Challenging Case Studies in Laboratory Diagnosis: A focus on anemia

Margaret A. Fitzgerald, DNP, FNP-BC, NP-C, FAANP, CSP
President, Fitzgerald Health Education Associates, Inc., North Andover, MA
Family Nurse Practitioner, Adjunct Faculty, Family Practice Residency
Greater Lawrence (MA) Family Health Center
Editorial Board Member
The Nurse Practitioner Journal, *Prescriber's Letter, American Nurse Today*

Anemia defined

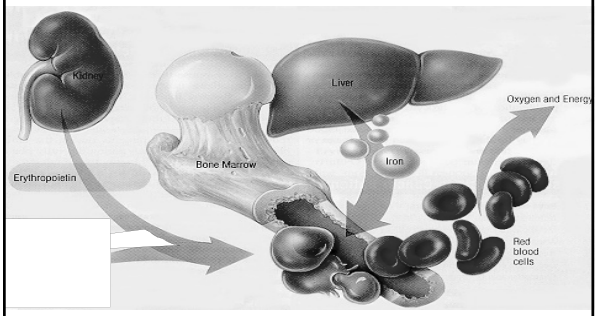
- A complex of signs and symptoms characterized by decreases in numbers of RBCs or Hb content caused by blood loss, deficient erythropoiesis, excessive hemolysis, or a combination of these changes.

When does anemia occur?

- With insult severe enough
  - Disturb normal homeostatic mechanisms
  - Exceed reserves

Normal erythropoiesis

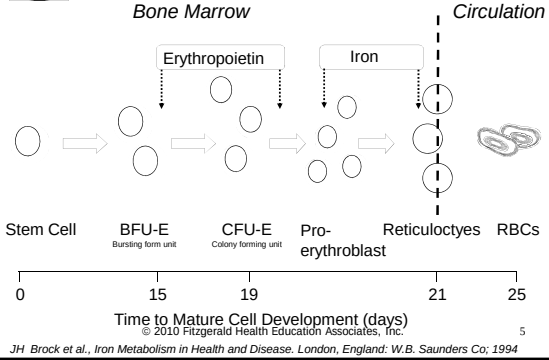
A decrease in oxygen tension of the renal blood perfusion serves as a signal to the kidney to begin producing EPO



Adapted from Schott et al. US Pharmacist, 1997;22:HS5-HS12

RBC Development

Stem cells, upon exposure to erythropoietin, proliferate and differentiate to form red blood cells



JH Brock et al., Iron Metabolism in Health and Disease. London, England: W.B. Saunders Co; 1994

Conditions needed for RBC formation

Functional erythropoietin mechanism	Erythropoietin source= 90% renal, 10% hepatic. Erythropoietin supply diminished in renal failure (Typically GFR < 49 mL/ min)
Uncompromised DNA synthesis	DNA synthesis impaired by presence of chronic inflammation such as found in infection, autoimmune disorders including systemic lupus erythematosus, rheumatoid arthritis.

Conditions needed for RBC formation

Hemoglobin synthesis unimpaired by lack of iron, vitamin or globin production	Adequate nutrition (iron, B vitamins, vitamin C, protein, others) and absorption

Conditions needed for RBC formation

Intact marrow micro-environment	Revealed in the production of reticulocytes, young RBCs that contain residual RNA. In health= 1-2% of TRBC. The reticulocyte count reflects ability of bone marrow to produce RBCs. Anticipated response in anemia= Reticulocytosis. Absence of reticulocytosis or reticulocytopenia= Inadequate bone marrow response

Causes of anemia

- Blood loss
  - Acute from hemorrhage
    - In adult >1 liter before drop in hemoglobin
    - Most likely cause of sudden, dangerous drop in hct
  - Chronic from erosive gastritis, heavy menses, GI malignancy
    - Iron from RBC wasted via blood loss cannot be recycled

Causes of decreased RBC counts

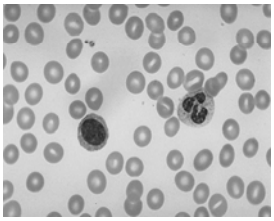
- Reduced RBC production
  - Nutritional (vitamin B12, folic acid, iron deficiency), anemia of chronic disease (ACD), bone marrow suppression
- Premature destruction
  - Hemolysis, shortened lifespan (<90 d, NL RBC lifespan= 90-120 d)

Laboratory evaluation of the person with suspected anemia

What are H and H, RBC?

