CLINICAL LABORATORY TESTING:

BLOOD CHEMISTRY & CBC ANALYSIS

FROM A FUNCTIONAL MEDICINE PERSPECTIVE

Part 5 of 8
Clinical Approach to Anemia

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8 PART SERIES

Clinical Approach to Anemia

- Comprehensive patient history
- Physical examination
- Skilled use of laboratory interpretation

Remember that the signs and symptoms of anemia are a function of its severity, its rapidity of onset, and the age of the patient.

Clues from the Patient History (aside from ongoing blood loss)

- History of anemia that dates back to **childhood** suggests a hereditary disorder: such as congenital hemolytic anemia.

- The **sudden onset of pancytopenia** in an otherwise healthy individual may be explained from the occupational history and/or environmental toxin exposure history or the affects of a new medication prescribed as opposed to gradual onset seen in marrow disorders.

- **Race:** hemoglobinopathies and enzyme deficiency states follow ethnic lines

The laboratory diagnosis of anemia generally starts by **assessing routine hematology tests**.

The key to any anemia patient is the **clinician's skill in applying and interpreting** the results of the lab tests according to the clinical presentation.

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Laboratory Tests Used to Diagnosis Anemia

Complete Blood Count

- RBC count
- Hemoglobin
- Hematocrit
- RBC indices
- Reticulocyte count
- Platelet count
- RBC morphology
- WBC count

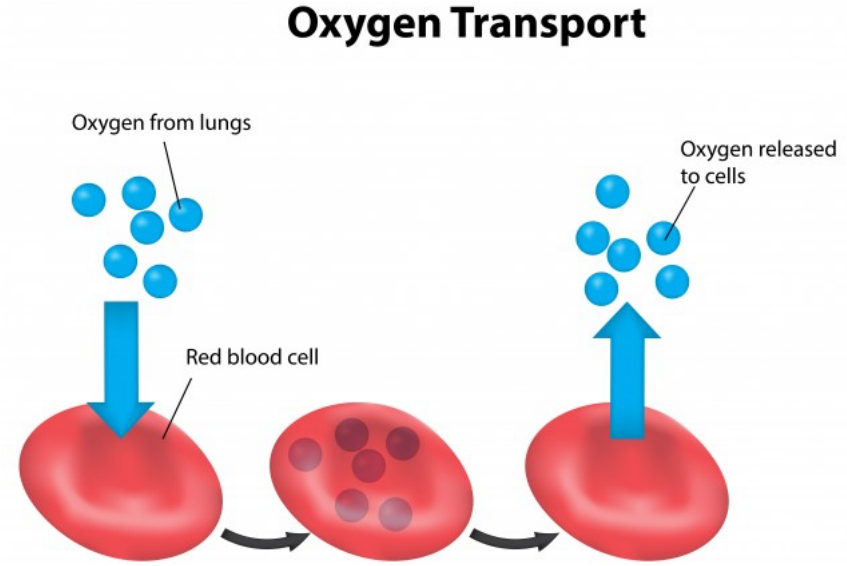
Iron Studies

- Iron transport: serum iron, total iron bind capacity
- Iron Storage: serum ferritin, marrow iron stain

Red Blood Cell Production

Since RBCs are the ‘trucks’ that carries hemoglobin and therefore oxygen, it’s important to know the factors that influence RBC production.

The rate of production of new RBCs varies according to the rate of RBC destruction and tissue oxygen requirements.



