Anemia ... a challenging diagnostic workout

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13-03-2012

Definition of anemia

a reduction in one or more of the major RBC measurements

- hemoglobin concentration
- hematocrit
- RBC count

WHO's hemoglobin thresholds used to define anemia

<table>
<thead>
<tr>
<th>Age or gender group</th>
<th>Hb threshold (g/dL)</th>
<th>Hb threshold (mmol/L) (5 g/dL = 0.6206 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (0.5–5.0 yrs)</td>
<td>11.0</td>
<td>6.8</td>
</tr>
<tr>
<td>Children (5–12 yrs)</td>
<td>11.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Teens (12–15 yrs)</td>
<td>12.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Women, non-pregnant (&gt;15yrs)</td>
<td>12.0</td>
<td>7.4</td>
</tr>
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<td>Women, pregnant</td>
<td>11.0</td>
<td>6.8</td>
</tr>
<tr>
<td>Men (&gt;15yrs)</td>
<td>13.0</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Ethnic differences in Hb level

Distribution of Hb in blacks and whites

Age-specific normative red blood cell values

<table>
<thead>
<tr>
<th>Age at Measurement</th>
<th>Mean Hb (g/dL)</th>
<th>Mean hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>13.6</td>
<td>47.6</td>
</tr>
<tr>
<td>6 months-1 year</td>
<td>13.4</td>
<td>47.3</td>
</tr>
<tr>
<td>1-5 years</td>
<td>13.2</td>
<td>46.9</td>
</tr>
<tr>
<td>5-10 years</td>
<td>13.0</td>
<td>46.6</td>
</tr>
<tr>
<td>10-18 years</td>
<td>12.9</td>
<td>46.4</td>
</tr>
<tr>
<td>19+ years</td>
<td>12.7</td>
<td>46.2</td>
</tr>
</tbody>
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Causes of anemia

- **Kinetic approach**
  - decreased RBC production
  - increased RBC destruction
  - blood loss

- **Morphologic approach** (RBC size)
  - macrocytic anemia
  - microcytic anemia
  - normocytic anemia

Red blood cell indices

- **‘old’ parameters**
  - RBC
  - Hb
  - Hct
  - MCV
  - MCH
  - MCHC
  - RDW

- **‘new’ parameters**
  - Reticulocytes (%)
  - Reticulocytes (10^9/L)
  - Immature reticulocytes fraction
  - Reticulocyte hemoglobin content
  - Reticulocyte volume

ANEMIA

- macrocytic anemia (MCV >100)
- normocytic anemia (MCV 80-100)
- microcytic anemia (MCV <80)

Retic count

- high reticulocyte count
- normal reticulocyte count

Reticulocyte count

- increased
- not increased

BM smear/biopsy

- normal
- abnormal

Hypo-/aplastic anemia

- BM infiltration
- dyserythropoietic anemia

Endocrine function?

- Addison’s
- hypothyroidism
- eunuchoidism
- panhypopituitarism

Retic count

- increased
- not increased

BM smear/biopsy

- normal
- abnormal

Reticulocyte count

- increased
- not increased

Megaloblastic changes

- yes
- no

Megaloblastic changes in BM?

- yes
- no
Regulation of iron balance

- Body iron content: 3-4 g
  - Most body iron (65-70%) is present in hemoglobin in circulating red cells
  - Storage (ferritin and hemosiderin)
  - Transferrin transports iron into the cells

- Iron homeostasis is strictly regulated:
  - Daily intake: absorption via duodenum (10% of daily iron is absorbed : 1-3 mg daily) Fe^{2+} better absorbed than Fe^{3+}
  - Daily small loss of iron via urine, faeces, skin and nails and in menstruating females as blood (1-2 mg daily)

