Overview of Anemia: Diagnostic Testing in the Clinical & Laboratory Medicine

Monet N. Sayegh, M.D.
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Anemia World Statistics

Global

- Iron Deficiency Anemia is the World’s most common micronutrient deficiency
- As many as 4-5 billion people (66-80% of population) may be iron deficient
- Categorized by WHO as one of the top ten most serious health problems in the modern world
- 1.62 billion people (>30% population) are anemic
- 818 million women worldwide (both pregnant and non-pregnant) and young children suffer from anemia and over half of these, approximately 520 million, live in Asia
- United States:~4% of men and 8% of women have values lower than normal values

Prevalence of Anemia by Age Group in Industrialized and Developing Countries, 1998

US Percent of Persons with Iron Deficiency

National Health and Nutrition Examination Survey 2003-2006

- **Children**
  - Age 1 - 3 years: 14%
  - Age 3 - 5 years: 4%

- **Females**
  - Age 12 - 19 years: 9%
  - Age 20 - 49 years: 9%
Anemia

Definition
Reduction in blood transport of oxygen due to a deficiency in red blood cells

- Anemia is defined by the WHO as a hemoglobin concentration of <12 g/dL for women and <13 g/dL for men.

Parameters

- Hematocrit – Percentage of blood volume as RBCs
- Hemoglobin – Concentration of hemoglobin in blood
- Mean Corpuscular Volume (MCV) – Average size of RBC
- Mean Corpuscular Hemoglobin (MCH) – Average hemoglobin content of RBC
- Mean Corpuscular Hemoglobin Concentration (MCHC) – The amount of hemoglobin relative to the size of the cell (hemoglobin concentration) per red blood cell
- RDW – Red cell distribution width
- Reticulocyte Hgb Content (CHr)
- %Hypo

Groups at High Risk

- Menstruating women
- Pregnant and breastfeeding women
- Babies, especially if premature
- Children going through puberty
- Vegetarians
- People with cancer, stomach ulcers and some chronic diseases (Renal Dialysis)
- GI surgery
- People on fad diets
- Athletes
Red Cell Production (Erythropoiesis)

Effective Erythropoiesis Requires 20mg of Iron per day
AA, Vitamin B₁₂, 6, Folic Acid, Ni, Co, EPO

Bone Marrow

Erythroid Stem Cell – Rubriblast - 1%

Prorubricyte 1 - 4%

Rubricyte 10 - 20%

Metarubricyte 5 - 10%

Reticulocyte
released from BM after 2-3 days
and 1% circulate for additional 1-2 days

Multi-potent Stem Cell

RNA Protein Synthesis

3 - 4 DAYS

Peripheral blood

Mitochondria and Ribosomes

RBC survival ~120 days

Kidney

Epo mRNA

Oxygen Sensor

Blood Vessel

Three Main Causes of Anemia

Increased RBC Destruction (Blood loss/hemolysis)
- Elevated reticulocyte count

Red cell maturation defects (ineffective erythropoiesis)
- Slight to moderately elevated reticulocyte count
- Macrocytic or microcytic anemia

Decreased RBC Production (hypoproliferation)
- Marrow production defects
- Low reticulocyte count
- Little or no change in red cell morphology (a normocytic, normochromic anemia)
Some Etiologies of Anemia

**Nutritional**
- Iron deficiency
- Vitamin B-12 deficiency
- Folate deficiency
- Starvation and generalized malnutrition

**Physical**
- Trauma
- Burns
- Frostbite
- Prosthetic valves and surfaces

**Chronic disease and malignant**
- Renal disease
- Hepatic disease
- Chronic infections
- Neoplasms
- Collagen vascular diseases

**Infectious**
- Viral - Hepatitis, infectious mononucleosis, cytomegalovirus
- Bacterial - Clostridia, gram-negative sepsis
- Protozoal - Malaria, leishmaniasis, toxoplasmosis

Some Genetic Causes of Anemia

- Hemoglobinopathies
- Thalassemia's
- Enzyme abnormalities of the glycolytic pathways
- Defects of the RBC cytoskeleton
- Congenital dyserythropoietic anemia
- Rh null disease
- Hereditary xerocytosis
- Abetalipoproteinemia
- Fanconi anemia

