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Clinical Pearls: How I Test

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I have no financial relationships to disclose.

AND

I will **NOT** include discussion of off-label or investigational use of any products in my presentation.

Evaluation of Anemia

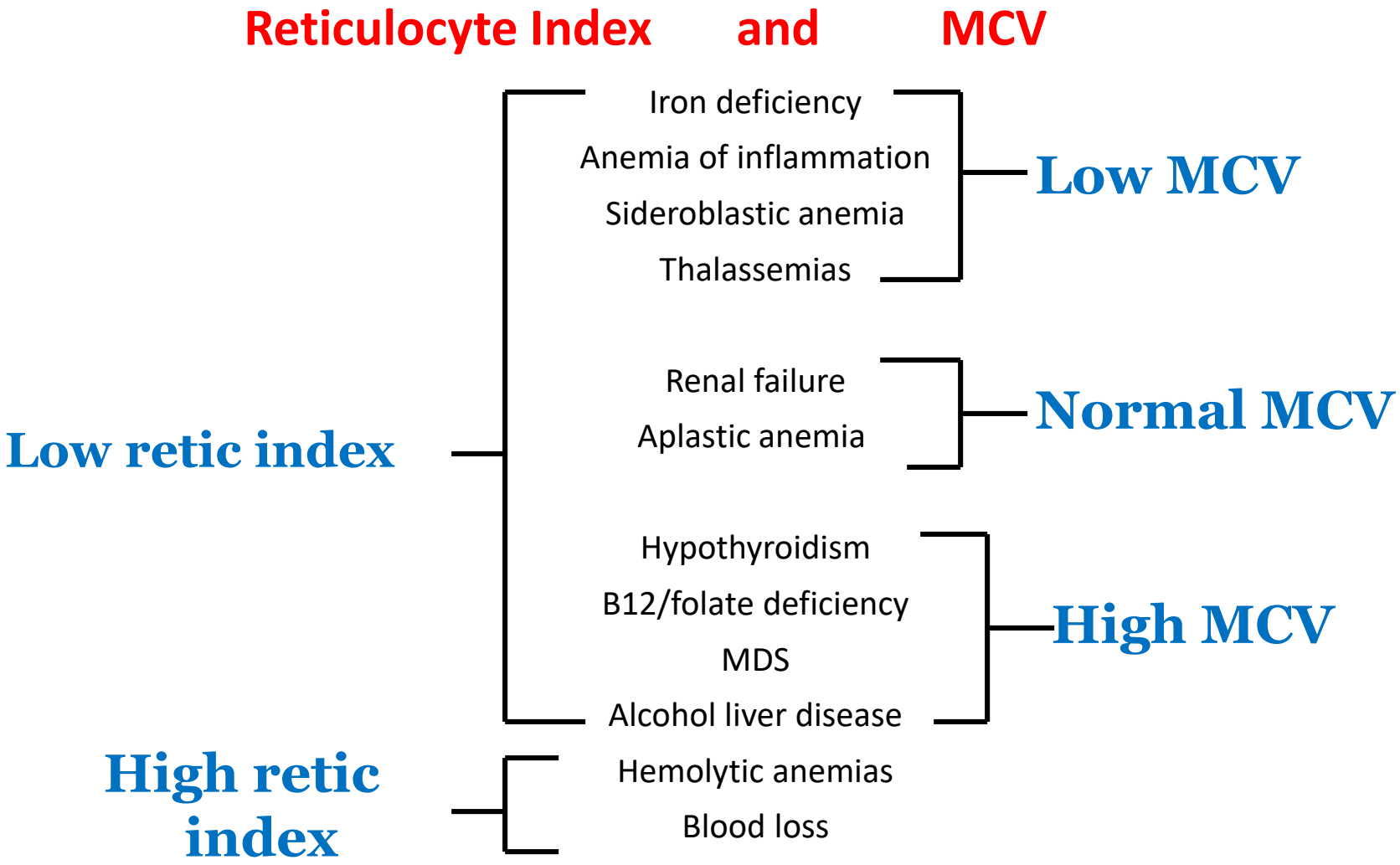
Reticulocyte count

Corrected Reticulocyte Count/Reticulocyte Index

$$\text{Reticulocyte Index} = \text{Retic count} \times \frac{\text{Hematocrit}}{\text{Normal Hct (45)}}$$

Reticulocyte Index in a normal healthy adult is between 1 and 2

Evaluation of Anemia



Evaluation of Hypoproliferative Anemia

Iron Studies: Fe/TIBC/Ferritin
Erythropoietin level
B12, folate
CRP/ESR

- Important for interpretation of ferritin, as it is an acute phase reactant
- In true iron deficiency, one cannot raise ferritin to over about 100

