The Abnormal CBC

Presented by: Guy W. Tillinghast, MD

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TPMG Cancer Care

Guy Tillinghast, MD

- Board certified in Internal Medicine and Medical Oncology
- Joined TPMG November 2013
- Riverside: June 2005 – November 2013
- Attended 3 year Heme/Onc fellowship at NIH
  - Opted out 6 month hematology-only hospital rotation in lieu of further bench research
  - Did not find the cure (sorry!)

Established Nov. 12, 2013

Office
Mary Immaculate Pavilion
12720 McManus Blvd.
Suite 307
Newport News, VA 23602

Monday through Friday
Disclosures

No drug or laboratory testing company affiliations
Goals

• The hematologist’s perspective: Giving a sense of this

• Enable primary care physicians to be hematologists, if they want to be

• Orders Module: Discuss anemia work-up order sets
Lecture Outline

I. The traditional workup, based on RBC size

II. Workup based on patient history

III. An integrated approach that includes RBC biology
Coulter Counter
RBC Statistics Review

- **MCV**: (Mean Corpuscular Volume) -- How big
- **RDW**: (Red cell Distribution Width) -- How varied
- (RDW = SD of RBC width/MCV x 100)
- **MCH**: Mean Corpuscular Hemoglobin (Hgb x 1/RBC)
- **MCHC**: Mean Corpuscular Hemoglobin Concentration
- (Hgb x 1/Hct): a normalized version of the MCH
- Hct can be calculated by multiplying the RBC and the MCV....makes sense!
Reasons Behind These:

- **HGB + HCT**: *Purpose*: So you can divide Hct by 3 and see if Hgb production problem
- **MCV**: *More on next slide*
- **MCH**: Usually low when MCV is (iron def./thal.) and high (folate/B12 def.)
- **MCHC**: *It’s only purpose for being*: dx Hereditary Spherocytosis
  
  (Nearly pathognomonic if elevated, esp. if Coombs negative)
  (But not always elevated in Hereditary Spherocytosis)
- **RDW**:
  
  o Can be used to dx. iron def. from thalassemia (high in iron def.)
  o Can be used to distinguish presence of two anemias
    (micro + macro)
  o Great statistical power because huge numbers
    (i.e. 200 billion RBCs made each day)
MCV:  
- Reflects underlying biology/what cells do in the marrow  
- A connection to biology makes MCV a powerful tool  
- So powerful that the mainstay for anemia workup for a half century  

**PROBLEM:** There are 400 anemias, not 9

The MCV thermometer

<table>
<thead>
<tr>
<th>MCV Value</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55</td>
<td>Thalassemia 3 genes missing</td>
</tr>
<tr>
<td>55-60</td>
<td>Thalassemia 2 genes missing</td>
</tr>
<tr>
<td>61-65</td>
<td>Thalassemia 1 gene missing</td>
</tr>
<tr>
<td>66-70</td>
<td>Sideroblastic Anemia</td>
</tr>
<tr>
<td>71-81</td>
<td>Iron deficiency hemolytic anemia</td>
</tr>
<tr>
<td>82-92</td>
<td>Anemia of Chronic Disorders drug effect</td>
</tr>
<tr>
<td>93-100</td>
<td>Megaloblastic anemia</td>
</tr>
<tr>
<td>100&gt;</td>
<td>Thyroid disorder</td>
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<tr>
<td></td>
<td>Liver disorder</td>
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<td></td>
<td>Alcohol toxicity</td>
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<tr>
<td></td>
<td>Bone marrow disorder</td>
</tr>
</tbody>
</table>
### Initial CBC

<table>
<thead>
<tr>
<th>Test</th>
<th>Result 1</th>
<th>Result 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC LH</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>RBC LH</td>
<td>4.2</td>
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<tr>
<td>HGB LH</td>
<td>10.7</td>
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<tr>
<td>HCT LH</td>
<td>35.5</td>
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<tr>
<td>MCV LH</td>
<td>84</td>
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<tr>
<td>MCH LH</td>
<td>25</td>
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<tr>
<td>MCHC LH</td>
<td>30</td>
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<tr>
<td>RDW LH</td>
<td>18.8</td>
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<tr>
<td>PLT LH</td>
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<td>NE%</td>
<td>64.9</td>
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<tr>
<td>LY%</td>
<td>26.0</td>
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<tr>
<td>MO%</td>
<td>6.8</td>
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<tr>
<td>EO%</td>
<td>1.8</td>
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<td>BA%</td>
<td>0.5</td>
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<td>Plt est</td>
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<td>Plt Morph</td>
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<td>Anisoc</td>
<td>1+</td>
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<td>Hypo</td>
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<td>Macro</td>
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<tr>
<td>Poikylo</td>
<td>1+</td>
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### Further Workup

