parameters that physicians can use to aid in differentiation of various thrombocytopenic states
  ▶ Treatment for the different classes of thrombocytopenia can differ drastically
  ▶ Knowing the class of thrombocytopenia helps direct management
  ▶ IPF parameters are automated, easy to perform at the same time as the CBC, and provide standardized results
  ▶ Inexpensive and available 24 hours a day in the hospital setting.
  ▶ Can also reduce diagnostic costs for the patient
This is the new hematology, constantly providing the clinician with better tools for making diagnoses and treating patients

Platelet counts alone and MPVs are out

*Make room for the new kid on the block; the IPF is in!*
What’s In and What’s Out?
New CBC Parameters for Clinical Diagnosis

Part 3: Beyond the CBC and Reticulocyte Count: Early detection of iron deficiency anemia

Becky Socha, MS, MLS, (ASCP)CM BB CM
Objectives

- Define Reticulocyte, Reticulocyte hemoglobin (RET-He) and Immature reticulocyte Fraction (IRF)
- Discuss how the RET-He can be used in early detection of iron deficiency and iron deficiency anemia
- Discuss how the RET-He can be used to monitor the patient response to iron therapy
- Analyze CBC results with reported advanced clinical parameters and correlate with diagnoses

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Advanced Clinical Parameters

- New test availability
- An extension of the traditional CBC and automated differential
- Direct cellular measures of hematopoiesis
- New tools for patient care
- Can provide a wealth of knowledge to physicians in making diagnoses
  - RET-He
  - IPF#, IPF%
  - IG#, IG%
Reticulocyte Counts

Reticulocyte counts are the quantity of the youngest red blood cells released from the bone marrow into the peripheral blood.

Reticulocytes are reported as a %.

Absolute reticulocyte count is calculated by multiplying the Retic% by the RBC.
Reticulocyte Hemoglobin Equivalent

- Reflects the **quality** of the newly formed reticulocytes
- Amount of hemoglobin in newly formed RBCs
  - Indicates the amount of iron available for incorporation into red cells.
  - Measured at cellular level
- Can monitor early changes in iron status and hemoglobin incorporation into the RBC
- More sensitive than indirect chemical measurements
- Used in early detection of iron deficiency
Reticulocyte Hemoglobin Equivalent

- There are two different hematology systems that report reticulocyte hemoglobin content.
  - RET-He - Sysmex
  - CHr - ADVIA
- Studies have been done that demonstrate their equivalence\(^1\)

\(^1\)Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret-He) and assessment of iron-deficient states. *Clinical Laboratory Hematology* 2006;28:303 - 308.
The immature reticulocyte fraction (IRF) tells us about the rate of production of reticulocytes.

- Reticulocytes are the youngest RBCs.
- IRF is the youngest of the young.
- Depends largely on the ability of the bone marrow to respond to erythropoietin.
RET Channel
Scattergram on Normal Pattern

FSC = Forward Scattered Light
SFL = Side Fluorescent Light
LFR = Low Fluorescing Retics
MFR = Medium Fluorescing Retics
HFR = High Fluorescing Retics
IRF = Immature Retic Fraction = MFR + HFR
Case Studies

The views expressed in the case studies are those of the presenter. Results of case studies are not predictive of other cases and results may vary.
Case Study 1

A 29 year old female was seen by her gynecologist reporting a history of heavy menstrual bleeding with current bleeding lasting 15 days. A CBC was performed. CBC results are shown below.