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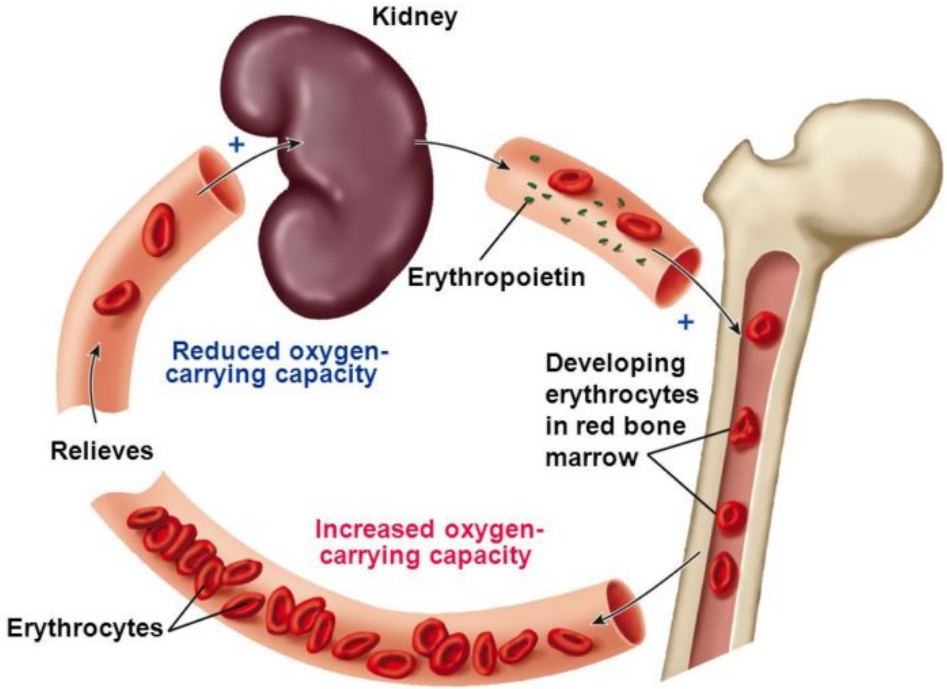
The **kidneys** stimulate an increased production in the erythropoietin in response to decreased oxygen saturation of hemoglobin, pulmonary dysfunction, and a low level of hemoglobin (i.e. anemia). **cytokines**.

Other factors influencing the level of erythropoietin include the mass of erythroid marrow and **the level of inflammatory cytokines**.



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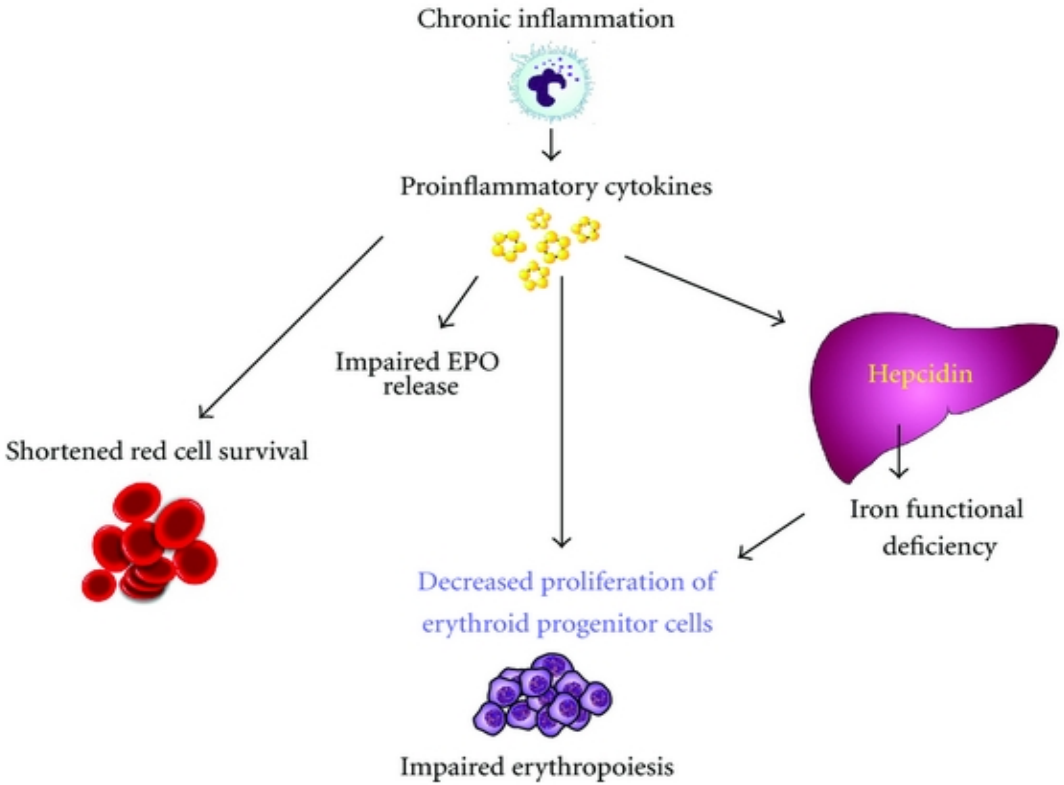
Inflammatory cytokines such as interleukin-1, interleukin-6, tumor necrosis factor alpha (TNF-α), and transforming growth factor beta cause a decrease in erythroid marrow response leading to an anemic state.



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Chronic inflammatory states will to lead anemia; and generally, takes on some of the same manifestations of iron deficiency.

For example, an individual with active rheumatoid arthritis will have a significant amount of the inflammatory cytokine TNF-α, which leads to a decrease in erythroid marrow response.