<table>
<thead>
<tr>
<th>Test</th>
<th>Result 1</th>
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</thead>
<tbody>
<tr>
<td>Ferritin, Serum</td>
<td></td>
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<tr>
<td>Ferritin</td>
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<tr>
<td>Folate (Folic Acid), Serum</td>
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<tr>
<td>Folate</td>
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<tr>
<td>Haptoglobin</td>
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<td>Haptoglobin</td>
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<td>Hep A Ab, Total</td>
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<tr>
<td>Hep A Ab, Total</td>
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<td>LDH</td>
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<tr>
<td>LDH</td>
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<tr>
<td>Reticulocyte Count</td>
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<tr>
<td>RET %</td>
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<tr>
<td>Sedimentation Rate-Western ESR</td>
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<tr>
<td>ESR</td>
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<tr>
<td>TIBC Panel</td>
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<tr>
<td>Iron AU</td>
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<td>Sat % AU</td>
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<td>TIBC AU</td>
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<td>UIBC AU</td>
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<td>UA Microscopic</td>
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<td>UwEC</td>
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<td>UrRBC</td>
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<td>UbAct</td>
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<td>UEpi</td>
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<td>UCasts</td>
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<td>UMucus</td>
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<tr>
<td>Uric Acid, Serum</td>
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<td>URIC f</td>
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<tr>
<td>Uric Acid, Serum AU</td>
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<tr>
<td>URIC AU</td>
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</tr>
<tr>
<td>Vitamin B12</td>
<td></td>
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<tr>
<td>Vit B12</td>
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<tr>
<td>Vitamin D, 25-Hydroxy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>33.8</td>
<td>129.00</td>
</tr>
</tbody>
</table>

**CASE #1**

- Ferritin: Low
- Folate: Slightly High
- Haptoglobin: Inappropriately Normal
- LDH: Normal
- TIBC Panel: High
- UA Microscopic: Normal
- Vitamin D, 25-Hydroxy: Normal
Case 1 Teaching Points Reinforced

• Iron deficiency affects hemoglobin production first

• Ratio of Hgb to HCT should be 1:3, if Hgb falls short → Hgb production problem

• RDW frequently abnormal in iron deficiency anemia

• ...but here, high RDW indicates a macrocytic anemia (B12 deficiency) mixed with a microcytic one (Normocytosis resulting from averaging)

✓ **IMPORTANT LESSON 1**: Always check B12 in iron deficiency

✓ **IMPORTANT LESSON 2**: Nutritional deficiencies track together
Case 1 More Teaching Points

• The “hemolysis” tests here (LDH, bilirubin, Haptoglobin and uric) negative

• The elevated ESR here points to ACD (Anemia of Chronic Disorders)

• Total: 3 causes for anemia

• Vitamin D done as a check for a nutritional deficiency

• **Vitamin D dosing:**
  • 1000mcg (1mg) IM daily x 5, weekly x 4, then monthly.
  • Afterwards: can transition to 2000mcg PO daily, but will need to check/monitor B12 levels.
Iron deficiency not even a quarter of the cause

Iron deficiency and Acute bleeding have to be combined to equal the “inflammation/neoplasia/chronic disease” category

Source: Cleveland Clinic
Data Summary

- There are over 400 causes of anemia
- 3.4 million Americans have anemia (1-2%)
- Over 4,000 TPMG patients have anemia
A Conflict of Two Paradigms, Two Dogmas

**Simple Approach**

- Because iron deficiency so often, triage mentality justified
  - First treat with iron ~ Investigate those not responding
  - Simple does not mean stupid

**Sophisticated Approach**

- With so many anemia causes: aggressive evaluation upfront
Part 1: The Traditional Workup

The MCV thermometer

- **<55**
  - Thalassemia 3 genes missing
  - Thalassemia 2 genes missing

- **55-60**
  - Thalassemia 1 gene missing
  - Sideroblastic Anemia

- **61-65**
  - Iron deficiency hemolytic anemia

- **66-70**
  - Anemia of Chronic Disorders drug effect

- **71-81**
  - Megaloblastic anemia
  - Thyroid disorder
  - Liver disorder
  - Alcohol toxicity

- **82-92**
  - Bone marrow disorder

- **93-100**
  - Megaloblastic anemia

- **100+**
  - Megaloblastic anemia
  - Thyroid disorder
  - Liver disorder
  - Alcohol toxicity
The Traditional Workup: Microcytic Differential

- Iron deficiency
- Thalassemia trait
- ACD
- Sideroblastic anemia (more in menstruating women)
- Pregnancy in menstruating women (check for pregnancy)
- Other hemoglobinopathies (more in children)
- Lead toxicity (more in children)
- Zinc excess
- Copper deficiency

**Std Tests**

- Iron Panel
- Ferritin (and ESR)
  * Recommend B₁₂
## The Traditional Workup: Macrocytic