**Is this patient iron deficient?**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Flags</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>7.23</td>
<td>4.5-10.5 K/CMM</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>4.38</td>
<td>3.70-5.30 M/CMM</td>
<td></td>
</tr>
<tr>
<td>HGB</td>
<td>12.0</td>
<td>12.0-15.5 GM/DL</td>
<td></td>
</tr>
<tr>
<td>HCT</td>
<td>35.2</td>
<td>36.0-46.0 %</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>80.4</td>
<td>80-100 FL</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>27.4</td>
<td>27.0-34.0 PG</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>33.1</td>
<td>32.0-36.0 %</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>243</td>
<td>150-450 K/CMM</td>
<td></td>
</tr>
<tr>
<td>MPV</td>
<td>11.0</td>
<td>9.6-12.0 FL</td>
<td></td>
</tr>
<tr>
<td>RDW</td>
<td>12.5</td>
<td>0-15.1 %</td>
<td></td>
</tr>
</tbody>
</table>
Case Study 1

This CBC showed no abnormal flags. Based on patient history and presentation, the physician was concerned about iron deficiency despite essentially normal hemoglobin and hematocrit, and indicies. She ordered a reticulocyte profile on the same specimen with the following results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Flags</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retic</td>
<td>1.87</td>
<td>0.5</td>
<td>0.5-2.0 %</td>
</tr>
<tr>
<td>Abs Retic</td>
<td>0.082</td>
<td>H</td>
<td>0.0391-0.057 M/CMM</td>
</tr>
<tr>
<td>Imm Retic Frac</td>
<td>14.9</td>
<td></td>
<td>2.3-15.9 %</td>
</tr>
<tr>
<td>RET-Hé</td>
<td>24.6</td>
<td>L</td>
<td>30-35 PG</td>
</tr>
</tbody>
</table>
Case Study 1

- Retic% and IRF are within normal ranges, absolute retic count is high.
- RET-He less than 29 pg in an adult is indicative of iron deficiency.
- Normal Hgb, Hct and low RET-He is an early indication of iron deficiency.
- With the absence of sufficient iron, this patient would eventually develop a microcytic, hypochromic anemia.
- RET-He can measure and indicate inadequate hemoglobin production before the hemoglobin and hematocrit decrease.
- Allows earlier intervention.
Case Study 2

The patient is a 76 year old woman diagnosed with Myelodysplastic Syndrome (MDS). She received 5 rounds of chemotherapy over the course of 9 months, with no significant response. CBC results are below:

<table>
<thead>
<tr>
<th>Patient results</th>
<th>Reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC 35.62</td>
<td>4.5-10.5 x 10³/μL</td>
</tr>
<tr>
<td>RBC 3.39</td>
<td>3.7-5.3 x 10⁶/μL</td>
</tr>
<tr>
<td>Hgb 8.7</td>
<td>12.0-15.5 g/dl</td>
</tr>
<tr>
<td>Hct 26.2</td>
<td>36.0-46.0 %</td>
</tr>
<tr>
<td>MCV 77.2</td>
<td>80-100 fl</td>
</tr>
<tr>
<td>Plt 142</td>
<td>150-450 x 10³/μL</td>
</tr>
</tbody>
</table>
Case Study 2

- Mercy Medical Center reflexes a RET-He when the Hgb is <9.0 g/dl and the MCV is <78.
- Given Aranesp (darbepoetin alfa) injections for anemia support.
- Aranesp is a man-made erythropoiesis stimulating protein which can be used to treat anemia associated with (MDS).

<table>
<thead>
<tr>
<th></th>
<th>Patient Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET-He</td>
<td>22.2</td>
<td>30.0-35.0 pg</td>
</tr>
</tbody>
</table>

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Case Study 2

- Functional iron deficiency may occur before IDA
  - Failure to release iron fast enough to keep up with demand
  - Patient can have iron stores and still have functional deficiency
- Classical chemical markers- Serum iron, transferrin and ferritin
  - Low Ferritin levels are indicative of iron deficiency
  - Markers are disturbed during an acute phase response in patients with severe disease
    - Interpretation of results is difficult in these cases
    - Ferritin may be normal or increased in iron deficient patients with other disease
Case Study 2

- Measuring RET-He tells us how much iron is available to make RBCs
- IDA is important to diagnose in oncology patients to determine treatment
  - ? Erythropoiesis stimulating agents
  - ?? Iron supplements
  - ??? Transfusion
- Recommended to investigate for ID in cancer patients if Hgb < 11g/dl