Depending on the severity, the symptoms of anemia may include:

- Pale skin
- Fatigue
- Weakness
- Tiring easily
- Breathlessness
- Orthostatic hypotension – this may happen after acute blood loss, like a heavy period
- Frequent headaches
- Racing heart or palpitations
- Becoming irritated easily
- Concentration difficulties
- Cracked or reddened tongue
- Loss of appetite
- Strange food cravings

Red = In severe anemia
Complications of Severe Anemia

The most serious complications of severe anemia arise from:

- Tissue hypoxia
- Shock
- Coronary insufficiency
- Pulmonary insufficiency
- Death


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Initial Evaluation

History and Physical Exam

• Eating ice or clay (Pica)
• Dyspnea
• Chest pain
• Medications
• Conjunctival pallor

Laboratory Evaluation

• CBC with differential
• Peripheral smear
• Reticulocyte hemoglobin content
• Iron Studies (iron, TIBC, transferrin, ferritin)
• Vitamin B12 and Folate
• Evaluation for hemosiderinuria, hemoglobinuria, and pulmonary hemosiderosis
• Hemoglobin electrophoresis and measurement of hemoglobin A2 and fetal hemoglobin
Suggested Algorithm for the Diagnosis of Anemia

Low Hb = Anemia

MCV

MCV < 80 = Microcytic

Fe Deficient

IDDA

Fe Normal

Anemia of Chronic Disease or Hemoglobinopathy

MCV 80-100 = Normocytic

Ferritin

Fe Deficient

IDA

Fe Normal

Anemia of Chronic Disease or Hemoglobinopathy

MCV > 100 = Macrocytic

Vitamin B12 & Folate

Normal

Obvious Cause

Consider BM MDS, Liver Dz, Chemo Myxedema

Low

Establish Cause

ACD, CRF, Aplastic anemia, Protein energy malnutrition or Marrow Failure

Reticulocyte Count

High

Low

Hemolysis or Blood Loss

## Daily Recommended Amount of Iron (mg) by Gender and Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&gt;18 years</td>
</tr>
<tr>
<td></td>
<td>&gt; 50 Years</td>
</tr>
<tr>
<td></td>
<td>19 - 50 Years</td>
</tr>
<tr>
<td>Females</td>
<td>Pregnant</td>
</tr>
<tr>
<td></td>
<td>Breastfeeding</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>Females</td>
</tr>
<tr>
<td>Children</td>
<td>4 - 8 Years</td>
</tr>
<tr>
<td></td>
<td>1 - 3 Years</td>
</tr>
<tr>
<td></td>
<td>7 Months - 1 Year</td>
</tr>
<tr>
<td></td>
<td>0 - 6 Months</td>
</tr>
</tbody>
</table>
Dietary Sources of Iron

- Red meat > poultry & fish
- In U.S., 20 mg iron added/lb of flour
- Baked bread contains ~28 mg iron/kg (equivalent to the iron content of beef)
- Iron cooking pots
- Plants are generally not good sources because of oxalate, phytate, tannins, etc.
- Spinach has a lot of iron, but has ~780 mg oxalate/100 g

Note: Heme iron absorption from diet not affected by ascorbate or phytate
http://ods.od.nih.gov/factsheets/iron-08-24-07
Iron Supplementation in Special Populations

Pregnant Women

• During the last two trimesters, daily iron requirements increase to 5 - 6 mg to maintain normal level

• Normal-term infants are born with sufficient iron stores to prevent iron deficiency for the first 4 - 5 months of life

• Thereafter, enough iron needs to be absorbed to keep pace with the needs of rapid growth

• Nutritional iron deficiency is most common between 6 and 24 months of life

What Causes Iron Deficiency?

