It's not Black and White: Unraveling the puzzles of Hematology

Becky Sotha MS, BB, MLS(ASCP)
Jenny Medical Center, Baltimore, MD
beckysotha@jmc.com
www.jennymedicalblog.com

Disclosures

- I am receiving an honorarium from Sysmex for preparing and delivering this presentation.
- The views expressed in this presentation are those of the presenter and their healthcare facility and provided for illustrative purposes only. Results of case studies are not predictive of other cases and results may vary. Prior to using these devices, please review the manufacturer’s Instructions for Use.

Objectives

At the end of this presentation, the attendee will be able to:

- List common preanalytical issues with hematology specimens
- Determine if a sample has a true abnormal value or a spurious result
- State the steps to resolving problematic samples, which include clumped platelets, cold agglutinins, critical low or high counts, and abnormal distribution/scattergrams
- Correlate cells seen on slides with numerical values of parameters
- Describe how to select, implement, and review delta check practices
- Investigate and interpret delta check alerts
Health Care Changes

**Old model**
- Payment for volume
- More tests = lower costs = more revenue
- Medically unnecessary testing was performed

**New model**
- Payment for value
- More tests, more procedures = higher costs and less profit
- Motive for labs to work with physicians to develop and better utilize tests
- Everything must be productive

Volume to Value Shift

- Everything must be productive
- Motive for labs to work with physicians to develop and better utilize tests

- The right test at the right time
  - Decreases cost
  - Helps physician to choose best therapy for patient
  - Improves patient care
  - Patients are more satisfied

- Focus on early diagnosis
  - Immediate access to lab test results improves patient outcomes
  - Use new parameters on analyzers to help physicians in making diagnoses
  - If we can detect disease earlier, easier to treat

What can labs do?
Attributes of Innovative Labs

- New technologies in diagnostics
- Automation
- New quality standards: six sigma, LEAN
- Efficient workflow
- Use of middleware
- Reduced turn around time
- Collaborate with providers to improve patient outcomes
- Eliminate ‘non value-added’ tests, methods, procedures
- Patients are more satisfied

- Improves outcomes
- Improves patient care
- Helps physician to choose best therapy for patient
How Can We Add Value??

Create
- Create an efficient workflow
  - Instrument ANC reported with CBC w/autodiff
  - nRBCs reported with the CBC w/autodiff

Encourage
- Encourage physicians to order automated differential
- Encourage physicians to order automated differential

Use
- Use the tools given to us!
  - Automation
  - Middleware
  - Cellavision
  - XBarM, BCQM
- Trained technologists

Use “Best Practices”
- Use “Best Practices”

What are ‘Best practices’?
- “A best practice is a method or technique that has been generally accepted as superior to any alternatives because it produces results that are superior ... or because it has become a standard way of doing things”
- Best practices
  - Complying with requirements
  - Used to maintain quality
  - Can be based on self-assessment or benchmarking
- Goals
  - Reduced TAT
  - Following SOPs - the standard way of doing things!
  - Accurate and Precise results

What are Clinical Laboratory Scientists?
- CLS or MLT
- “Diagnostic detectives”
- We can’t be good detectives without looking at all the clues!
- Evaluate the results of laboratory analyses for accuracy and validity, and correlate laboratory data to disease processes
- Use best practices!
Hematology is not always black and white. Standard Operating Procedures are our GPS.

**BUT**
- We can't just blindly follow.
- If we just follow our GPS we don't learn.
- We need to follow SOPs, but if we don't look at the whole picture, we could end up going down the wrong path!

Hematology is not always black and white. Standard operating procedures (SOP)
- When we get a sample in hematology, we are looking at more than the results.
- Operator alerts give us more detailed direction.
- Attention to detail.
- Is specimen adequate, patient age, location, deltas,
- Deductive reasoning.
- We are Diagnostic Detectives! Use all those things you learned in school!

**Instrument Flags**

- **NEGATIVE judgement**
  - No flags generated
  - Results may not be normal, but results usually reported without review
  - Use autocommerce

- **Asterisk (*) next to a parameter**
  - Results may be unreliable
  - Confirm according to your laboratory SOP

- **Flags: Positive judgement**
  - Flags can be on Count, Morph, Diff
  - Recognize why the flag exists
  - Use histograms and scattergrams
Abnormal vs. Spurious Results

- In most cases, automated analyzers give quick and accurate results for both normal and abnormal samples.
- When flags exist, investigate!
- Abnormal or spurious result?