Interpretation of Iron Studies

	Iron deficiency	Anemia of Inflammation
Serum Fe	Low	Low
TIBC	High	Low
Transferrin saturation	Low	Low
Ferritin	Very low	N/High

Anemia of Inflammation

Characterized by low serum Fe/TIBC in setting of elevated ferritin

Associated with a wide variety of clinical disorders

- Infections (bacterial endocarditis)
- Rheumatologic Disease (rheumatoid arthritis, SLE)
- Organ dysfunction (CHF, chronic renal failure)
- Malignancy (MDS, NHL)

Pathophysiology

- Impaired EPO responsiveness of hematopoietic stem cell
- shortened red cell survival
- impaired iron mobilization ⟹ iron-limited erythropoiesis related to overexpression of hepcidin

Erythropoietin and Anemia of the Elderly

Epo secretion and Epo responsiveness of HSCs may be altered with age

Epo levels rise with age in healthy, non-anemic individuals

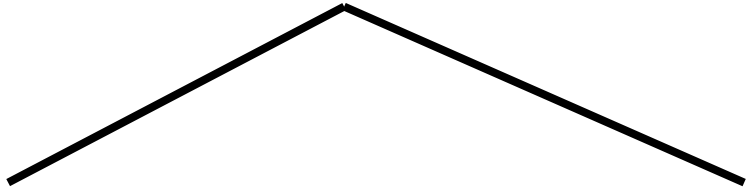
- Slope of the rise greater in those without diabetes or hypertension
- Anemic individuals had a lower slope of rise
- Hypothesis: Anemia reflects failure of a normal compensatory rise in Epo levels, reflecting age-related co-morbidities

Even in the setting of a normal creatinine, EPO may help interpret anemia, especially in elderly patients

Ferrucci et al; Blood 2010; 115: 3810

Evaluation of Anemia with ↑Reticulocytes

Hemolytic Anemias



Hereditary

1. Defects in RBC membrane
2. Defects in RBC metabolism (enzymopathies)
3. Defects in Hemoglobin (hemoglobinopathies)

Acquired

1. Immune HA
2. Non-immune HA

Autoimmune Hemolytic Anemia

	Warm AIHA	**Cold AIHA**
Direct Coombs	IgG or IgG & C3	C3 only
Antibody	IgG	IgM
Etiology	1. Drugs: Methyldopa, PCN, Sulfa 2. Malignancy: CLL, NHL 3. Infection	1. Drugs: Quinidine 2. Malignancy: NHL 3. Infection: Mycoplasma 4. Paroxysmal cold hemoglobinuria
Treatment	Steroids +/- Danazol Rituximab Splenectomy	No role for steroids Warm pt Rituximab +/- fludarabine

Leukocytosis: Differential Diagnosis

SECONDARY TO OTHER ILLNESSES

- Infection
  - Acute: Demargination/release storage pool
  - Chronic: Granulomatous dx (leukoerythroblastic)
- Stress
- Drug-induced (steroids, β-agonists, lithium)
- Chronic inflammation
- Post-splenectomy
- Non-hematologic malignancy
- Marrow stimulation (ITP, hemolysis, CMT)

PRIMARY HEMATOLOGIC DISEASE

- CML
- Other MPD

Evaluation of Leukocytosis

Neutrophilia is usually reactive, indicative of a normal functioning bone marrow. Bone marrow evaluation is often unnecessary

- Repeat WBC to R/O factitious or artifactual elevation
- Evaluation for acute/chronic infection or inflammation
- FISH for bcr-abl
- Bone marrow exam: r/o granulomatous dx, fungus

Neutropenia: Differential Diagnosis

Congenital Neutropenia

- Duffy-null associated neutrophil count (DANC)
- Familial neutropenia
- Severe congenital neutropenia
- Cyclic neutropenia
- Other rare disorders

Acquired Neutropenia

- Autoimmune neutropenia
- Drug-induced neutropenia
- Chronic idiopathic neutropenia
- Primary marrow failure syndromes (MDS, aplasia)

Evaluation of Neutropenia

For Congenital Neutropenia:

- Molecular Diagnosis for ELANE, HAX1
- Some advocate for testing for Duffy antigen negative phenotype in suspected Duffy-null associated neutrophil count (DANC, formerly benign ethnic neutropenia