<table>
<thead>
<tr>
<th>Macroblastic (Involves (B_12) or folate)</th>
<th>Non-megaloblastic</th>
<th>False Macrocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophic gastritis</td>
<td>Alcohol (though may evolve to (B_12) or folate deficiency)</td>
<td>Cold agglutinins</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Medications (zidovudine/retrovir)</td>
<td>Hyperglycemia</td>
</tr>
<tr>
<td>HIV treatment</td>
<td>Myelopdysplasia</td>
<td>Marked leukocytosis</td>
</tr>
<tr>
<td>Seizure medications (a folate depletion)</td>
<td>Hypothyroidism</td>
<td>Std Tests</td>
</tr>
<tr>
<td>Some bone marrow disorders</td>
<td>Liver disease</td>
<td>- B12</td>
</tr>
<tr>
<td>Nitrous oxide use</td>
<td>Hemolytic anemia</td>
<td>- Folate</td>
</tr>
<tr>
<td>Inherited</td>
<td>Hemorrhage</td>
<td>- Reticulocyte</td>
</tr>
<tr>
<td></td>
<td>COPD</td>
<td>- TSH</td>
</tr>
<tr>
<td></td>
<td>Splenectomy</td>
<td>- CMP</td>
</tr>
<tr>
<td></td>
<td>Renal disease</td>
<td>- GGT</td>
</tr>
<tr>
<td></td>
<td>Reticulocytosis</td>
<td>If all tests neg., drug or etoh not implicated:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone marrow</td>
</tr>
</tbody>
</table>

**Notes:** Folate levels fluctuate over time • RBC folate gives the 120 day average, but the extra expense not justified • On \(B_12\) level, if result 185-500, must reflex to a methylmalonic acid • Alternative: Look for megaloblastic changes on the peripheral smear • If \(B_12\) deficient, follow-up with “intrinsic factor assay” to diagnose pernicious anemia
The Traditional Workup: Macrocytic

**Notes:**

- Folate levels fluctuate over time, and may be normal just when you test, whereas macrocytic RBC may last 120 days.

- RBC folate gives the 120 day average, but the extra expense not justified.

- RE: B12 levels, if result 185-500, must reflex to a methylmalonic acid.

- Methylmalonic acid falsely elevated in renal failure and dehydration.

- Alternative: Look for megaloblastic changes on the peripheral smear.

- If B12 deficient, follow-up with “intrinsic factor assay” to diagnose pernicious anemia.
Comments

- **Medications** and **alcohol** are the most common causes of macrocytosis (at least in the hospital)

- **Notable Medications** causing macrocytosis (and by inhibiting DNA synthesis):
  - Chemotherapy, azidothymidine, azathioprine, **MTX**
  - **Bactrim**
  - **Anticonvulsants**
The Traditional Workup: Normocytic

**Differential (greatly abbreviated):**

- Blood loss
- Hemolysis (inherited, acquired)
- Decreased retics (primary, secondary: notably ACD)
- False (pregnancy, over-hydration)

**Std Tests**

- Marrow response: reticulocytes
- Hemolysis: CMP, (or add haptoglobin)
- Iron status: iron panel, ferritin

**Std Algorithm**

- Hemolysis tests and reticulocytes positive: reflex to Coombs test
- Coombs test negative: reflex to RBC enzyme or membrane defect
When Coombs Negative

• This is the time to have a smear reviewed: can (almost) give the diagnosis:
  • Hereditary Spherocytosis
  • Hereditary Eliptocytosis

• Look at the MCHC: if elevated: (almost) pathognomonic for hereditary spherocytosis

• Urine hemosiderin: if positive, suggests cardiac valves or hemangiomas

• Flow cytometry for PNH

• RBC enzyme tests:
  • G6PD levels
  • Pyruvate kinase levels
CASE #2

- 44 year old woman
- Previous gastric resection and Bilroth I
- Recurrent gastric ulceration and bleeding
- Converted to Bilroth II
- Developed chronic anemia
- Treated with parenteral iron, B12, folate, no response
- Required RBC transfusion every 2 months
Bone Marrow Findings

- Dyserythropoiesis, dysmyelopoiesis, ringed sideroblasts, hemosiderin in plasma cells
- Cytogenetics normal
- Diagnosed with MDS, RARS type
- **But!!! Is this the diagnosis?**
Copper Deficiency!!!