Abnormal results

- True reflection of patient status
- Correlate with patient history
- May autovalidate with no flags
- Flags
  - Results may be unreliable
  - If flags present, investigate
- Critical results flagged for notification

Spurious Results

- Erroneous results for any parameter
- Not a true reflection of patient status
- Typically Pre-analytical
  - Specimen collection or handling problems
    - Underfilled/overfilled tube
    - Clotted specimen
    - Hemolyzed
    - Lipemic
    - Wrong patient drawn
  - Contamination
  - Analytical-Instrument problems
    - Reagent
    - Check for instrument errors
Insufficient Blood Volume (short sample)

- What do you do?
  a) Reset alarm, ignore message
  b) Find the sample and rerun
  c) Sample looks ok, must be contaminated, call floor to request new sample
  d) Take a break

OR?

- Locate sample
- Check for clot, check volume, rerun if ok
- Visually check blood
- If suspect low hemoglobin
- Turn off aspiration sensor
- Remix & rerun in manual mode

Retic Abnormal Scattergram

- Abnormal scattergram ok to report if no asterisks (*)
  - You’re done!
- Flag indicates interfering substances
- Dilute sample and rerun
- Dilutions >1:5 should not be used
- Adequate particles needed for accurate gating
  - Turn aspiration sensor off
- Diluted RBC count must not be <0.50 x 10⁶/µL
  - If < 0.5 make lower dilution

*Sysmex users
*Sysmex XN Flagging Guide
Retic Abnormal Scattergram

- Dilutions
- % retic, IRF are %/ratio, RET-H measured at cellular level
- no multipliers needed
- Only use multiplier for absolute
- If (*) does not go away
  - Review smear for polychromasia, parasites, NRBCs, HJ Bodies, basophilic stippling
  - If present, add comment ‘may be affected by interfering substances’
  - Perform reticulocyte by an alternate method

RBC Indices

- Maxwell Wintrobe developed first reliable Hematocrit measurement, 1929
- Spun Hct, Wintrobe tube
- Investigated relationship of normal RBC measurements
- RBC, Hemoglobin, Hematocrit
- Indices
  - MCV
  - MCH
  - MCHC
- Math?

“Rules of 3”

- Quick shortcuts
  - Hemoglobin x 3 = Hematocrit +/- 3%
  - RBC x 3.3 = Hemoglobin +/- 1.5 g/dL
  - RBC x 9 = Hematocrit +/- 3%
- Used to tell us
  - Normal RBCs?
  - Abnormal RBCs?
  - Analytical error?
Rules of 3 are ‘history’!

Current technology can automatically calculate indices

Not every sample will follow "rules of 3"

They only work when the RBCs are normal!!

Normal Hgb content
Normal size

Counting and sizing RBCs

- Classic impedance counting
  - Curves are not Gaussian
  - Inaccurate pulses if cells do not pass directly through center of aperture
  - Some cells pass through together
  - Some cells recirculate and are counted twice

- Impedance + Hydrodynamic focusing
  - Eliminates inaccurate pulses
  - Directs cells singly through center of aperture
  - Cells remain close to normal, not deformed
  - Accurate determination of Hct and MCHCs in both normal and abnormal samples

- RBC counting technology can influence MCHC results

MCHC

- 95% of normal values will be within 2SD of mean
- 5% of normal healthy individuals have MCHC results <32.0 or >36.4 g/dL
- MCHC at high end of normal or slightly increased
  - Normally healthy young males with Hgb at high end of normal
- Low MCHC, Low MCV
  - Microcytic, Hypochromic anemia
- Normal MCHC, Low MCV
  - Hemoglobinopathies
- High MCHC also seen in patients with HgS, HgbSC, HgbC
  - Hyperdense RBCs
High MCHC

<table>
<thead>
<tr>
<th>Low Sodium affecting Hct? Electrolyte Imbalance</th>
<th>Lipemia, Icterus, Abnormal protein or severe leukocytosis</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low or Normal MCV, High MCHC</td>
<td>Low or Normal MCV, High MCHC</td>
<td>Low or Normal MCV, High MCHC</td>
</tr>
<tr>
<td>Dilute 1:5 with CELLINK DCL, correct affected results for dilution factor</td>
<td>1:5 dilution; Correct results for dilution factor</td>
<td>Spurious! Recollect sample</td>
</tr>
</tbody>
</table>

**Did you know??**

It’s not always a Cold Agglutinin!