Acquired Neutropenia

- Stop possible offending drugs
- Flow cytometry for clonality, LGL
- Serologic studies for ANA
- Anti-neutrophil antibodies are not recommended
- R/O MDS: NGS or bone marrow examination

Tests of the Coagulation Cascade

- PT (***prothrombin time***, initiate with tissue factor, phospholipid, calcium)
- PTT (***partial thromboplastin time***, initiate with kaolin or silica, calcium, limited in phospholipid)
- TT (thrombin time, initiate with *thrombin*)
- FDP (Fibrin(ogen) degradation products, non-sp.)
- D-dimers (specific fibrin degradation)
- Factor XIII Screen (clot dissolution)
- 1:1 MIXING STUDY

Causes of ↑ PT

Elevated PT:

- Less than 30% of VII (the sole *“extrinsic pathway only”* protein), X, V, II (common pathway) or fibrinogen
- Inhibitors of fibrin polymerization (FDPs)
- Inhibitors of II or X
- Heparin in vast excess

Most Common Causes

- Vitamin K deficiency
- Warfarin Therapy
- Liver Disease

Causes of ↑ PTT

Elevated PTT:

- Any factor level less than 30% except VII,XIII
- Inhibitors of fibrin polymerization (FDP)
- Other inhibitors (lupus anticoagulants)
- Heparin (and warfarin, to lesser degree)
- Most Common Causes
- Congenital factor deficiency
- Acquired factor Inhibitors
- DIC
- Dysfibrinogenemia
- Lupus anticoagulant

Interpretation of Mixing Studies: deficiency vs inhibitor

	PTT Pt Plasma	PTT Nml Plasma	PTT 1:1 Mix
Factor Deficient	70 sec	30 sec	33 sec
Inhibitor	70 sec	30 sec	70 sec

Lupus Anticoagulant

Discovered in lupus patients in early 1960s
Unrelated to bleeding in vast majority of cases (exception: some patients have LA plus antibody to prothrombin and long PT and PTT).
LA is *defined* by

- Prolongation of PTT
- Behavior as an inhibitor in a mixing study
- Neutralization with excess phospholipid

In terms of pathophysiology, it is usually an antiphospholipid antibody, but not all APLA act as lupus anticoagulants
LA is a risk factor for THROMBOSIS, not bleeding

- The PTT can't be corrected with plasma or other products, and should not be.

VTE

Risks for hypercoagulable states

- Inherited
- Acquired: more common
  - 35% US adults are obese, OR of 2.3 for VTE
  - <10% have an inherited thrombophilia
- Mixed: all are additive or synergistic

“Provoked” vs “Unprovoked”

- Clear precipitating factor vs idiopathic or unidentified risk factor
  - Transient vs persistent provoking factor
  - Unprovoked = idiopathic

The “Hypercoagulable Workup”

Test for **Factor V Leiden** mutation

PCR for **Prothrombin G20210A** mutation

Functional assay of **Antithrombin**

Functional assay of **Protein C**

Functional assay of **Protein S**

- Free Protein S Antigen
- Total Protein S Antigen (free + bound to C4bp)

WHAT NOT to test:

Homocysteine:
FVIII
XIII polymorphisms, IX, XI,XII
PAI-1 4G/5G promoter, PAI-1

APLAS work-up

Tests for **Antiphospholipid Antibodies**

- **Lupus anticoagulant**:
  - Screen: functional clotting assays
    - Sensitive PTT
    - DRVVT
    - Kaolin clotting time
  - Confirmatory: remove APLA
    - Platelet neutralization test
    - Hexagonal phase phospholipids
- Anticardiolipin and β2-glycoprotein I antibodies
  - IgG and IgM only
  - No diagnostic role for other tests

Who should be tested?

Indications of possible inherited hypercoagulable state:
Age of onset < 50 years
Recurrent thrombosis
Positive family history in 1st degree relative
Unusual location/site

However:
• Avoid indiscriminate testing in the inpatient or ER setting
• There is **no** need to know immediately—require at least 3 months anticoagulation for VTE regardless of thrombophilia status

Thrombophilia Testing Remains Controversial

Why the controversy?

- There are no data that results should affect care
- ASH Choosing Wisely Campaign 2013: “do not test in the setting of provoked VTE due to strong risks”

Misinterpretation of the significance of results

- Over treatment in the case of positive results
  - Duration of therapy determined by provoked vs unprovoked VTE
- False sense of security with negative results
  - Studies demonstrate increased VTE risk for patients with a family history of VTE despite negative results

When does it not change care?

- Provoked VTE
- Antiphospholipid syndrome
- Malignancy