- Cu <10 mcg/dL (normal 70-155)

**Management**: IV copper chloride 2.5 mg daily x 14 days

**Clinical course**: Her Hgb normalized by 6 weeks, and she was placed on oral copper sulfate 3mg TID

- Repeat bone marrow showed reversal of previous findings
Copper Deficiency

- Though often macrocytic, the MCV can vary from micro to normocytic. One larger series reported the range 70.3 – 114.1

- **Leukopenia** reported in more than 50%

- Occasionally there can be **thrombocytopenia**

- Bone marrow findings variable, can mimic MDS, frequently with **ringed sideroblasts**

- Cytoplasmic vacuolization a common finding

- **Dorsal and lateral column spinal cord dysfunction**
CASE #3

- 46 year old obese woman
- History of gastric bypass surgery
- Presented with progressive fatigue and numbness in hands/feet
- Taking Chinese herbal medicines and iron
- Exam unremarkable

<table>
<thead>
<tr>
<th>CBC with Differential</th>
<th>Collection Date &amp; Time</th>
<th>02/07/2014 10:37</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC LH</td>
<td>1.4</td>
<td>Low</td>
</tr>
<tr>
<td>RBC LH</td>
<td>1.9</td>
<td>Low</td>
</tr>
<tr>
<td>HGB LH</td>
<td>6.8</td>
<td>Low</td>
</tr>
<tr>
<td>HCT LH</td>
<td>19.9</td>
<td>Low</td>
</tr>
<tr>
<td>MCV LH</td>
<td>83</td>
<td>Normal</td>
</tr>
<tr>
<td>MCH LH</td>
<td>16</td>
<td>Normal</td>
</tr>
<tr>
<td>MCHC LH</td>
<td>28</td>
<td>Normal</td>
</tr>
<tr>
<td>RDW LH</td>
<td>19.0</td>
<td>Normal</td>
</tr>
<tr>
<td>PLT LH</td>
<td>147</td>
<td>Normal</td>
</tr>
<tr>
<td>NE%</td>
<td>35.0</td>
<td>Normal</td>
</tr>
<tr>
<td>LY%</td>
<td>60.0</td>
<td>Normal</td>
</tr>
<tr>
<td>MO%</td>
<td>1.0</td>
<td>Normal</td>
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<tr>
<td>EO%</td>
<td>4.0</td>
<td>Normal</td>
</tr>
<tr>
<td>BA%</td>
<td>0.0</td>
<td>Normal</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RET%</td>
<td>1.10</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Diagnosis: Zinc excess !!!

- Retic% 1.1%, Iron, ferritin, B12, folate normal
- Cu <1.57 umol/L (13-24)
- Ceruloplasmin <18 mg/L (170-540)
- Serum Zinc 39 (9-20)

**NOTE**: Patient did not wear dentures (another source for zinc)

Clinical Course

- Told to stop taking zinc
- After copper sulfate 1 mg IV weekly, her anemia, leukopenia, sensory neuropathy resolved by 3 months
31 year old woman hospital inpatient with cytopenias, schistocytes on smear, thought to have TTP and Coombs-negative hemolytic anemia.

Bone marrow showed hypoplasia, iron stores reduced.

Treated for TTP by plasma exchange but no improvement.

CASE #4
Subsequent Hospital Course

- RBC and later required platelet transfusions for vaginal bleeding

- Given iron, folate and B12 supplementation

- *Diagnosis* ___________________________
Diagnosis: Paroxysmal Nocturnal Hemoglobinuria (PNH)

**DX test:** PNH panel positive

**YES!** Its rare! 1-2 persons per million

**BUT!** (and this makes all the difference) There’s a treatment!*

*Eculizumab

**PREDICTION:** True incidence higher
Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Acquired, **life-threatening**
- **Young** adults (median age of diagnosis 35-40 years)
- **Symptoms**: severe abdominal pain crises, severe headaches, back pain, excessive weakness, fatigue and recurrent infections
- **Classic symptom**: of bright, red blood in urine (hemoglobinuria) in 50%
  - Frequently urine dark tea-color
  - Typically in the morning, precipitated by infections, alcohol, exercise, stress or certain medications
- **Hemolytic anemia, blood clots**, impaired bone marrow function
Paroxysmal Nocturnal Hemoglobinuria (PNH)

- **Blood clots** (thrombosis)
  - Exclusively in veins, as opposed to arteries
  - The leading cause of death in PNH
  - The most common sites:
    - Hepatic vein thrombosis (Budd-Chiari syndrome)
    - Sagittal vein (in the head)

- **Median survival**: 10 years;
  (however, some patients can survive for decades with only minor symptoms)

- 3-5% risk of developing leukemia
PNH: A History

- First confused with paroxysmal cold hemoglobinuria (PCH)
- An enigma for 130 years
- Its study led to Ham test and sucrose hemolysis test (the main diagnostic tests for decades)
- Then complement binding proteins (CD55, CD59) discovered absent - Led to flow cytometry for PNH (gold standard for diagnosis)
- Mutations in PIG-A gene cause PNH
- Treatment: Eculizumab
Per ASH: Settings for PNH Testing

1. Unexplained bone marrow failure
2. Hemolysis associated with bone marrow failure
3. Unexplained thrombosis
4. Paradoxical combination of hemolysis with iron deficiency
5. ...or hemolysis precipitated by iron therapy
Summary 1

- **The most common hematology office consultation:**
  “Unexplained Anemia”
  (Anemia persisting after months of iron therapy)

- **Hematology perspective:** Anemia frequently multiple causes
  (3 or 4 quite common!)