Check Pattern of Results

Look at MCV and MCHC

Other interferences may affect Hct and cause >MCHC

**Cold Agglutinin**

- MCHC is >37.5 g/dL
- Suspect, Turbidity/HGB interference?
- Turbidity may be present in the diluted and lysed sample
- May interfere with Hgb detection and falsely increase Hgb
- Other interferences may affect Hct and cause >MCHC
- Asterisks (*) appear next to the HGB, MCH and MCHC

**Did you know??**

- MCHC is >37.5 g/dL
- Suspect, Turbidity/HGB interference?
- Turbidity may be present in the diluted and lysed sample
- May interfere with Hgb detection and falsely increase Hgb
- Other interferences may affect Hct and cause >MCHC
- Asterisks (*) appear next to the HGB, MCH and MCHC
Note Indicies: High MCV, Low MCHC

- Severe hypernatremia
- Anemias = microcytic/hypochromic cells or microcytic/normochromic
- Generally not macrocytic/hypochromic
- Indices on this patient do not make sense.
- Dilute, equilibrate for 15 minutes
- RBC measurements change

<table>
<thead>
<tr>
<th></th>
<th>A11</th>
<th>A12</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>3.82</td>
<td>2.54</td>
</tr>
<tr>
<td>RBC</td>
<td>2.50</td>
<td>2.54</td>
</tr>
<tr>
<td>Hgb*</td>
<td>7.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Hct</td>
<td>25.9</td>
<td>22.9</td>
</tr>
<tr>
<td>MCV*</td>
<td>103.2</td>
<td>89</td>
</tr>
<tr>
<td>MCH</td>
<td>28.8</td>
<td>31.9</td>
</tr>
<tr>
<td>MCHC</td>
<td>27.8</td>
<td>31.4</td>
</tr>
</tbody>
</table>

Platelet Counts

- Platelets: first line of defense in controlling bleeding
- Thrombocytopenia can lead to:
  - Easy bruising
  - 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 treat

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
    - Erythrocyte fragments
    - Schistocytes
    - Microcytic RBCs
    - Can lead to falsely increased count
Platelet Counting Methods

Fluorescent Platelet
- Platelet specific dye
- Measures RNA of platelets
- Used for plt abnormal distribution flags or low counts
- PLT-F is more reliable, counting time 6x
- Eliminates errors with other methods

Platelet Scenario-platelet clump flag

No patient history
PLT-132, clump flag, reflex F ~ F-132, clump flag
Slide maker alarm: short sample error
Time consuming phone calls
Occurrence report
Resolve alarms before verifying any results!

Tricky Platelets- Follow SOPs!
- SOPs can’t cover every scenario
- Our procedure is all flagged results are repeated with PLT-F, check for clot, review smear
- PLT under 100 repeated with PLT-F, checked for clot and slide review
- Even if no short sample alarm, always check tube if clump flag or according to SOP BEFORE validating
- First time patient with low normal count for no apparent reason
- IPF and MPV with *. Follow SOPs for reporting results that cannot be measured due to potential interference
Reporting platelet counts - clumped

- Review smear under scope at feathered edge
- Giant or small platelets
- Clumps
- Fragmented, microcytic RBCs
- Parasites
- “Clumped platelets, appear to be within normal range”
- “Clumped platelets, appear to be decreased”
- “Clumped platelets, appear to be increased”
- Add 4th comment?
- May be beneficial to estimate >100 or <100
- OB, L&D, NICU, OR patients may have different critical values

Thrombocytopenia

True Thrombocytopenia
- Check volume in tube
- Clot check
- Review deltas
- slide review, low estimate, no clumping
- report per SOP

Pseudothrombocytopenia
- Clumping due to preanalytical error-recollect sample
- Clumping due to patient condition or therapy
- Patient may be thrombocytopenic, even after clumping is resolved
- Low PLT count without hematologic disease, family history, and/or bleeding tendency identified
- PTCP should be considered

EDTA induced thrombocytopenia
- Presence of EDTA dependent IgM/IgG autoantibodies
- Causes of in vitro agglutination of platelets
  - EDTA induced pseudothrombocytopenia (EDTA-PTCP) is the most common cause.
  - Other causes: cold agglutinins, multiple myeloma, infections, antiphospholipid antibodies, high immunoglobulin levels, abciximab therapy
  - Clumping not resolved? Validate alternate anticoagulant
    - Na citrate, ACD, CTAD
    - Add Amikacin
  - Pitfalls to manual platelet counts

(Ref: Nakashima, 2020)
How Else Can We Add Value to Laboratory Tests?

The difficulty with Differentials

- Automated vs. Manual Differential
- Which to accept and report?
- Benefits of automated differential
- The innovative lab - encourage physicians to order auto diff
- Sometimes we still need to do manual diffs!
  - Autovalidation allows us to spend more time on those specimens that need it

Manual Differentials

100 Cell Manual Differential

- Enumerates percentage of each cell type
- Detects presence of abnormal cells
- Labor intensive, slow, expensive
- Subjective; imprecise, inaccurate
- Absolute counts needed to be calculated

Sometimes we still need to do manual diffs!
How can we add value to Lab tests?