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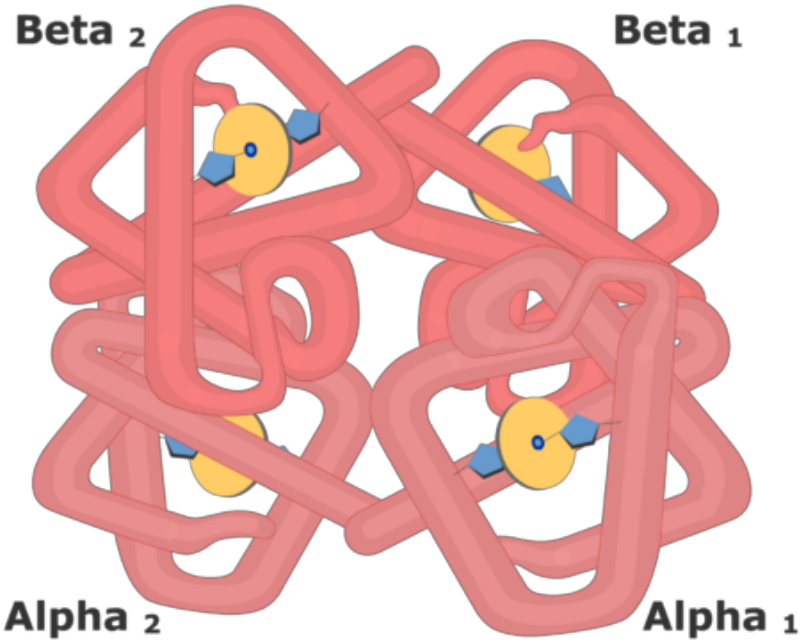
Conditions Associated with a Reduced Erythropoietin Response

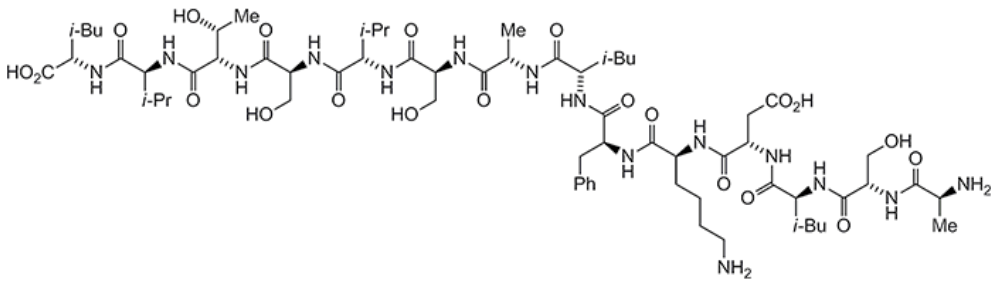
Inflammatory states	• Acute and chronic bacterial infections • Collagen vascular disorders • AIDS • Malignancies
Renal disease	• Nephritis • End-Stage renal disease
Hypometabolic states	• Protein deprivation • Nutritional deficiencies
Endocrine disorders	• Hypothyroidism • Hypopituitarism • Hyperparthyroidism

Hemoglobin

4 polypeptide chain:
each chain a heme group that can bind oxygen

Normal conditions:
arterial heme saturated > 97% O2; venous heme saturated 75 – 80 %





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Hemoglobin < 9 – 10 g/dL

Changes in regional blood flow, cardiac output, and blood volume

Residential elevation above sea level and smoking will increase hemoglobin and must be taken into account.

Hemoglobin Value (g/dL) at Sea Level

Population (Age)	Non-Anemia	Mild Anemia	Moderate Anemia	Severe Anemia
6 – 59 months	≥ 11.0	10.0 – 10.9	7.0 – 9.9	< 7.0
5 – 11 years	≥ 11.5	11.0 – 11.4	8.0 – 10.9	< 8.0
12 – 14 years	≥ 12.0	11.0 – 11.9	8.0 – 10.9	< 8.0
Non-pregnant women ≥ 15 years	≥ 12.0	11.0 – 11.9	8.0 – 10.9	< 8.0
Pregnant women	≥ 11.0	10.0 – 10.9	7.0 – 9.9	< 7.0
Men ≥ 15 years	≥ 13.0	11.0 – 12.9	8.0 – 10.9	< 8.0

Altitude Adjustment to Measured Hemoglobin

Altitude in meters above sea level	Measured Hb adjustment (g/dL)
< 1000	0
1000	- 0.2
1500	- 0.5
2000	- 0.8
2500	- 1.3
3000	- 1.9
3500	- 2.7
4000	- 3.5
4500	- 4.5

Adjustment to Measured Hemoglobin Value in Smokers

Smoking Status	Measured Hb Adjustment (g/dL)
Non-smoker	0
Smoker (all)	- 0.03
½ - 1 pack/day	- 0.03
1 – 2 packs /day	- 0.05
≥2 packs/day	- 0.07

The Diagnosis of Anemia

Hemoglobin value of the patient compared to a 'normal' reference range

Anemia has also been defined as **a reduction in the total circulating RBCs** and **a reduction in the hematocrit** (packed red blood cell volume) value.