Are these values proportionally decreased?

- Hemoglobin to hematocrit ratio= 1:3
  - 10 g= 30%
  - 12 g= 36%
  - 15 g= 45%



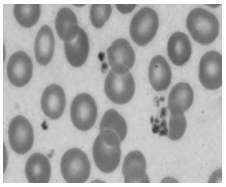
What is the cell size?

Wintrobe's classification

- Mean corpuscle volume (MCV)
  - Microcytic (small cell)
    - MCV < 80 fl
  - Normocytic (normal size cell)
    - MCV 80-96 fl
  - Macrocytic (Abnormally large cell)
    - MCV > 96 fl

What is the cell's hemoglobin content?

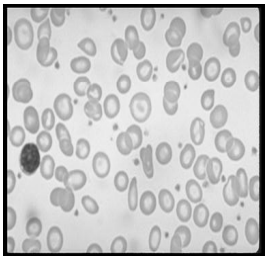
- Reflected by mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC)

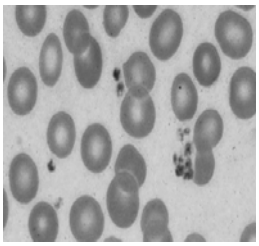


What is the cell's hemoglobin content?

- Hemoglobin=color
- Color=chromic
  - Normochromic= Normal color
    - MCHC = 31- 37 g/dL
  - Hypochromic= Pale
    - MCHC < 31 g/dL

Hypochromic vs. normochromic

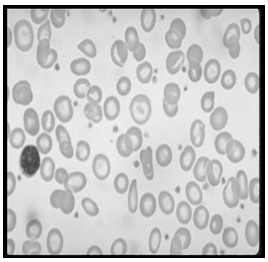


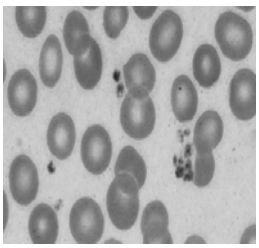


What is the RDW (RBC volume distribution width)?

- An index of variation in RBC size
  - RDW NL= 11.5-15%, significant report>15%
  - Quantitative report of anisocytosis
    - New cells differ in size when compared to older cells
- One of the most reliable markers of deficient erythropoiesis in evolving macro/ microcytic anemia

Elevated RDW vs. NL RDW





What is the reticulocyte count?

- Is the body able/ attempting to correct the anemia?
- In health
  - 0.5-1.5% of TRBCs
- NL response in anemia
  - Reticulocytosis (>1.5%)
    - Since retic MCV> 96 fl, marked reticulocytosis= Elevated RDW

Reticulocyte count

Per Ferri, one of the most helpful tests in evaluating person with anemia

- Usually elevated
  - Hemolytic anemia, hemorrhage, with anemia therapy (folate, B12, iron therapy as needed and directed by underlying disease process)

Reticulocyte count

Per Ferri, one of the most helpful tests in evaluating person with anemia

- Usually decreased
  - Aplastic anemia, bone marrow suppression, chemotherapeutic agents, anemia of chronic disease

Lab assessment in anemia

- What is the RPI?
  - Reticulocyte production index
    - <2= Inadequate response
    - >3= Adequate response

Corrected retic ct, RPI

- Retic% corrected
  - Retic% reported X (pt's hct)/45
- RPI
  - Retic% corrected/ Correction factor

RPI

How fast do RBC mature?

Patient's hct (%)	Correction factor
40-45	1.0
35-39	1.5
25-34	2.0
15-24	2.5
<15	3.0

Reticulocyte production index calculator
http://cpsc.acponline.org/enhancements/227rpiCalc.html

42 yo woman with an CU-IUD

- Heavy menses for the past 5 years
- CC= Fatigue, decreased exercise tolerance X 3 months
- Concomitant hx
  - Gastric bypass > 10 years ago (BMI preop= 57, now 34)
  - HTN, on lisinopril with HCTZ

42 yo woman with an CU-IUD

Physical exam reveals pale conjunctiva and a grade II/VI systolic ejection murmur over the precordium without radiation.