- Use automated differentials and autovalidation
- Fully automated systems handling CBC, differentials, reticulocyte measurement and preparation of smears
- Less subjective
- Less hands on time
- Improved TAT
- Auto diff can count thousands of cells
- Counting more cells gives a more accurate differential
- Statistically superior
- Sysmex 6 part diff can separate IG from Neuts and bands and thus ANC can be reported at same time as CBC
- Improved TAT
- Better patient care

Case Study: What Would You Do?!

- Instrument reported ANC (IANC) = ANC 0.49 (called as critical value)
- ANC is important because risk of infection is higher when ANC is below 500 (with chemotherapy)
- 0.5 is “cutoff” used to determine if patient will get chemo
- Manual diff performed, ANC = 0.53
- Discrepancy??
- How to resolve?
IANC vs. ANC

- WBC = 83,000
- Neut% 0.77
- Lymph% 93.9
- Eos% 0.03
- Baso% 0.02
- Mono% 3.1
- IG% 2.2
- IANC 0.64

Tech reclassified cells in CellaVision
- 115 cells counted
- Neuts 0 0%
- Lymphs 106 92.2%
- Monos 6 5.2%
- Myelos 3 2.6%

No neuts seen, so ANC is now 0!
100 more cells counted, 2 neuts seen
ANC = .83

Resolving IANC Discrepancy

- Watch lower counts
- With normal range IANC, close match not as critical
- ex: IANC 3.42
- Validate CBC on these and release IANC
- OK even if man diff is done and ANC = 3.0 or 4.0
- Cell confirm, if appropriate
- If manual diff matches auto diff, use auto diff
- If ‘discrepancy’, or manual diff required, count more cells!
- Manually check slide for neutrophils

Use Technology: CellaVision

- Manual Diffs laborious and time consuming
- CV Automation saves time finding cells, simplifies
  - Counts 115 cells
- If very low WBC, putting same slide on counts same cells again
- Can make additional slides and merge
- If unusual cells seen, check previous diffs in CV
- In LIS you don’t see cells
  - Pictures in CV
- Can see path reviews, actual cells reviewed
- Use cell confirm if appropriate
- Know when to reclassify and perform manual differential
Tools & Resources: CellaVision
- Cells can be enlarged for better viewing
- More experienced techs can review a slide
- Share common database across sites
- Remote access
- CV allows collaboration
- Can have pathologist review slides without transporting slides

Other benefits
- Barcoded samples reduce errors
- Better ergonomics
- Can use as a teaching tool!

Smudge cells: Artifact or clinically significant?
- "basket cells", Gumprecht shadows
- Remnants of leukocytes
- No cytoplasm, sometimes all that can be seen are smashed nuclei
- Formed from leukocytes
- Fragile
- Typically lymphocytes
- In vitro phenomenon?

Smudge cells
- Originally thought to be artifact of slide making
- Number of smudge cells seen may be clinically significant
- Not diagnostic of CLL, but ...
  - Newly diagnosed CLL, larger % of smudge cells is a better prognostic factor
  - Vimentin is protein important in lymphocyte rigidity
  - Low vimentin can mean more smudge cells and better survival rates
- Presence of smudge cells should not be ignored
A Differential has 3 parts!

- WBC differential
- RBC morphology: CV Pre-classifies RBCs based on size, color, shape into 6 morphologies
- Platelet estimate

Must address all 3 tabs before you can sign out slide.
New Resource:
Automated Advanced RBC Morphology Application

- Adds consistency to RBC morphology
- Less subjectivity
- Time saving
- Semiquantitative and quantitative
- Auto locates and presents images of RBCs
- Pre-classifies RBCs based on size, color, shape, 21 morphologies
- Displayed as overview and individually
Improving Lab Operations

- CBC and diff are the highest volume tests in clinical lab
- Creating good workflow is vital for efficient lab operations
- SOPs ensure everyone does things the same way
- Less subjectivity
- Use resources
  - Middleware or onboard rules: Use rules and Op alerts to make things consistent
- Use Automation and Technology
- Teamwork: flags, alarms, communication
- Review instrument features and continual training
- Trained techs are one of our best resources
- Use interesting or problem samples as teaching moments

“"It is by riding a bicycle that you learn the contours of a country best, since you have to sweat up the hills and coast down them. Thus you remember them as they actually are, while in a motor car only a high hill impresses you, and you have no such accurate remembrance of country you have driven through as you gain by riding a bicycle."" - Ernest Hemingway

Thank you! Questions?