Anemia can be classified as mild, moderate or severe based on the hemoglobin value.

Hemoglobin values for moderate and severe anemia are **7-10 g/dL and > 7 g/dL**, respectively.

Signs and Symptoms of Anemia (Non-Specific)

- Headaches
- Dyspnea
- Vertigo
- Light-headedness
- Muscle weakness
- Lethargy
- Hypotension
- Tachycardia

Classification of Anemia

Functional (hypoproliferative) – failure of RBC production

Maturation disorders – nutritional deficiencies [B12, folate, iron] defects in globin chain synthesis [thalassemia]);

Increased RBC destruction (blood loss, autoimmune disease, hemoglobinopathy [sickle cell anemia]

Morphological (changes of RBCs based on size and color)

Clinical (associated cause – blood loss, iron deficiency, hemolysis, infection, bone disease)

Quantitatively (blood tests)

Most anemias encountered in clinical practice are hypo proliferative caused by chronic illness (inflammatory response) and iron deficiency

State of Anemia	Associated Conditions
Hypoproliferative Low Hemoglobin Normocytic Retic Index < 2 ↓ Indirect –unconjugated bilirubin (↓ production)	• Marrow damage • ***Iron deficiency*** • Deceased stimulation (renal disease, **inflammation**, metabolic disease)
Maturation Disorders Low Hemoglobin Macrocytic or microcytic Retic Index < 2 ↑ Indirect –unconjugated bilirubin (Ineffective production)	• **Nuclear maturation defects (B_{12}, folate deficiency)** • ***Iron deficiency*** • **Sideroblastic** (stem cell disorder) • **Thalassemia** (reduction in the amount of normal globulin chain produced – inherited defect in globulin chain synthesis – microcytic anemia)
Hemorrhage/Hemolysis Low hemoglobin Normocytic Retic Index > 3 ↑ Indirect –unconjugated bilirubin (↑ destruction)	• Blood loss • Intravascular hemolysis • Autoimmune disease • **Hemoglobinopathy** (amino acid substitutions in the globin chain) • Membrane defect (e.g. **hereditary spherocytosis**) • Intracellular metabolic defects (e.g. pyruvate kinase deficiency, glucose-6-phosphate isomerase deficiency, glucose-6-phosphate dehydrogenase deficiency

First Step: Classification of the physiological mechanism

Once anemia has been identified, classification of the physiologic mechanism is the most useful first step.

- **Blood loss**
- **Hemolysis (RBC destruction)**
- **Underproduction**

More than one category can exist, and that one category can lead to another.

Anemia: Due to Bone Marrow Damage

- Drug toxicity
- Autoimmune disease
- Infections
- Environmental toxins (e.g. benzene), radiation, and malignancy.
- Malignancy: infiltration of the marrow.
- Idiopathic type (aplastic anemia): may be due to an autoimmune process
- Medications (drugs)

Potential Causes of Bone Marrow Anemia

Stem cell/ marrow structural damage	• Chemotherapy • Radiation • Gaucher disease • Myelofibrosis
Autoimmune disease	• Rheumatologic disorders • Viral infections • Graft-host disease • Idiopathic aplastic anemia • Pure red cell aplasia
Congenital disorders	• Fanconi anemia • Diamond-Blackfan anemia • Shwachman-Diamond syndrome • Dyskeratosis congenital

Hemoglobinopathies: genetic disease of hemoglobin
'ABNORMAL HEMOGLOBIN MOLECULE'

The inherited hemoglobin disorders are the **most common single gene defect** in humans

The frequency of the carrier state has been estimated to be 270 million with about 400,000 annual births a year of infants with serious hemoglobinopathies.

Hemoglobin is a tetrameric protein with four peptide chains, two α and two non-α-globulin chains.