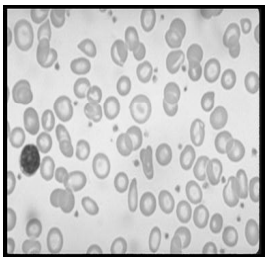
42 yo woman with an CU-IUD

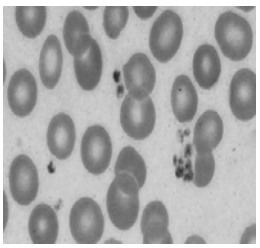
- Hg=6.6 g (12- 14 g) (660 g/L {120-140 g/L})
- Hct=21.4% (37- 45%) (.214 proportion {.37-.45 proportion})
- RBC= 2.7 mil (4.2-5.4 mil)
- WBC= 4,400 mm3 (6,000-10,000 mm3)
  - NL differential

42 yo woman with an CU-IUD

- MCV=65.5 fl (80- 96 fl)
  - Mean corpuscle volume
- MCHC=30.5 g/dl (31- 37 g/dl) (305 g/L {310-370 g/L})
  - Mean corpuscle hemoglobin concentration
- MCH= 20 pg/cell (27-33)
  - Mean corpuscle hemoglobin

Hypochromic vs. normochromic

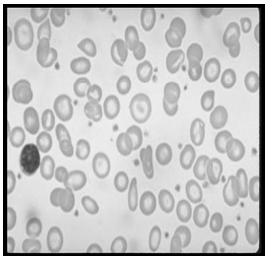


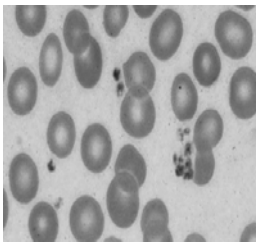


42 yo woman with an CU-IUD

- RDW=22.9% (11.5- 15%) (.229 proportion {.115-.15 proportion})
  - New cells differ in size when compared to older cells
  - Per Ferri, best indicator of evolving micro- or macrocytic anemia

Elevated RDW vs. NL RDW





RDW in IDA

- "This is reflected in the red blood cell distribution width (RDW); thus, the earliest evidence of the development of an iron-deficient erythropoiesis is seen in the peripheral smear and by an increased RDW."
  - Source- Conrad, M. Iron deficiency anemia, available at www.emedicine.com, accessed 4.29.10.

Mean Corpuscle Hemoglobin vs Mean Corpuscle Hemoglobin Concentration

- MCH
  - Average mass of hemoglobin per RBC
- MCHC
  - Measure of the concentration of hemoglobin in a given volume of packed RBC

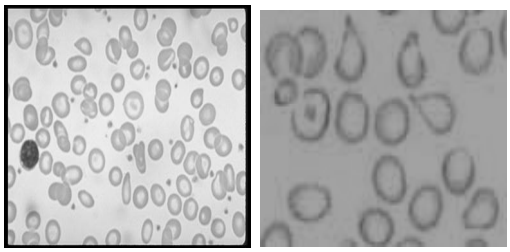
42 yo woman with an CU-IUD

- Reticulocytes
  - Reported= 5% (.05 proportion)
  - Retics (corrected)= 2.4% (.024 proportion)

42 yo woman with an CU-IUD

- RBC Morphology
  - 3+ hypo (pale)
  - 1+ aniso
    - Anisocytosis= Varied size
  - Marked poik
    - Poikilocytosis= Varied shape

Aniso, poik



42 yo woman with an CU-IUD

- Platelets= 670,000 mm3
  - Reactive or clonal?
    - Evidence of previous elevation?
    - Splenomegaly?
    - Evidence of source (profound anemia, infection, inflammation)

42 yo woman with an CU-IUD

- Ferritin= 2 ng/ml (10- 322) (4.5 pmol/L {22.47-723.53 pmol/L})
  - Highly sensitive and specificity
- Iron= 75 ug/dl (30- 160) (13.4 umol/L {5.37-28.64 umol/L})
  - Lower sensitivity and specificity
  - Why WNL?