- **Hematology solutions:** Go beyond the traditional, RBC-sized based algorithm:
  - Know when micro-, normo-, macro- paradigm breaks down
  - Go back to patient medical history
  - Go back to the CBC and think cell biology.....what’s going on in the marrow?

- **Hematology changes over the years:**
  - Surgical changes (gastric reduction)
  - Patient habits (folate supplementation, antacids, herbal medicines, dentures, zinc use)

*(Infectious disease similar: Changes reflecting new resistance from antibiotic use)*
The Abnormal CBC: Etiology-based Differential

- **Nutritional** (always acquired)
- **Hemolytic** (mostly Normocytic)
  - Hereditary: Enzymopathy, Hemoglobinopathy (thalassemia), Membrane
  - Acquired: Autoimmune, PNH, HUS/TTP, drug induced
- **Aplastic** (mostly normocytic)
  - Hereditary: Fanconi
  - Acquired: PRCA, sideroblastic anemia
- **Other**: Methemoglobinemia, sulfhemoglobinemia

*Note:* 400 causes fit within this framework
Nutritional Anemias

- Iron
- Folate
- B12
- Copper
- Zinc excess
- Lead excess (test only in children?)

The nutritional anemia test panel: iron panel, ferritin/ESR, folate, B12, copper, zinc, (lead), retic

Clinical thinking I: If testing for one, should test for them all

Clinical thinking II: If find a nutritional deficiency, screen for celiac and carcinoid syndrome
• **Celiac Screening:**
  Go to LabCorps
  Scroll to “celiac screen”

• **Carcinoid Screening:**
  Go to LabCorps
  Scroll to “5-HIAA, Quant., 24 Hr Urine”
  *(YES, it’s a 24 Hr Urine collection test)*
Malabsorption

**Infective agents:** HIV, parasites, intestinal tuberculosis

**Structural defects:** Blind loops, IBC/Crohn’s, diverticulae, infiltrations (amyloidosis, lymphoma, eosinophil gastroenteritis), radiation, enteritis, systemic sclerosis, short bowel syndrome

**Surgical changes:** Gastrectomy, bariatric surgery

**Mucosal abnormality:** Celiac, milk intolerance

**Digestive failure:** Pancreatic (cystic fibrosis, chronic pancreatitis, pancreatic cancer), Zollinger-Ellison syndrome, bile malabsorption (obstructive jaundice, bacterial over-growth)

**Systemic diseases:** Hypo/hyper-thyroidism, Addisons, Diabetes, Carcinoid, abeta-lipoproteinemia

**Clinical thinking:** presence of any of these with anemia = think the nutritional anemia test panel
The Abnormal CBC: An Etiology-based Differential

- **Nutritional** (always acquired)

- **Hemolytic** (mostly Normocytic)
  - **Hereditary**: Enzymopathy, Hemoglobinopathy (thalassemia), Membrane
  - **Acquired**: Autoimmune, PNH, HUS/TTP, Drug induced

- **Aplastic** (mostly normocytic)
  - **Hereditary**: Fanconi
  - **Acquired**: PRCA, sideroblastic anemia

- **Other**: Methemoglobinemia, sulfhemoglobinemia

*Note*: 400 causes fit within this framework
Etiology-based: Hemolytic

- **A hemolysis screening test panel**: CMP, LDH, Uric, haptoglobin, urine hemosiderin (plus retic)  
  (also consider RBC fragmentation on the smear)

- **NOTE**: Haptoglobin detects intravascular hemolysis  
  (as does indirect bilirubin)

- **NOTE**: elevated retic + hemolysis markers normal = bleeding

- **NOTE**: urine hemosiderin detects chronic hemolysis, such as  
  from cardiac valves, varices, tumor vasculature
Summary 2:

**Two anemia workup tools**

1. **The nutritional anemia test panel**
   - If positive, reflex to screens for celiac and carcinoid

2. **The hemolysis test panel**
   - If positive, reflex to Coombs, PNH flow cytometry

**But should we use more refined diagnostic tools based on disease states?**
The Abnormal CBC and Patient History: Disease Associations with Anemia

Crohn’s Disease
4 potential causes at once:
• Bleeding
• Iron deficiency
• B12 deficiency
• ACD

Therefore, the Crohn’s Anemia Panel
✓ Iron panel, ferritin, ESR, B12, retic
✓ Versus the nutritional anemia test panel
The Abnormal CBC and Patient History: Disease Associations with Anemia

**Chronic Liver Disease**

5-6 potential causes

- *Hypersplenism?* Trilineage decrease
- *Varices/bleeding?* Iron deficiency
- *Alcoholism?* Direct toxic effect on the bone marrow
- *Folate/B12 Deficiency?* Behavior change in alcoholic: screen B12
- *Autoimmune hepatitis?* Screen immune hemolytic anemia, or even aplastic anemia
- *Thrombosis?* Screen for PNH