**Increased Iron Needs**
- Rapid growth in infancy or adolescence
- Pregnancy
- Erythropoietin therapy
- Acute or chronic Blood loss
- Heavy menstrual periods
- Frequent blood donation
- Some stomach and intestinal conditions (food sensitivity, hookworms)
- Phlebotomy as treatment for Polycythemia Vera

**Decreased Iron Intake and Absorption**
- Lack of heme iron sources in the diet (e.g., vegetarian diets)
- Low absorption
- Taking antacids beyond the recommended dose or medicine used to treat peptic ulcer disease and acid reflux can reduce the amount of iron absorbed in the stomach
- Malabsorption from disease (Celiac sprue, Crohn's disease)
- Malabsorption from surgery (post-gastrectomy)
- Acute or chronic inflammation

MMWR, April 03, 1998 / 47(RR-3):1-36
Systemic Manifestation of Iron Deficiency Anemia

Koilonychia (spooning of the fingernails)

Angular Cheilosis (fissures at the corners of the mouth)

Glossitis (soreness of the tongue, or inflammation with depapillation of the dorsal surface of the tongue)

Esophageal Web (thin membranes of normal esophageal that can partially obstruct) Plummer-Vinson Syndrome
Iron Deficiency Anemia

- Hypochromic red cell
- Microcytic cell
- Target cell (Codocytes) in sever cases
- Anisocytosis
- Poikilocytosis
- Mild thrombocytosis

Types of Iron Deficiency

**Absolute Iron Deficiency:**
Inadequate production of erythropoietin by kidney disease and decreased total Fe body content

**Chronic renal failure**

**Hemodialysis** (Average iron loss 57-78 mg/month)

One fifth of patients starting dialysis have absolute iron deficiency

**Functional Iron Deficiency:**
Iron stores are present but cannot be mobilized rapidly enough to maintain maximal erythropoietin-driven erythropoiesis

**Serum ferritin** may be normal or elevated

% hypochromic RBC raised (> 5%)

**Reticulocyte hemoglobin content low (CHr)**

**Transferrin saturation** usually < 20%, but variable

**Erythrocyte Indices** normal

**Seen in CRF who undergo Tx with epoetin**

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Data suggests that high levels of pro-inflammatory cytokines and increased oxidative stress are common features that may contribute to malnutrition, rHuEPO resistance and anemia.

Iron Deficiency Anemia in ESRD Patients

- It is largely the result of insufficient production of Erythropoietin by non-functioning kidney.
- End result – very poor quality of life.

Anemia (i.e. hemoglobin levels < 11 - 12 g/dL) is almost universal feature of patients with end-stage renal disease on dialysis.

Iron Loss in End Stage Renal Disease

- Increased external iron losses in transfusions:
  - Blood loss by the hemodialysis filter & lines
  - Frequent phlebotomy for serial monitored iron status
- Increased internal iron loss
  - Gastrointestinal bleeding

Decreased availability of body’s storage iron

Deficit in intestinal iron absorption

Iron Deficiency Anemia in ESRD Patients

- Increased iron losses in transfusions:
  - Blood loss by the hemodialysis filter & lines
  - Frequent phlebotomy for serial monitored iron status

End result – very poor quality of life.


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Measurement of Body Iron Status

Biochemical

- Serum iron (Fe)
- Total iron binding capacity (TIBC)
- Percentage transferrin saturation (%TSAT)
- Soluble circulating transferrin receptor (sTfR)
- Transferrin receptor ratio (TfR/FRT)
- Ferritin
- Haptoglobin

Hematological

- Hgb, MCV, MCH, RDW
- Erythrocyte zinc protoporphyrin (ZPP)
- Reticulocyte Hgb Content (CHr)
- % Hypochromic RBCs (% HYPO)
- Bone marrow iron store

## Stages of Iron Deficiency

<table>
<thead>
<tr>
<th>Depleted Iron Stores</th>
<th>Compromised Delivery</th>
<th>Iron Deficiency Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Serum ferritin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ TSAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ Erythrocyte ZPP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ Hb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ MCV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Hypo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ CHr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Differential Diagnosis using Iron Panel

<table>
<thead>
<tr>
<th></th>
<th>MCV</th>
<th>Serum Fe</th>
<th>TIBC</th>
<th>% Saturation</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe Deficiency</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>ACD</td>
<td>N to↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Sidroblastic</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