1Steinmetz HT, Ther Adv Hematolog, 2012
Case Study 2

Subsequent results, 1 month later and 4 months later

<table>
<thead>
<tr>
<th></th>
<th>Patient results 10/18</th>
<th>Patient results 01/19</th>
<th>Reference Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>32.20</td>
<td>7.92</td>
<td>4.5-10.5 x 10^3/μL</td>
</tr>
<tr>
<td>RBC</td>
<td>2.32</td>
<td>2.41</td>
<td>3.7-5.3 x 10^6/μL</td>
</tr>
<tr>
<td>Hgb</td>
<td>7.0</td>
<td>5.4</td>
<td>12.0-15.5 g/dl</td>
</tr>
<tr>
<td>Hct</td>
<td>23.6</td>
<td>17.5</td>
<td>36.0-46.0 %</td>
</tr>
<tr>
<td>MCV</td>
<td>77.4</td>
<td>75.4</td>
<td>80-100 fl</td>
</tr>
<tr>
<td>Plt-F</td>
<td>102</td>
<td>46</td>
<td>150-450 x 10^3/μL</td>
</tr>
<tr>
<td>Retic</td>
<td>5.64</td>
<td></td>
<td>0.5-2.5 %</td>
</tr>
<tr>
<td>IRF</td>
<td>3.9</td>
<td></td>
<td>2.3-15.9%</td>
</tr>
<tr>
<td>RET-He</td>
<td>15.4</td>
<td></td>
<td>30.0-35.0 pg</td>
</tr>
<tr>
<td>IPF</td>
<td>0.9</td>
<td></td>
<td>1.0-7.0%</td>
</tr>
</tbody>
</table>

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Case Study 2

- Iron Studies done on the patient (Jan 2019) results below
- In IDA by itself you would expect to see high TIBC, low iron and low ferritin
- Ferritin is of limited value as an indicator of iron status in patients with cancer
- Low or normal TIBC, normal to high ferritin and low to normal iron may suggest inflammation or chronic disease

<table>
<thead>
<tr>
<th></th>
<th>Patient results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>142</td>
<td>50-174 mcg/dl</td>
</tr>
<tr>
<td>TIBC</td>
<td>180</td>
<td>250-450 mcg/dl</td>
</tr>
<tr>
<td>Iron Sat</td>
<td>86.9</td>
<td>15-50%</td>
</tr>
<tr>
<td>Ferritin</td>
<td>&gt;1650 (2460)</td>
<td>10-120 ng/ml</td>
</tr>
</tbody>
</table>

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Case Study 2

Patient was diagnosed with refractory anemia due to MDS

- Aranesp injections for anemia support continue
- Transfusions, as needed
- Pancytopenia, with accompanying anemia and infections

Pancytopenia common in MDS

- Note Low PLT + Normal/Low IPF
- Consistent with production disorder
Case Study 2

- Left shift and blasts seen on peripheral smear prompted flow cytometry studies.
  - A myeloblast phenotype was detected representing 27% of the leukocytes.
  - Diagnosis: Acute monoblastic/monocytic leukemia
- Situation was discussed with patient and family
- Patient chose more conservative and palliative treatment options over further chemotherapy
Other Clinical Uses of RET-He

- Screening for and prevention of IDA before surgery
  - Correct anemia before surgery
  - Helps improve surgical outcomes
  - Better blood management
- Screening for IDA in infants and toddlers
  - Hgb with RET-He
  - Children with RET-He >29 pg have low probability of being iron deficient
RET-He: Conclusions

- Early screening for iron deficiency
- Screening can rapidly rule out IDA
- Very sensitive for early detection of IDA
  - More sensitive than Hgb and MCV
  - Less variation than Iron studies
- Used to monitor response to treatment
- Reduce unnecessary testing
  - Cost savings

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RET-He: Conclusions

- Interpret results with other tests and clinical presentation of patient
- Screening measure, used to reflex for iron studies
- Education programs should be arranged to help clinicians effectively use RET-He
- Physicians and laboratorians can develop guidelines for reflex testing to improve patient care
Thank you!
Questions?