Iron homeostasis

Iron deficiency stages

**Prelatent:** reduction in iron stores **W**ITHOUT reduced serum iron levels

**Latent:** iron stores are exhausted, but Hb-LEVEl remains **N**ORMAL

**Iron deficiency anemia:** Hb-CONCENTRATION FALLS below lower limit of normal

<table>
<thead>
<tr>
<th></th>
<th>PRELATENT</th>
<th>LATENT</th>
<th>IDA</th>
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</thead>
<tbody>
<tr>
<td>Hb</td>
<td>nl</td>
<td>nl</td>
<td>↓</td>
</tr>
<tr>
<td>MCV</td>
<td>nl</td>
<td>nl (RDW ↑)</td>
<td>↓</td>
</tr>
<tr>
<td>ferritin</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>marrow iron</td>
<td>↓</td>
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**chronic bleeding**
- menorrhagia
- peptic ulcer
- stomach/intestinal cancer
- ulcerative colitis
- **increased intake**
- **juvenile age**
- pregnancy
- lactation

**Iron deficiency anemia**
- Reduced iron availability
  - IDA
    - Reduced dietary iron
    - Increased requirements
    - Blood loss
    - Iron sequestration
  - ACD
- Reduced heme synthesis
  - Sideroblastic anemia
  - Lead poisoning
- Reduced globin production
  - α-thalassemia
  - β-thalassemia

**Iron deficiency anemia of chronic disease**

**Iron deficiency stages**

- **Prelatent:** reduction in iron stores **W**ITHOUT reduced serum iron levels
- **Latent:** iron stores are exhausted, but Hb-LEVEl remains **N**ORMAL
- **Iron deficiency anemia:** Hb-CONCENTRATION FALLS below lower limit of normal
Reticulocyte

- immature red blood cell
- about 0.5-1.5% of red cells in human body (non-anemic)
- develop and mature in bone marrow
- circulate for about 1 day in blood stream before developing into mature red blood cell

Reticulocyte counting

- Microscopic counting (supravital staining of cytoplasmic ribosomal RNA)
  - rather imprecise
  - different procedures
  - staining variations
  - distributional variability of quality blood film
  - inter- and intra-observer variations
- Automated counting (available in the mid-1990's)
  - increasing measurement precision by analyzing a much greater number of cells
  - Elimination of variability of pre-analytic staining, dilution and incubation, variability due to subjective elements
  - also measurement of additional parameters (such as the mean reticulocyte volume (MRV) and reticulocyte hemoglobin content (CHr, Ret He),...)

BLOOD CONCENTRATION OF RETICULOCYTES REPRESENTS A QUANTITATIVE MEASURE OF ERYTHROPOIESIS
RETICULOCYTE PARAMETERS PROVIDE REAL-TIME INFORMATION ABOUT THE QUALITY OF THE ERYTHROPOIESIS

Retic counting on Sysmex XE-5000

- dye (polymethine)
- penetration of the cell membrane
- staining of RNA (retics) and DNA/RNA (nucleated cells)
- measurement of forward scatter and side fluorescence
- separation of retics from mature RBC and nucleated cells
- retics divided in 3 ranges: LFR, MFR, HFR
- NOTE: PLT-O also determined
- no interference from NRBC, WBC parasites Howell-Jolly bodies

To be useful reticulocyte count must be adjusted for patient’s hematocrit. When hematocrit is low(er), reticulocytes are released earlier from the marrow so one can adjust for this phenomenon.

⇒ Reticulocyte correction

- Correction for the degree of anemia
  CORRECTED RETICULOCYTE COUNT

- Correction both for Hct and maturation time
  RETICULOCYTE PRODUCTION INDEX (RPI)
  Correction for the longer life span of prematurely released reticulocytes in the blood (a phenomenon of increased RBC production).