# Diagnostic Value of Iron Indices in Hemodialysis Patients Receiving Epoetin in Predicting a Response to Iron

<table>
<thead>
<tr>
<th>Serum Ferritin Threshold</th>
<th>Epoetin Dose Change of = 30 U/kg/week</th>
<th>Epoetin Dose Change = 60 U/kg/week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True Positives</td>
<td>True Negatives</td>
</tr>
<tr>
<td>&lt;100 ng/mL</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>&lt;200 ng/mL</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>&lt;300 ng/mL</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>&lt;400 ng/mL</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transferrin Saturation Threshold</th>
<th>Epoetin Dose Change of = 30 U/kg/week</th>
<th>Epoetin Dose Change = 60 U/kg/week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True Positives</td>
<td>True Negatives</td>
</tr>
<tr>
<td>&lt;12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>&lt;16</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>&lt;20</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>&lt;24</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>&lt;28</td>
<td>37</td>
<td>4</td>
</tr>
</tbody>
</table>

Treatment of Iron Deficiency

- Red blood cell transfusion
- Oral iron therapy
  - Ferrous sulfate
  - Ferrous fumarate
  - Ferrous gluconate
- Parenteral iron

The Prevalence of Vitamin B-12 Deficiency

Vitamin B12 deficiency is a global health problem

• In Canada, approximately 5% are B12-deficient.
• In the USA, 3-6% of adults are estimated to have vitamin B12 deficiency (Total B12 <148pmol/L).
• In the UK, B12 deficiency (Total B12 ≤150pmol/L) is estimated at 5% of those aged 65-74y and 10% in those aged ≥75y.
• Marginal depletion (Total B12 of 148-221pmol/L) is more common, occurring in >20% of those aged >60y.

Ability to absorb vitamin B12 decreases with age-associated gastric atrophy therefore prevalence will increase as the global population ages.


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Megaloblastic Anemia

- Due to impaired DNA synthesis
- Affects cells primarily having relatively rapid turnover, especially hematopoietic precursors and gastrointestinal epithelial cells
- Cell division is sluggish, but cytoplasmic development progresses normally, so megaloblastic cells tend to be large, with an increased ratio of RNA to DNA.
- Megaloblastic erythroid progenitors tend to be destroyed in the marrow
- Marrow cellularity is often increased but production of red blood cells (RBC) is decreased


Macrocytic RBC
Hypersegmented Neutrophils
Pernicious Anemia

- Most common cause of cobalamin deficiency
- Caused by the absence of IF
- Atrophy of the mucosa
- Autoimmune destruction of parietal cells
- Seen in individuals of northern European descent and African Americans
- Men and women are equally affected
- Disease of the elderly, the average patient presenting near age 60

Causes of Megaloblastic/Macrocytic Anemia

**Vitamin B\textsubscript{12} Deficiency**
- Inadequate intake: Vegans (rare)

**Malabsorption**
- Defective release of cobalamin from food
- Gastric achlorhydria-atropic gastritis
- Long term drug use that block acid secretion
- Partial gastrectomy
- Inadequate production of intrinsic factor (IF)
- Pernicious anemia
- Total gastrectomy
- Terminal ileal resection (> 100 cm), decreases the site of absorption of B\textsubscript{12}-IF complex
- Pancreatic insufficiency

**Disorders of Terminal Ileum**
- Celiac (Sprue) disease
- Regional enteritis
- Crohn’s disease

**Competition for Cobalamin**
- Fish tapeworm (Diphyllobothrium latum)
- Bacteria: "blind loop" syndrome
- Drugs: p-aminosalicylic acid, colchicine, neomycin

Causes of Megaloblastic/Macrocytic Anemia

Vitamin $B_{12}$ Deficiency
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Clinical Manifestations of Vitamin B$_{12}$ Deficiency

**Hematologic**
- Macrocytic anemia
- Pancytopenia

**Gastrointestinal**
- Glossitis
- Anorexia
- Diarrhea

**Psychiatric**
- Mild irritability and forgetfulness to severe dementia or frank psychosis

**Neurologic**
(found in 3/4th of individuals with pernicious anemia)
- Paresthesia in the extremities
- Peripheral neuropathy
- Combined systems disease (demyelination of dorsal columns and corticospinal tract)
- Weakness, ataxia, sphincter disturbances