The classification of abnormal hemoglobin synthesis includes:

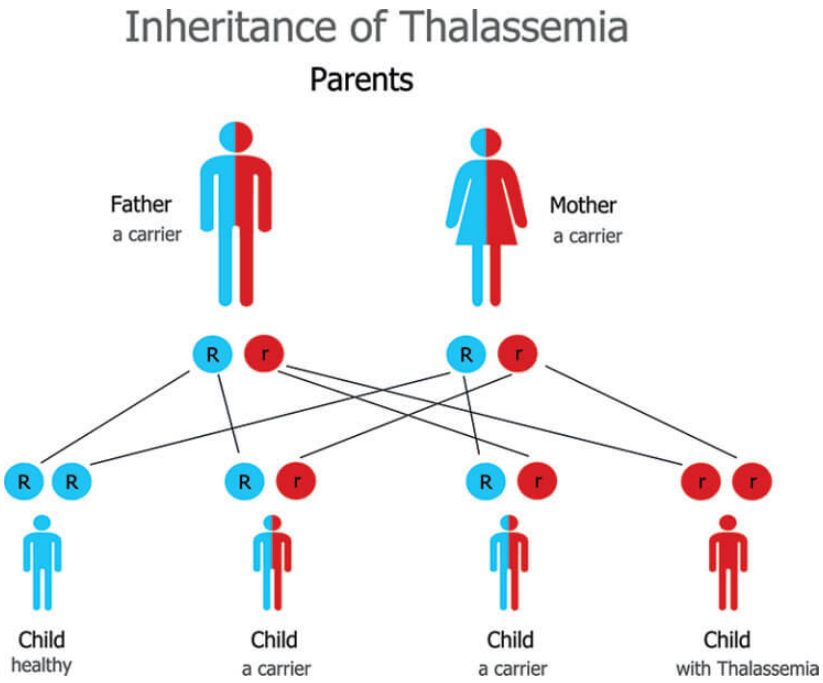
- **Production of structurally normal**, but **decreased amounts of globulin** chains (the thalassemia- failure of synthesis)
- **Production of structurally abnormal globulin chains**: (e.g. Hemoglobin S, Hemoglobin C, and Hemoglobin E)

Thalassemia

Genetic (inherited**)** **decrease in globin synthesis** leading to microcytic/hypochromic anemia (iron deficiency anemia is also microcytic/hypochromic).

The most clinically relevant thalassemia's are α and β-thalassemia.

Erythropoietic profiles of thalassemia and iron deficiency can help to differentiate the between the two conditions.



Erythropoietic Profile of Thalassemia

	Thalassemia	Iron deficiency
Anemia	Minor to severe	Minor to severe
MCV fl	< 70	< 80
Blood smear	Microcytic, hypochromic with prominent targeting	Normal to microcytic, hypochromic
Reticulocyte Index	< 2	< 2
Serum iron	Increased	Very low
TIBC	Normal	Increased
Serum ferritin ug/L	> 100	< 15
% Saturation	> 50	< 10
Bilirubin	Increased	Normal
LDH	Increased	Normal

Hemoglobinopathy

Hemoglobinopathies present with a structural point mutation of an amino acid in one of the globulin chains.

Sickle cell disease is a frequent and clinically relevant hemoglobinopathy.

It presents in early life as severe hemolytic anemia. The clinical features include pallor, jaundice, fatigue, and poor growth. It is diagnosed by the presence of Hemoglobin S/S.

The blood profile consists of low hemoglobin, high reticulocyte count, high bilirubin and high LDH

Erythropoietic Profile of Hemoglobinopathy

Blood Smear	Normocytic, normochromic to slightly microcytic/ sickle cells, target cells
MCV fL	70 - 80
Polychromasia	Present to prominent
Reticulocyte Index	> 3
Bilirubin	Increased
LDH	Increased
Serum Iron	Normal to increased
TIBC	Normal
Serum Ferritin	> 100 ug/L

Chronic Blood Loss

Chronic blood loss leading to iron deficiency is usually caused by gastrointestinal blood loss; however some individuals may present with a defect in iron absorption, secondary to small bowel disease.

The presentation of low hemoglobin, low RBC count and a low MCV is suspicious for possible blood loss and therefore required further investigation such as a stool test for blood loss (i.e. GI work-up) and/or gynecological work-up.