42 yo woman with an CU-IUD

- TIBC= 479 ug/ dl (228- 428) (85.7 umol/L {40.8-76.6 umol/L})
  - Lower sensitivity and specificity

42 yo woman with an CU-IUD

- Vitamin B12= 121 pg/ml (180-914) (89.3 pmol/L {132.8-674.5 pmol/L})
  - Dietary source?
  - Contributor to low levels?
  - Does this contribute to marked microcytosis?

Order of change in IDA

- Ferritin ↓
  - Iron stores
- Marrow ↓
  - Most sens, spec test but seldom needed
- Serum iron ↓
  - Drug level
- RDW ↑
  - New cells are smaller, paler
- TIBC ↑
  - Open spots for iron to bind
- Hb, hct ↓
- Indices ↓
  - Small, pale cells

Vitamin B12 replacement: True or False?

- Traditionally, doses of vitamin B12 (cobalamin) 1000 mcg per injection have been used. A cobalamin dose of more than 100 mcg in a single injection exceeds the binding capacity of transcobalamin II, however; the excess is excreted via the kidney and wasted.

Vitamin B12 supplementation options

- Vitamin B_{12} nasal gel
  - Weekly at a dose of 500 mcg
- Vitamin B12 oral tablets
  - 1000 mcg daily
- Cobalamin injection
  - 100 -1000 mcg monthly

Same patient also mentions…

- New onset restless legs over p 6 months
  - Could this be attributable to IDA?

Iron deficiency anemia
Most common form of anemia worldwide

- "It is important economically because it diminishes the capability of individuals who are affected to perform physical labor, and it diminishes both growth and learning in children."
  - Source- Conrad, M. Iron deficiency anemia, available at www.emedicine.com, accessed 4.30.10.

IDA tx= Fe plus B complex with C

- Iron forms
  - Oral forms
    - Ferrous sulfate
    - Ferrous gluconate
    - Enteric coated Fe
  - Parenteral Fe

Treatment of IDA

- Advise with oral Fe
  - Possible chelation effect
  - Spacing of doses
- How long should you treat?
  - Correct Hb plus at least 2 months
- What about routine Fe supplementation?

Anticipated results with IDA treatment

- Reflects marrow response
  - Reticulocytes peak @ 6 d
- If not actively losing blood
  - Hg increase at 2 g/ q 3 weeks
  - Hct increase q 6% q 3 weeks
- Stores replenished
  - NL ferritin at 3-6 months after hg to norm

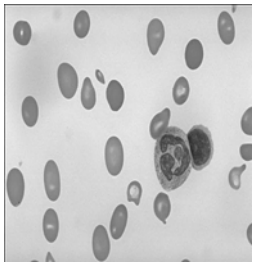
Example 32 year-old woman who is taking phenytoin

- Hg= 12 g (12-14 g)
  - 120 g/L (120-140 g/L)
- Hct= 37% (36-43%)
  - .37 proportion (.36-.43 proportion)
- RBC= 4.2 million (4.2-5.4 mil)
- MCV= 105.5 fl (81-99 fl)
- MCH= 31 pg (27-33 pg)
- RDW= 12.8% (11.5-15%)
  - .128 proportion (.115-.15 proportion)

Macrocytosis

(MCV>96 fl, >102 fl in elder)

- Abnormally large cell (macrocytic) due to altered RNA:DNA ratio, hemoglobin content WNL (normochromic), new cells larger than old cells (elevated RDW)



Drug-induced macrocytosis usually without anemia

- Etiology
  - Use of select medications such as carbamazepine (Tegretol), zidovudine (AZT), valproic acid (Depakote), phenytoin (Dilantin), alcohol, others
  - Reversible when use of offending medication is discontinued

Drug-induced macrocytosis

- Abnormally large (macrocytic) cell due to altered RNA:DNA ratio, hemoglobin content WNL (normochromic), new cells usually same size as old cells (NL RDW)