- HCV causes anemia by itself

**Therefore, a Liver Disease Anemia Panel?**

- ✓ Iron panel/ferritin/ESR/folate/B12/retic/Coombs/hemolysis/HCV
- ✓ Consider flow cytometry for PNH if thrombosis or hemolysis
The Abnormal CBC and Patient History: Disease Associations with Anemia

**Anemia with Renal Failure**
4 potential causes
- High rates of **iron deficiency**: iron panel, ferritin/ESR
- Inflammation frequently in renal disease (**ACD**)  

**Renal Anemia Test Panel**
- Iron panel, ferritin/ESR, creatinine
- Not EPO (*because procrit won’t be reimbursed based on*)
The Abnormal CBC and Patient History: Disease Associations with Anemia

**Anemias with Arthritis**
4 potential causes
- By definition: **ACD**
- NSAIDs use causes iron loss from *GI bleeding*?
- Immune dysregulation leads to immune **hemolytic anemia**?
- **Felty’s syndrome** with neutropenia? Screen with ANCA

**Anemia Test Panel**
- Iron panel, ferritin/ESR, hemolysis panel, ANCA
- Add a **multiple myeloma test panel**?
  (SPEP, UPEP, plasma free light chains, B2M)
- Add **ANA, RF** if not done already?
When Multiple Medical Problems: A “Shotgun” Test Panel?

**The Nutritional Anemias**
1. B12  
2. Folate  
3. ferritin  
4. Copper  
5. Zinc

**The Hemolysis Tests**
6. Bilirubin/CMP  
7. LDH  
8. Haptoglobin  
9. Urine hemosiderin  
10. Retic count  
11. Coombs test

**An Inflammation Screen**
12. ESR

**An Endocrine-related Test**
13. TSH

**Multiple Myeloma Test**
14. SPEP  
15. FLC  
16. UPEP

**Infection-related Tests**
17. HIV  
18. HCV  
19. Flow cytometry for PNH? Hgb electrophoresis?
Summary 3: What We Have Reviewed So Far!

• **The traditional anemia workup** based on RBC size (i.e. MCV)

• Anemia **work up based on HPI/PMH**

• Next: an **integrated workup** incorporating RBC biology
- A shared cellular ancestry with all CBC members
- The lymphocyte lineage breaks away first
- A tighter association with platelets and neutrophils, basos, eos, monos
RBC Biology: Making Its Way Into The Clinic

African-American Population

Usual range for WBC and ANC

**Adult Men:**
- WBC: 3.1-9.9
- ANC: 1.3-6.6

**Adult Women:**
- WBC: 3.4-11
- ANC: 1.4-7.5

- **Note:** WBC and ANC usually both low, along with lower RBC and platelet count
- **Note:** Lymphocyte count not normally low in African-Americans
- This is just as predicted based on cell lineage biology

More RBC Biology #2

- RBC production begins with large precursors (known as stem cells)

- With each division, descendants get smaller

Two signals regulate the number of cell divisions

1. Cell cycle arrest (from the nucleus)
2. The heme signal (from the cytoplasm)
What We See In The Clinic

**Macrocytosis**
- **Drugs** damage DNA: cell cycle arrest: **big cells**
- **Folate/B12 def.** limit DNA production: cell cycle arrest: **big cells**
- **Leukemic mutations** = damaged DNA: cell cycle arrest: **big cells**

**Microcytosis**
- **Iron deficiency:**
  - No problem making DNA, no cycle arrest;
  - slowdown making heme:
  - divisions eventually stop: **smaller cells**

- **Thalassemia:**
  - No problem making DNA, no cycle arrest;
  - slowdown making heme (and also less cytoplasm production):
  - divisions eventually stop: **smaller cells**
RBC Biology and RDW

• **Thalassemia**
  • **Uniformity**: All cells shave the same genetic blueprint
  • An *equal problem* making DNA, (and also less cytoplasm production)
  • Uniform number cell divisions/*uniform RDW*

• **Iron Deficiency**
  • **Non-uniformity**: Each cell has variable amounts of iron
  • *Unequal* numbers of cell divisions downstream
  • More *variable RDW*

High RDW distinguishes iron deficiency from thalassemia
More RBC Biology #3

The Four Stages of RBC Production

1. **Before EPO dependence**: stem cells dividing on their own
2. **EPO Stage**: Further division based on renal EPO
3. **Terminal differentiation** (when Hgb accumulates)
4. **Reticulocyte maturation** (in the peripheral blood, where precursors lose mitochondria and run on glycolysis and pentose phosphate pathway)