⇒ Absolute reticulocyte count = reticulocyte count × # RBC
Calculations

1. **CORRECTED RETICULOCYTE COUNT**
   \[ \text{patients reticulocyte count (%)} \times \left( \frac{\text{patients Hct}}{\text{normal Hct}} = 45 \right) \]

2. **RETICULOCYTE PRODUCTION INDEX (RPI)**
   \[ \text{RPI: corrected reticulocyte count / maturation time} \]

### Example:
- Patient has reticulocyte count of 5%, Hb of 7.5 g/dL, and Hct of 25%
- \[ \text{RPI} = \left( 5 \times \frac{25}{45} \right) / 2 = 1.38 \]

<table>
<thead>
<tr>
<th>Hct (%)</th>
<th>Retic survival (days)</th>
<th>Maturation correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-45</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>26-35</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>16-25</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

### Reticulocyte Production Index

- Normal RPI = 1 (for non-anemic patients)
- RPI ≥2 suggests an effective erythropoiesis response
- RPI <2 suggests an ineffective response

### Reticulocyte maturation parameters, such as **immature reticulocyte fraction (IRF)**, provides the same clinical significance as the RPI

### Immature reticulocyte fraction (IRF)
- Replaces need for "corrected" reticulocyte count
- Originally called 'reticulocyte maturity index' (RMI)
- To indicate the less mature reticulocyte fraction
- Some divide the reticulocytes into 3 distinct populations (eg. Sysmex XE), others into only 2 based on RNA content
  - Sysmex: \( \text{IRF} = \text{HFR} + \text{MFR} \) (ref. range: 5-21%)
- Reflects rate of erythropoietic activity; early + sensitive index for erythropoiesis

### Most commonly used automated reticulocyte analyzers + parameters

<table>
<thead>
<tr>
<th>Company</th>
<th>Instrument</th>
<th>Method</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>CELL DYN Sapphire</td>
<td>Fluorescence detection</td>
<td>Cyanine dye</td>
</tr>
<tr>
<td>Beckman</td>
<td>Coulter UniCel DxC 8000</td>
<td>Light scattering, impedance and conductivity (VCS technology)</td>
<td>New methylene blue</td>
</tr>
<tr>
<td>Siemens</td>
<td>ADVIA 2120</td>
<td>Absorbance and optical light scatter detection</td>
<td>Oxazine 750</td>
</tr>
<tr>
<td>Sysmex</td>
<td>XE5000, XT4000i</td>
<td>Fluorescence and light scattering</td>
<td>Polymethine X</td>
</tr>
<tr>
<td>Sysmex</td>
<td>XE2100, XT2000i</td>
<td>Fluorescence and light scattering</td>
<td>Auramine O</td>
</tr>
</tbody>
</table>

### Changes in RBC parameters (Hb, Retic, IRF) with stimulated erythropoiesis

- Hemoglobin
- Retic Count
- IRF

![Graph showing changes in RBC parameters](https://via.placeholder.com/150)
Clinical applications of IRF

- early identification of BM regeneration after chemo (prior to increase # neutrophils and total reticulocyte count)
- early identification of hematopoietic engraftment (Tx)
- monitor EPO therapy (eg. renal failure)
- monitoring renal engraftment (EPO production)

Patterns of IRF and retic counts in anemia

<table>
<thead>
<tr>
<th>Clinical conditions</th>
<th>Reticulocyte count</th>
<th>IRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplastic anemia</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>Hypoplastic anemia</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>BM regeneration</td>
<td>low / WNL</td>
<td>high / WNL</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>low / WNL</td>
<td>WNL / high</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>low / WNL</td>
<td>high</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>WNL / high</td>
<td>WNL / high</td>
</tr>
<tr>
<td>Folate/B12 deficiency</td>
<td>low / WNL</td>
<td>high</td>
</tr>
<tr>
<td>MDS</td>
<td>any level</td>
<td>WNL / high</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Blood loss</td>
<td>WNL / high</td>
<td>high</td>
</tr>
</tbody>
</table>