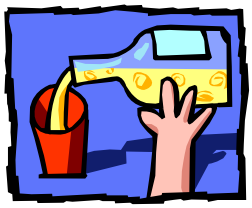
Tamara

35 year-old woman who drinks 5-9 beers/d

- Hg=11.4 g (114 g/L)
- Hct= 34.1% (.341 proportion
- MCV=103 fl
- MCHC=32 g/dl
- RDW= 18% (.18 proportion)
- Reticulocytes
  - Reported=1.5% (.15 proportion)
  - Corrected= 1.95% (.195 proportion)
- RPI=0.73

Tamara

- WBC- 3,200/ mm3
  - 5-10 K
- Neuts= 40%
  - 50-70%
- Lymph= 55%
  - 24-44%
- Plts= 96K
  - 130-400K



Macrocytic anemia evaluation

- Degree of macrocytosis typically in proportion to degree of anemia
- Serum folate= 10 ng/mL (3-20 ng/ml)
  - "Drug" level, reflects dietary intake over p 48-72 h
- Vitamin B12= 398 pg/mL (NL= 180-914 pg/mL)

Tamara

- RBC folate= 160 ng/ml (NL= 280-790 ng/ml)
  - Incorporated in erythrocytes during cell development, remain unchanged throughout RBC lifespan (90-120 d), not influenced by diet
  - Potentially falsely elevated in person with rapidly developing folate deficiency
  - Also low in about 50% who have vit B12 (cobalamin) deficiency

FAD risks

- Inadequate dietary intake
  - Elders, alcoholics, impoverished
- Decreased ability to absorb folic acid
  - Malabsorption syndromes such as sprue and celiac disease
- High demand state
  - Lactation (due to transfer to milk)

FAD risks

- When cell division is high
  - Pregnancy
  - Childhood growth spurts
  - Hemolytic anemia
  - Inflammation

FAD risks

- In select at-risk populations
  - Fish tapeworm infestation
    - Potent risk for IDA, FAD, PA
  - Dietary FAD less common in women than in past due to FA added to wheat flour

Treatment FAD anemia

- Modify underlying cause
- Folic acid supplementation
  - 0.5 mg/day to 1 mg/day to 5 mg/day, with the usual dose being 1 mg/day

Treatment FAD anemia

- Counsel about diet
  - Most fruits, vegetables rich in folic acid
  - In particular, asparagus, broccoli, spinach, lettuce, lemons, bananas, melons, liver, and mushrooms
- Overcooking food destroys most of folic acid content.

Response to folic acid therapy

- Bone marrow response
  - Reticulocytosis=7-10 d into therapy
- If not actively losing blood
  - Hct rises by 4-5%/ wk
- Leukopenia, thrombocytopenia
  - Resolve within 2- 3 d tx

If dx in question

- B12, folate
  - Critical to intracellular biochemical reactions
- Deficiency leads to buildup of substrates
  - Homocysteine (5.1-13.9 uM, FAD)
  - Methylmalonic acid (MMA) (73-271 u, PA)

You advise a person who is a vegan to supplement the diet with:

A. Vitamin A.
B. Iron.
C. Vitamin B12.
D. Folic acid.

66 yo woman presents

- 6 mo hx
  - Increasing fatigue
  - Worsening numbness of hands and feet
- Health history
  - Type 2 DM, dyslipidemia, HTN, all at treatment goal

66 yo woman presents

- Current medications (daily doses)
  - Metformin 2 g
  - Gliperimide 4 mg
  - Atorvastatin 20 mg
  - ASA 81 mg
  - Lisinopril 20 mg
  - HCTZ 12.5 mg

66 yo woman presents

- Hg= 11.2 g/ dl (12-14 g)
  - 112 g/L (120-140 g/L)
- Hct= 33% (36-43%)
  - .33 proportion (.36-.43 proportion)
  - 1: ratio with NL hydration
- RBC= 3.2 million (4.2-5.4 mil)
  - Proportionally decreased when compared with H & H

66 yo woman presents

- MCV= 112 fl (81-96 fl)
  - Does RBC size or color change over cell's life span?
- MCHC= 34.8 g/dL (31-37 g/dL)
  - What is the RBC lifespan?
- RDW= 19% (11.5-15%)
  - .19 proportion (.115-.15 proportion)
  - New cells different size (likely larger) when compared to old cells

Macrocytic anemia evaluation
True or false?