**Step 1** *(independent stem cells)*
- Affects WBC and platelets (Steps 2-4 do not)

**Step 2** *(cell community participation)*
- Complex cellular signaling:
  - EPO: pro-survival
  - FasL/Trail: pro-death (apoptosis)
  - *(Multiple myeloma* causes RBC precursor death/anemia, via FasL/Trail)*
The CBC in Multiple Myeloma

<table>
<thead>
<tr>
<th>CBC with Differential</th>
<th>02/17/2014 13:56</th>
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<tbody>
<tr>
<td>WBC LH</td>
<td>8.4</td>
</tr>
<tr>
<td>RBC LH</td>
<td>2.9</td>
</tr>
<tr>
<td>HGB LH</td>
<td>7.6</td>
</tr>
<tr>
<td>HCT LH</td>
<td>23.9</td>
</tr>
<tr>
<td>MCV LH</td>
<td>84</td>
</tr>
<tr>
<td>MCH LH</td>
<td>27</td>
</tr>
<tr>
<td>MCHC LH</td>
<td>32</td>
</tr>
<tr>
<td>RDW LH</td>
<td>16.8</td>
</tr>
<tr>
<td>PLT LH</td>
<td>418</td>
</tr>
<tr>
<td>NE%</td>
<td>60.0</td>
</tr>
<tr>
<td>LY%</td>
<td>26.0</td>
</tr>
<tr>
<td>MO%</td>
<td>2.0</td>
</tr>
<tr>
<td>EO%</td>
<td>1.0</td>
</tr>
<tr>
<td>BA%</td>
<td>1.0</td>
</tr>
</tbody>
</table>

[Image of a computer screen with a table of CBC results]
Four Common Anemia Patterns Based on RBC Biology

1. Anemia with abnormal platelet or leukocyte counts
2. Anemia with increased reticulocytes
3. Chronic life-long anemia
4. Anemia with inappropriately low reticulocytes

An integrated approach to the abnormal CBC, incorporating:

- Clinical patterns
- What’s happening in the marrow and spleen
Pattern 1: Anemia with Abnormal Platelet or Leukocytes Counts

Low Hct/Hgb with Low Platelets or WBC?

Yes

Splenomegaly?

Yes

Hypersplenism
Liver disease
Lymphoma
SLE

No

Myelodysplasia
Multiple Myeloma
Aplastic Anemia

No

Increased Platelets or WBC?

Yes

Increased WBC
Inflammation
Acute Leukemia
Chronic Leukemia
Myelofibrosis

Increased Platelets
Iron Deficiency
E.T. + myelofibrosis

No

Low Hct/Hgb: Are reticulocytes increased?
Pattern 2: Anemia with Reticulocytosis

1. Low Hct/Hgb: Are reticulocytes increased?
   - Yes
     - Hemolysis?
       - Yes: Bilirubin, LDH, Haptoglobin
       - No: Blood smear
         - Yes: Fragmented RBCs?
           - Yes: Microangiopathy
           - No: Pos. DAT?
             - Yes: AIHA Cold Aggl.
             - No: Membrane Defect (HS; PNH) Enzymopathy (G6PD, PK) Hemoglobinopathy
         - No: Blood Loss
           - GI; GU
           - Blood donor
   - No: Yes
Pattern 3: Life-long Chronic Anemia

**Thalassemia**
- The most frequent cause of life-long undiagnosed chronic anemia
- The most common worldwide cause of congenital anemia
- Deficient RBC production with preservation of RBC number

**Alpha-thalassemia trait:** missing 2 of 4 alpha-globin chains
- **Mild** microcytic anemia
- Diagnosed at birth **hgb Barts** (gamma-globin tetramers)
- If not diagnosed at birth, distinguish from iron deficiency by:
  1. Normal RDW
  2. Normal or elevated RBC number
  3. Normal ferritin

**Note:** The Hgb electrophoresis is normal
- **Alpha-globin gene sequencing** at specialty labs
Pattern 3: Life-long Chronic Anemia (Continued)

**Beta-thalassemia**

- Diagnosed at birth by presence of **only fetal Hgb**
- Later: **Decreased Hgb and RBC number**
- **But** Beta-thalassemia intermedia: resemble alpha-thal
  - (with **increased RBC number**)
  - Detected by increased Hgb A2 on electrophoresis:

<table>
<thead>
<tr>
<th>Hemoglobin A</th>
<th>92.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin A2</td>
<td>6.6%</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

- **Others**: RBC enzyme and membrane deficiencies
Hemoglobin Electrophoresis

Migration patterns:

**Note:** HbA (alpha-beta)  
HbA2 (alpha-delta)
## Hgb Electrophoresis Patterns

<table>
<thead>
<tr>
<th>Normal</th>
<th>B-Thalassemia Minor with Iron Deficiency</th>
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</thead>
<tbody>
<tr>
<td>Hemoglobin A</td>
<td>Hemoglobin A</td>
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<tr>
<td>95-98%</td>
<td>97%</td>
</tr>
<tr>
<td>Hemoglobin A2</td>
<td>Hemoglobin A2</td>
</tr>
<tr>
<td>1.5-3.5%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>Hemoglobin F</td>
</tr>
<tr>
<td>&lt;2%</td>
<td>0.9%</td>
</tr>
<tr>
<td>Hemoglobin C</td>
<td>Hemoglobin C</td>
</tr>
<tr>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin S</td>
<td>Hemoglobin S</td>
</tr>
<tr>
<td>0%</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Beta Thalassemia Trait</th>
<th>Beta Thalassemia Intermedia</th>
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<tbody>
<tr>
<td>Hemoglobin A</td>
<td>Hemoglobin A</td>
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<tr>
<td>92.7%</td>
<td>0-80%</td>
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<tr>
<td>Hemoglobin A2</td>
<td>Hemoglobin A2</td>
</tr>
<tr>
<td>6.6%</td>
<td>up to 7%</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>Hemoglobin F</td>
</tr>
<tr>
<td>0.7%</td>
<td>20-100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sickle/B+ Thalassemia</th>
<th>Sickle Homozygous</th>
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<tbody>
<tr>
<td>Hemoglobin A</td>
<td>Hemoglobin A</td>
</tr>
<tr>
<td>30%</td>
<td>0%</td>
</tr>
<tr>
<td>Hemoglobin A2</td>
<td>Hemoglobin A2</td>
</tr>
<tr>
<td>4.7%</td>
<td>2-4%</td>
</tr>
<tr>
<td>Hemoglobin F</td>
<td>Hemoglobin F</td>
</tr>
<tr>
<td>5.3%</td>
<td>2-20%</td>
</tr>
<tr>
<td>Hemoglobin S</td>
<td>Hemoglobin S</td>
</tr>
<tr>
<td>60%</td>
<td>80-90%</td>
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</table>

<table>
<thead>
<tr>
<th>Sickle Carrier</th>
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<tbody>
<tr>
<td>Hemoglobin A</td>
<td>Hemoglobin A</td>
</tr>
<tr>
<td>60-65%</td>
<td>0%</td>
</tr>
<tr>
<td>Hemoglobin A2</td>
<td>Hemoglobin A2</td>
</tr>
<tr>
<td>2-4%</td>
<td>2-4%</td>
</tr>
<tr>
<td>Hemoglobin S</td>
<td>Hemoglobin S</td>
</tr>
<tr>
<td>35-40%</td>
<td>35-40%</td>
</tr>
</tbody>
</table>
Pattern 4: Anemia with Inappropriately Low Reticulocytes

Low Hct/Hgb: Are reticulocytes increased?

Yes

Hemolysis?
- ↑ Bilirubin, LDH
- ↓ Haptoglobin

Yes

Blood smear Fragmented RBCs?

Yes

Microangiopathy

Yes

AIHA Cold Aggl.

Yes

Membrane Defect (HS; PNH) Enzymopathy (G6PD, PK) Hemoglobinopathy

No

Blood Loss GI; GU Blood donor

No

Macrocytosis?

Yes

Hemoglobinopathy
- Thalassemia
- Congenital Anemia
- Fanconi; DBA

Blood donor

Yes

Fe Deficiency?
- ↓ Tf sat., Ferritin

Yes

Renal Failure
- Hypothyroidism
- Inflammation
- PRCA

Blood Loss GI; GU Blood donor

No

Previous normal Hct/Hgb?

Yes

Microcytosis?

Yes

Myelodysplasia
- Hypothyroidism
- Congenital Anemia
- Fanconi; DBA

No

Inflammation
- Thalassemia
- Sideroblastic Anemia

No

No

Pos. DAT?

Blood donor

No

Megaloblastic Anemia

No

Yes

Myelodysplasia
- Hypothyroidism
- Congenital Anemia
- Fanconi; DBA

No

Inflammation
- Thalassemia
- Sideroblastic Anemia

No

No

No
Pattern 4: Anemia with Inappropriately Low Reticulocytes

- **Microcytosis**
  - Iron Deficient?
    - Yes
      - Blood Loss
    - No
      - Inflammation
        - Thalassemia
        - Sideroblastic Anemia

- **Normocytosis**
  - Renal failure
    - Hypothyroidism
    - Inflammation
    - PRCA

- **Macrocytosis**
  - Decreased B12/folate?
    - Yes
      - Megaloblastic Anemia
    - No
      - MDS
        - Hypothyroid
        - Congenital Fanconi
Take Home Messages

• With so many anemia causes, the danger of finding one is hiding another

• There is no harm in testing for multiple causes of anemia

• There is no harm even in “shotgun” testing

• There may be harm by NOT “shotgun” testing

• Even in adults, a patient who never had a normal hgb/hct may have a congenital anemia; such as hemoglobinopathy, sideroblastic anemia, Fanconi
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