Diagnosis of Vitamin $B_{12}$ Deficiency

- Macrocytosis
- Peripheral blood smear
- Cobalamin levels
- Serum Vitamin $B_{12}$
- Elevated serum methylmalonic acid and homocysteine levels
- Serum Folate
- Schilling Test

## Current Routinely Laboratory Tests

<table>
<thead>
<tr>
<th>Four biochemical tests routinely used for assessment of Vitamin B12 status:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Total Serum B12</strong></td>
</tr>
<tr>
<td>▪ Measures all circulating B12, both inactive and active forms</td>
</tr>
<tr>
<td>▪ Significant indeterminate zone where B12 status cannot be confirmed</td>
</tr>
<tr>
<td>▪ Serious problem with IF-antibody interference</td>
</tr>
<tr>
<td>2. Methylmalonic Acid (MMA)</td>
</tr>
<tr>
<td>▪ B12 deficiency causes elevation</td>
</tr>
<tr>
<td>▪ Non-specific – also elevated in old age, with renal disease and with gut bacteria overgrowth</td>
</tr>
<tr>
<td>3. Homocysteine</td>
</tr>
<tr>
<td>▪ B12 deficiency causes elevation</td>
</tr>
<tr>
<td>▪ Non-specific – also elevated in old age, with renal disease and with folate deficiency</td>
</tr>
<tr>
<td>4. Active B12 (Holotranscobalamin or HoloTC)</td>
</tr>
<tr>
<td>▪ Transcobalmin transports Vitamin B12 from site of absorption (ileum) to tissues and cells</td>
</tr>
<tr>
<td>▪ Vitamin is internalized as Active B12 complex</td>
</tr>
</tbody>
</table>

[https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142735/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142735/)
Schilling Test

Schilling, Robert Frederick (born 1919), American hematologist. Schilling undertook research on the absorption and utilization of vitamin $B_{12}$, the mechanisms involved in the causation of anemia, and on the gastrointestinal absorption of nutrients. He introduced the Schilling test in 1953.

The Schilling Test

- Measures $B_{12}$ deficiency
- Detects IF deficiency
- Detects abnormal results in patients with genetic defects in $B_{12}$ absorption, bacterial overgrowth of the small bowel, resection/bypass of terminal ileum, and pancreatic insufficiency

Key Points of Vitamin B12 Diagnosis

1. Accurate diagnosis of vitamin B12 status
   • Diffused and varied signs and symptoms.
   • Potentially misleading hematological parameters.
   • Limitations of current front-line Total B12 test and other reflex tests.

2. Early diagnosis of B12 deficiency
   • Needed in order to initiate treatment before disease symptoms manifest and/or become irreversible (neurological).

Can Active-B12 address these needs?

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142735/
Active-B12 vs Vitamin B12

Vitamin B12 in the Circulation

- Active B12 represents 10-30% of Vitamin B12 circulating in the blood.
- Only form of Vitamin B12 taken up and used by the cells of the body.

Active-B12 vs Vitamin B12

• The current front-line test, Total B12, measures all of the Vitamin B12 in the blood, but not all circulating B12 can be used by the body.

http://www.axis-shield.com/active-b12/
Typical Stages in the Development of a Vitamin B$_{12}$ Deficiency

<table>
<thead>
<tr>
<th>Stage</th>
<th>Manifestation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Circulating serum B$_{12}$ levels depleted.</td>
<td>Patients are typically asymptomatic and can remain in this stage for several years.</td>
</tr>
<tr>
<td>II</td>
<td>Cellular stores of B$_{12}$ are depleted.</td>
<td>Patients can remain asymptomatic. This stage can also continue for several years.</td>
</tr>
<tr>
<td>III</td>
<td>Evidence of bio-chemical deficiency via increases in serum homocysteine and methylmalonic acid.</td>
<td>Vitamin B$_{12}$ is required for the conversion of these compounds.</td>
</tr>
<tr>
<td>IV</td>
<td>Clinical signs and symptoms apparent.</td>
<td>The spectrum of clinical manifestations is broad and the sequence of symptom development varies markedly.</td>
</tr>
</tbody>
</table>

Laboratory Test Results for the Progression of B-12 Deficiency

Adapted from Herbert V, Nutrition Science as a continually unfolding story: the folate and vitamin B12 paradigm
Correlation of Total B12 with HoloTC

- n=468 patients
- 67% of results are immediately indeterminate by Total B12
- More importantly, 180 patients (38%) have Total B12 levels >150pmol/L but are deficient by HoloTC.