- Degree of macrocytosis is typically in proportion to degree of anemia.
- Vitamin B12 and folate deficiency are causes of macrocytic anemia.

66 yo woman presents

- Cobalamin=100 pg/mL (190-914 pg/ml)
- Serum folate=8 ng/mL (3-20 ng/ml)
  - "Drug" level, reflects dietary intake over p 48-72 h

66 yo woman presents

- RBC folate = 380 ng/ml (NL= 280-790 ng/ml)
  - Incorporated in erythrocytes during cell development, remain unchanged throughout RBC lifespan (90-120 d), not influenced by diet

66 yo woman presents (continued)

- Potentially falsely elevated in person with rapidly developing folate deficiency
  - Also low in about 50% who have vit B12 (cobalamin) deficiency

Now what?

Vitamin B12 deficiency and metformin use

- Dose dependent response
  - Each 1 g/day metformin increment nearly triple vitamin B12 deficiency risk (odds ratio: 2.88; 95% CI, 2.2-3.9, P<0.001)
    - Ting R Z-W, Szeto CC, Chan M H-M, et al. Risk factors of vitamin B12 deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-9

Vitamin B12 deficiency and metformin use

- Time dependent response
  - On metformin for => 3 y had 2 X risk compared with those using the drug for less than three years (odds ratio: 2.4; 95% CI, 1.5-3.9, P=0.001)
    - Ting R Z-W, Szeto CC, Chan M H-M, et al. Risk factors of vitamin B12 deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-9

Vitamin B12 deficiency and metformin use

- Particular risk in vegetarians
  - Adjusted risk of developing vitamin B12 deficiency vegetarians who use metformin = 1600%
    - Ting R Z-W, Szeto CC, Chan M H-M, et al. Risk factors of vitamin B12 deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-9

Vitamin B12 deficiency and metformin use (continued)

- Advice with metformin use
  - Monitor for vitamin B12 deficiency
  - Vitamin B12 and B complex supplementation
    - Ting R Z-W, Szeto CC, Chan M H-M, et al. Risk factors of vitamin B12 deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-9

72 yo woman with HTN, dyslipidemia, rheumatoid arthritis

- Medications
  - Lisinopril 20 mg qd
  - Atorvastatin 20 mg qd
  - Methotrexate 17.5 mg weekly
- Presents with 2-d hx new onset fatigue and shortness of breath on exertion

She was seen about 6 weeks ago…

- … And had a normal hemogram and basic metabolic profile.
- About 10 days ago she was successfully treated for a UTI.
- PE today is non contributory other than a hemic murmur and conjunctival pallor.

Today's labs

- Hg= 8.8 g/ dl (12-14 g)
  - .88 g/L (120-140 g/L)
- Hct= 23% (36-43%)
  - .23 proportion (.36-.43 proportion)
- RBC= 2.3 million (4.2-5.4 mil)
- MCV= 89 fl (81-96 fl)
- MCHC= 34.8 g/dL (31-37 g/dL)
- RDW= 12% (11.5-15%)
  - .12 (.115-.15 proportion)

Today's labs

- WBC= 2,800 mm3
  - N= 30%
  - L= 60%
  - M= 5%
- Platelets= 60,000 mm3

Her abnormal laboratory results today are most likely due to:

A. The interaction between the methotrexate and the recently prescribed antibiotic.
B. The impact of the UTI on her overall health.
C. A hemolytic reaction.
D. Another cause not mentioned here.

How much will…

- ..the H and H drop in 1 week if no new RBCs are produced?
- In the absence of bleeding, how much increase in H and H should you anticipate with transfusion of 1 unit RBC?

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End of Presentation!

Thank you for your time and attention.

Margaret A. Fitzgerald, DNP, FNP-BC, NP-C, FAANP, CSP
www.fhea.com, e-mail: cs@fhea.com