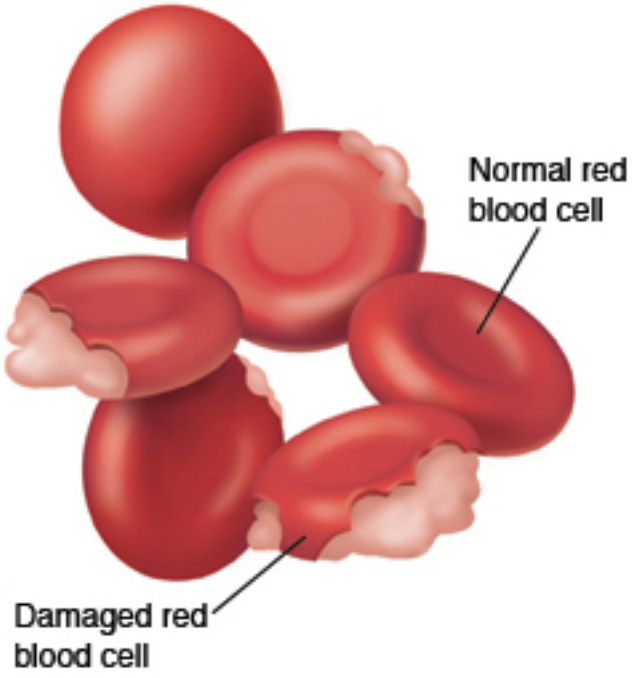
Hemolytic Anemia

Destruction or removal of red blood cells from circulation before their normal life span of 120 days.

Hemolysis presents as acute or chronic anemia, reticulocytosis, or jaundice.

There are two pathways of red blood cell destruction:

- intravascular
- extravascular



Overview of Hemolytic Anemias

Type	Etiology	Associations	Diagnosis	Treatment considerations
Acquired Immune-mediated	Antibody to red blood cell surface antigens	Idiopathic, malignancy, drugs, **autoimmune** disorders, infections, transfusions	Spherocytes and positive **direct antiglobulin test** (DAT)	Treat underlying cause: removal of offending drugs
Acquired Microangio-pathic	Mechanical disruption of red blood cell in circulation – RBC traverse an injured vascular endothelium	**Thrombotic thrombocytopenic purpura**, **hemolytic uremic syndrome**, **disseminated intravascular coagulation**, pre-eclampsia, **malignant hypertension**, prosthetic values	**Schistocytes (fragmented RBC on peripheral blood smear – helmet cells)**	Treat underlying disorder

Overview of Hemolytic Anemias

Type	Etiology	Associations	Diagnosis	Treatment considerations
Acquired: **Infection**	Malaria, babesiosis, Clostridium infections		Cultures, blood smears, serology	Treat infection
Hereditary Enzymopathies	G6PD deficiency	Infections, drugs, ingestion of fava beans	Low G6PD	Treat infection remove offending drug
Hereditary Membranopathies	Hereditary spherocytosis		Spherocytes, family history, negative **DAT**	
Hereditary **Hemoglobin-opathies**	Thalassemia and sickle cell disease		Hemoglobin electrophoresis Genetic studies	Folate, transfusions

Chronic Hemolytic Anemia Erythropoietic Profile

Blood Smear	Normocytic, normochromic, abnormal RBCs
Polychromsia (high immature RBCs)	Present
Reticulocyte Index	> 3
Serum iron	Normal
TIBC	Normal
Serum bilirubin	1 – 3 mg/dL
LDH	> 1000 IU/mL

Macrocytic Anemia

Macrocytosis: increase in MCV >100 fL.

The most common cause: vitamin B12 and folic acid deficiency, alcoholism and medication-induced.

Vitamin B12 and folic acid deficiency mostly due to **malabsorption** diseases such as celiac disease, Crohn disease, pancreatic insufficiency, and intrinsic factor deficiency (due to autoimmune disease or gastric surgery).

Other causes include: medication-induced (methotrexate, anticonvulsants, HIV drugs, chemotherapeutics, biguanides- e.g. metformin), liver disease, hypothyroidism, hemolysis, COPD, hyperglycemia, and splenectomy.

Testing for Vitamin B12 and Folic Acid Deficiency

Analyte – (normal range)	Vitamin B_{12} Deficiency	Folic Acid Deficiency
Serum B_{12} (> 200 pg/mL)	< 100	> 200
Serum Folate (> 4ng/mL)	> 4	< 4
Serum Methylmalonic Acid (< 270nM/L)	2 – 100 x normal	normal
Serum Homocysteine (< 16 nM/L)	2 – 20 x normal	2 – 10 x normal

Iron Deficiency Anemia

Iron is an essential element of heme protein (e.g. hemoglobin, myoglobin), as well as in numerous enzymes such as thyroid peroxidase.

Iron deficiency is one of the **most common** causes of **microcytic anemia** in adults and children.