Clinical utility of Ret-He/RET-Y/CHR

- Diagnosis of iron deficiency (absolute and functional)
  - Absolute iron deficiency: depletion of iron stores and absence of stainable iron in bone marrow
  - Functional iron deficiency: stored iron is sufficient
- Monitoring response on IV or oral iron substitution
  - Hb (>1 m)
  - Reticulocyte (5-10 d)
  - Ret-He (2-3 d)

Reticulocyte hemoglobin

- Provides hemoglobin content information on immature RBCs (retics)
- Hb and MCH
- 0-120 days (including all RBC)
- Ret-He (Sysmex), cfr. CHr < ADVIA Siemens
- An estimate of the recent functional availability of iron into the erythron

Clinical utility of Ret-He/RET-Y/CHR

- Evaluation of iron status in dialysis patients (planning of adequate r-HuEPO R/ and iron substitution)
- Iron deficiency in childhood (before anemia)

Pitfalls
- hemoglobinopathy: Ret-He ↓↓↓, normal Fe-parameters
- megaloblastic anemia: Ret-He normal, despite comonittant Fe-deficiency
Clinical utility of RET-He in the diagnosis of anemia

Case 1

- Man 69
  - Clinical info: oedemen, dyspepsie, melkene, epigastrische pijn
  - VIG: cardiovasculaire problematiek (ben pop bypass, angior pectoris, art. hypertensie), sigmoïddiverticulose

LABO

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>16.0 - 18.0</th>
<th>16.0 - 18.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocriet</td>
<td>0.43 - 0.46</td>
<td>0.43 - 0.46</td>
</tr>
<tr>
<td>MCV µ/L</td>
<td>80 - 100</td>
<td>80 - 100</td>
</tr>
<tr>
<td>MCH pg</td>
<td>27.0 - 32.0</td>
<td>27.0 - 32.0</td>
</tr>
<tr>
<td>RDW %</td>
<td>13.7 - 14.5</td>
<td>13.7 - 14.5</td>
</tr>
<tr>
<td>MCHC g/µL</td>
<td>31.6 - 33.5</td>
<td>31.6 - 33.5</td>
</tr>
</tbody>
</table>

Case 2

- 58-year female
  - extreme alcohol abuse, duodenal ulcer, liver cirrhosis

CBC:

- Hb 3.7 g/dL ➔ severe macrocytic anemia
- MCV 141 fl ➔ ineffective erythropoiesis
- reticulocyte count: 10.2 x 10⁹/µL ➔ ineffective erythropoiesis

Blood smear:

- macrocytes ➔ ineffective erythropoiesis
Case 2

serum parameters:
- increased LDH (1483 U/L)
- decreased vitamin B12 (104 pg/mL)
- decreased folic acid (2.57 ng/mL)
- CRP normal
- low normal ferritin (58 ng/mL)

bone marrow

[Images of different megaloblasts and vacuolisation in a proerythroblast]

megaloblastic anaemia
due to nutritional deficiency, malabsorption of vitamin B12 and folic acid

Case 2

Treatment:

1) transfusion of several units of PC
- Hb 7.0 g/dL
- RBC histogram: 2 distinct RBC populations
  - transfused RBC with a normal MCV (83.6 fL; RBC 1.14 x106/µL)
  - residual macrocytic cells (149.3 fL; RBC 0.69 x106/µL)

2) Cobalamin (vitamin B12) IV
- cumulative Hb (day 1: Hb 3.7 => day 18 : Hb 8.5)
- increase of reticulocytes (225.900/µL)
- increase of IRF (27%)
- RPI = 2.4
- normalisation platelets and leucocytes

Laboratory evaluation

- initial testing
  - CBC with differential, including RBC indices
  - reticulocyte count
  - peripheral blood smear
  - serial Hct or Hb
- iron deficiency / nutritional deficiency
  - iron studies / folate, vitamin B12
- hemolysis
  - serum LDH, indirect bilirubin, haptoglobin, coombs
- bone marrow examination
- others-directed by clinical indication
  - hemoglobin electrophoresis