Raw data provided by Prof. W Herrmann, Universitätskliniken des Saarlandes, Homburg, Germany
New Definition of Vitamin B12 Deficiency

- Use of MMA as the gold standard is flawed due to poor specificity.

- Innovative study in 2011 used Red Blood Cell B12 as a measure of the true B12 status in the tissues and allowed a direct comparison of the power of Active-B12, Total B12 and MMA to detect deficiency.
Performance of Markers vs RBC-B12

- Using this novel approach, Active-B12 has higher AUC and diagnostic performance than Total B12 and MMA.

### AUC
- No discrimination: 0.5
- Active-B12: 0.90
- Serum B12: 0.80
- MMA: 0.78

Valente et al. Clinical Chemistry 2001 57:6
Performance of Markers in Grey-Zone

- Potential clinical use was assessed by use of a gray-zone with limits of 60% for ruling-in deficiency (PPV) and 98% for ruling-out deficiency (NPV).

<table>
<thead>
<tr>
<th></th>
<th>MMA</th>
<th>HoloTC</th>
<th>Total B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey-zone</td>
<td>0.31 to 1.40 μmol/L</td>
<td>19.6 to 29.9 pmol/L</td>
<td>79 to 238 pmol/L</td>
</tr>
<tr>
<td>Samples in grey-zone (n)</td>
<td>349/700</td>
<td>96/699</td>
<td>313/700</td>
</tr>
<tr>
<td>Samples in grey-zone (%)</td>
<td>50%</td>
<td>14%</td>
<td>45%</td>
</tr>
</tbody>
</table>

- A high proportion of samples in the gray-zone restricts clinical utility.

Valente et al Clinical Chemistry 2001 57:6
Total B12: Interference from anti-IF Antibodies

- Total B12 test can give False Negatives due to assay interference, as seen in the following articles:
  - Failures of Cobalamin assays in Pernicious Anemia Carmel and Agrawal, NEJM 2012 367;4
  - Spurious Elevations of Vitamin B12 with Pernicious Anemia Yang and Cook, NEJM 2012 366;18
  - Falsely elevated cobalamin concentration in multiple assays in a patient with pernicious anemia: A case study van Rossum et al CCLM 2013 14:1-3
Can Active-B12 Replace Total B12?

If Active-B12 replaces Total B12 as the front line test, the following criteria should be met:

- **Must be a better marker of Vitamin B12 status.**
  - HoloTC measures only the biologically-available form of B12.
  - HoloTC appears to be a better measure of B12 status then Total B12 when using RBC-B12 as the true definition.

- **Must offer improved sensitivity and specificity.**
  - HoloTC appears to be more sensitive and more specific than Total B12.

- **Should have no interference from anti-IF antibodies.**
  - No interference.

- **Must be an early marker of deficiency.**
  - Changes in HoloTC levels occur earlier than Total B12 in Vitamin B12 depletion.
Active-B12 in Practice: Resolution of Grey-zone Total B12 Results

- Many users have started using the test as a reflex to Total B12 but as experience with the marker grows, they have stopped Total B12 testing and exclusively use Active-B12.

Adapted from Schneede J., Scan J Clin Lab Invest 2003; 63: 369 – 376

Note that all suggested cut-offs will be dependent on the population served by the laboratory
Active-B12 in Practice: Replacement for Total B12 Assay

Labs in Australia, Germany, Netherlands and the UK have adopted this approach and no longer use Total B12.