Iron deficiency anemia (IDA) results in RBCs that **are microcytic and hypochromic** due to a defect in hemoglobin synthesis.

Iron Deficiency Anemia - Three Stages

Stage 1 - Reduced storage – iron depletion characterized by a reduced serum ferritin and an increase in TIBC

Stage 2 - Iron deficient erythropoiesis – early iron deficiency anemia characterized by decreases in serum iron and transferrin saturation % - MCV and blood smear are normal

Stage 3 - Advanced iron deficiency – IDA characterized by microcytic/hypochromic RBCs with high levels of TIBC and low levels of serum iron, serum ferritin and transferrin saturation %.

Iron Study Analytes

Analyte	Description	Conventional Range	Optimal Range
Serum Iron	Measure of iron bound to transferrin in the serum. (Makes iron soluble; prevents iron-mediated free radical damage; and facilitates transport into the cells.)	60 – 150 ug/dL 10.7–26.9 umol/L	40 – 100 ug/dL 7.5-17.91 umol/L
TIBC	Total iron-binding capacity – the amount of iron that can be bound to transferrin. In IDA, TIBC is increased due to increased synthesis of transferrin, which leads to decreased % saturation of transferrin.	250-400 ug/dL 45-82 umol/L	250-400 ug/dL 45-82 umol/L

Analyte	Description	Conventional Range	Optimal Range
Serum Ferritin	Iron-protein complex. Iron stores found primarily on the macrophages. Parallels total body storage of iron. Liver disease and inflammatory conditions cause an increase in levels.	Adult Male 12-300 ng/mL or ug/L Adult Female 10 – 150 ng/mL or ug/L	Adult Male 30-190 ng/mL or ug/L Adult Female 20 – 130 ng/mL or ug/L
Transferrin % Saturation	Transferrin is synthesized in the liver and secreted in to the plasma. % Saturation is a calculated value of iron saturation of transferrin. **It is one of the first indicators of iron overload.**	15 – 50 %	20-35 %

Biomarkers in Iron Deficiency

	Normal	Iron depletion	Iron deficient erythropoiesis	IDA
Hemoglobin	Normal	Normal	Normal	**Microcytic Hypochromic**
Serum iron ug/dL	115±50	115	**< 60**	**< 40**
TIBC ug/dL	330±30	360	**390**	**410**
% Saturation	35±15	20 - 30	**< 15**	**< 10**
Ferritin ug/L	100±60	**< 40**	**< 20**	**< 15**

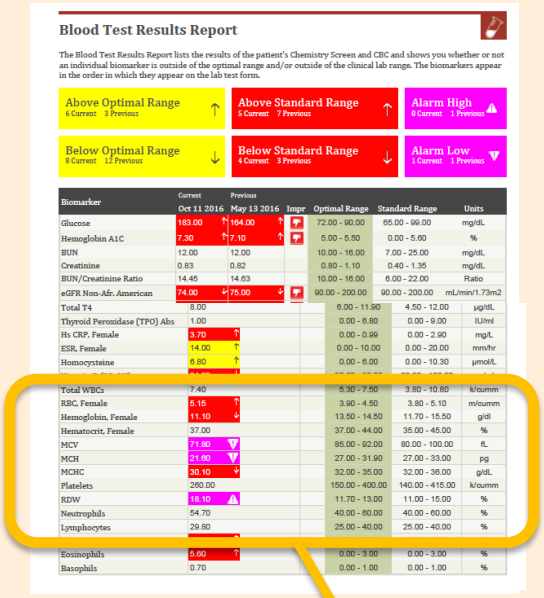
Conditions	Ferritin	Serum Iron	TIBC	% Saturation
Chronic blood loss	L	L	H	L
Acute blood loss	N	L	N	L
Iron deficiency	L	L	H	L
Hemolytic anemia	H	H	L	H
Chronic disease	H	L	L	L
Hemochromatosis	H	H	L	H
Pregnancy	L	L	H	L
Estrogen therapy	N	H	H	L
Acute inflammation	H	N	L	H
Iron toxicity	H	H	N	H
Iron excess	N	N	N	H (>45%)
Iron depletion	L	N	N	N
Iron deficiency without anemia	N/L	N/L	N/H	N/L

Anemia of Inflammation: Infections, Inflammation and Neoplastic disease

Any disease state with a major inflammatory component will be accompanied by a hypo-proliferative anemia.

Inflammatory cytokines and the up-regulation of hepcidin lower serum iron and inhibit the release of iron stores.

Laboratory tests can be used to differentiate pure iron deficiency from an inflammatory state.



Blood Test Results Report

The Blood Test Results Report lists the results of the patient's Chemistry Screen and CBC and shows you whether or not an individual biomarker is outside of the optimal range and/or outside of the clinical lab range. The biomarkers appear in the order in which they appear on the lab test form.

Above Optimal Range — 6 Current, 3 Previous
Above Standard Range — 5 Current, 7 Previous
Alarm High — 0 Current, 1 Previous
Below Optimal Range — 8 Current, 12 Previous
Below Standard Range — 4 Current, 3 Previous
Alarm Low — 1 Current, 1 Previous

Biomarker	Current Oct 11 2016	Previous May 13 2016	Impr	Optimal Range	Standard Range	Units
Glucose	183.00	184.00		72.00 - 90.00	65.00 - 99.00	mg/dL
Hemoglobin A1C	7.30	7.10		5.00 - 5.50	0.00 - 5.60	%
BUN	12.00	12.00		10.00 - 16.00	7.00 - 25.00	mg/dL
Creatinine	0.83	0.82		0.80 - 1.10	0.40 - 1.35	mg/dL
BUN/Creatinine Ratio	14.45	14.63		10.00 - 16.00	6.00 - 22.00	Ratio
eGFR Non-Afr. American	74.00	75.00		90.00 - 200.00	90.00 - 200.00	mL/min/1.73m2
Total T4	8.00			6.00 - 11.90	4.50 - 12.00	µg/dL
Thyroid Peroxidase (TPO) Abs	1.00			0.00 - 6.80	0.00 - 9.00	IU/ml
Hs CRP, Female	3.70			0.00 - 0.99	0.00 - 2.90	mg/L
ESR, Female	14.00			0.00 - 10.00	0.00 - 20.00	mm/hr
Homocysteine	6.80			0.00 - 6.00	0.00 - 10.30	µmol/L
Total WBCs	7.40			5.30 - 7.50	3.80 - 10.80	k/cumm
RBC, Female	5.15			3.90 - 4.50	3.80 - 5.10	m/cumm
Hemoglobin, Female	11.10			13.50 - 14.50	11.70 - 15.50	g/dl
Hematocrit, Female	37.00			37.00 - 44.00	35.00 - 45.00	%
MCV	71.80			85.00 - 92.00	80.00 - 100.00	fL
MCH	21.60			27.00 - 31.90	27.00 - 33.00	pg
MCHC	30.10			32.00 - 35.00	32.00 - 36.00	g/dL
Platelets	260.00			150.00 - 400.00	140.00 - 415.00	k/cumm
RDW	18.10			11.70 - 13.00	11.00 - 15.00	%
Neutrophils	54.70			40.00 - 60.00	40.00 - 60.00	%
Lymphocytes	29.80			25.00 - 40.00	25.00 - 40.00	%
Eosinophils	6.60			0.00 - 3.00	0.00 - 3.00	%
Basophils	0.70			0.00 - 1.00	0.00 - 1.00	%

FHR shows high Red Blood Cell (RBC) Index

The Functional Health Report highlights out-of-range analytes and then provides a summary of possible health conditions related to the results in multiple summary areas like, Health Improvement Plan, Functional Index Report and Clinical Dysfunctions Report.

Here is an example of the summary, from the **Functional Index Report** section of the FHR. In this case levels of measurable Hemoglobin, MCV, MCHC, RDW, and MCH are highly likely to show dysfunction.

Red Blood Cell Index

The RBC Index is a measure of the degree of anemia in your patient. The higher the index the more likely it is that your patient is dealing with an anemia and you'll need to examine the blood test further to identify the cause of the anemia. One of the main causes is nutrient deficiency: iron, B12/folate, vitamin B6, copper and vitamin C. You must also rule out other causes that are not nutritionally related. Based on this blood test, your patient's Red Blood Cell index is:

[92%] - Dysfunction Highly Likely. Much improvement required.

Rationale:
Hemoglobin, Female ↓, MCV ↓, MCHC ↓, RDW ↑, MCH ↓

Elements Considered:
RBC, Female, Hemoglobin, Female, Hematocrit, Female, MCV, MCHC, RDW, MCH

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Ask the Doctor

A **FREE** service available to all Evexia clients, accessed via your Evexia Clinician Portal.

Dr. Wayne Sodano, will **review test results, clinical conditions, further test recommendations or answer any other questions you may have via email**.

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Next lesson: Part 6 of 8

Integrative and Functional Medicine Perspective of Laboratory Interpretation – Patterns of Dysfunction