Subjects at risk of B12 deficiency

- HoloTC <35 pmol/L
  - Likely deficient

- HoloTC >35 pmol/L
  - Unlikely deficient*
  *Renal patients should be further investigated with creatinine and/or MMA

• Labs in Australia, Germany, Netherlands and the UK have adopted this approach and no longer use Total B12.

Note that all suggested cut-offs will be dependent on the population served by the laboratory.
Treatment of Vitamin B$_{12}$ Deficiency

- Replacement therapy

- Parenteral treatment given weekly intramuscularly for 8 weeks, followed by intramuscularly every month for the rest of the patient's life

- Daily oral replacement therapy

# Folate Deficiency

<table>
<thead>
<tr>
<th>Manifestations of Folate Deficiency</th>
<th>Stages of Folate Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>More often malnourished than those with cobalamin deficiency</td>
<td>Negative folate balance (decreased serum folate)</td>
</tr>
<tr>
<td>Gastrointestinal manifestations</td>
<td>Decreased RBC folate levels and hypersegmented neutrophils</td>
</tr>
<tr>
<td>More widespread and more severe than those of pernicious anemia</td>
<td>Macroovalocytes, increased MCV, and decreased hemoglobin</td>
</tr>
<tr>
<td>Diarrhea is often present</td>
<td></td>
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<tr>
<td>Cheilosis (a painful inflammation and cracking of the corners of the mouth)</td>
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<tr>
<td>Glossitis</td>
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<tr>
<td>Neurologic abnormalities do not occur</td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis of Folate Deficiency

- Peripheral blood and bone marrow biopsy look exactly like B₁₂ deficiency
- Plasma folate < 3 ng/ml – fluctuates with recent dietary intake
- RBC folate – more reliable of tissue stores < 140 ng/ml
- Only increased serum homocysteine levels but NOT serum methylmalonic acid levels

**Dietary Factors**
- Low iron, heme iron
- Excess phytate
- Excess tea or coffee
- Fad diets

**Demographic Factors**
- Elderly
- Teenager
- Female
- Immigrant
- Aborigine
- Widower

**Social, Physical Factors**
- Poverty
- Poor detention
- Alcohol abuse
- Candle burning
- GIT disease
- Depression

http://www.health-writings.com/folate-deficiency-anemia-caused/
Treatment of Folate Deficiency

Oral Replacement Therapy

Folate Prophylaxis

• Women planning pregnancy are advised to take 400 g folic acid daily before conception and until 12 weeks of pregnancy to prevent neural-tube defects (5 mg/day for women with a previous affected pregnancy).

• Folate fortification of cereal grains at 1 - 4 mg/kg has been made mandatory in the USA as an additional method of improving the folate status of the population.

• Prophylactic folate is also recommended in other states of increased demand such as long-term hemodialysis and chronic hemolytic disorders.

Inappropriate Treatment of Pernicious Anemia With Folate

Vitamin B$_{12}$ deficiency anemia can be temporarily corrected by folate supplementation.

However, this does not correct the neurologic deficits.

- Folate “draws” Vitamin B$_{12}$ away from neurologic system for RBC production and can exacerbate combined systems degeneration.

Take Home Message

• Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children.

• Anemia goes undetected in many people, and symptoms can be minor or vague.

• Most commonly, people with anemia report non-specific symptoms of a feeling of weakness, or fatigue, general malaise and sometimes poor concentration.

• Anemia is a disease with multiple causes, both nutritional (Iron, Vitamin B$_{12}$, Folate deficiencies are most common) and non-nutritional (infection) that frequently co-occur.

• Anemia is a laboratory diagnosis.

Take Home Message

• Vitamin B12 deficiency is common in General Practice populations
• Symptoms may be ill-defined and high index of suspicion is necessary
• Vitamin B-12 exists as an active and inactive form in the blood
• All standard B-12 assays measure active and inactive forms and are thus prone to false positives & negatives. Between 15-40% of patients with low B-12 results do not have B-12 deficiency
• A new "Active B-12" assay measures only active form in measurable in pmole/L and is thus an improvement for detecting early depletion and deficiency

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142735/
Questions?
References


References

22. MMWR, April 03, 1998 / 47(RR-3);1-36.
References

References


