Reticulocyte Hemoglobin (RET-He/CHr) as a Biomarker of Erythropoiesis and Anemia Assessment

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Presentation Objectives

- Describe biochemical and hematological markers used in the diagnosis of iron deficiency.
- Discuss applications of Reticulocyte Hemoglobin in (RET-He/CHr) identifying iron deficiency.
- Discuss applications of Reticulocyte Hemoglobin (RET-He/CHr) in assessing red cell production in response to erythropoietin (EPO).
- Recognize unique aspects of blood cell analysis in the context of improved clinical diagnosis and optimal patient care.
John T. Mather Memorial Hospital

Our Mission is to be the best community hospital in New York State

U.S. News & World Report's Best Hospital Rankings

Magnet Status

- 248 Bed Community Hospital established in 1929
- Located on Long Island, 60 miles from NYC
- Continually changing to meet the needs of the community

Anemia Background

- Is a sign of a wide range of underlying disorders
- Anemia is often under recognized and undertreated
- Associated with morbidity and increased risk of mortality
- Contributes to over utilization of blood transfusions
Anemia Prevalence

- 3.4 million people in United States
- 2 billion people globally (1/3 of population)
- Type of Anemias:
  - Iron deficiency
  - Anemia of Chronic Disease
  - Vitamin Deficiency Anemia
  - Aplastic Anemia
  - Hemolytic Anemia
  - Sickle Cell Anemia … and many others

Patients at Highest Risk of Anemia
Almost all people with cancer develop mild anemia.

Causes of Anemia
- Abnormal iron metabolism
- Cancer
- Cancer treatment
- Blood loss
- Deficiency in certain vitamins or minerals
- Major organ problems (severe heart, lung, kidney, or liver disease)

Functional Iron Deficiency (FID)
- Due to the retention of iron in macrophages
- The major cause of suboptimal responses to ESA therapy (rh-EPO)
- Traditional indirect Iron tests are uninformative and can be normal in the presence of functional iron deficiency
Iron Distribution in the Body

- Iron is an essential trace element
- Iron ions circulate bound to plasma transferrin
- Iron is stored in hepatocytes and reticuloendothelial macrophages
- Use of blood tests as a proxy for stored iron content

Iron Distribution in the Body

Laboratory Anemia Work-up
Diagnosis of Iron Deficiency

Biochemical parameters
- Serum iron
- Ferritin
- Transferrin, transferrin saturation (TSAT)
- Circulating transferrin receptor

Hematological parameters
- Hb, MCV, RDW
- Erythrocyte zinc protoporphyrin
- Reticulocyte Hb content (CHr / RET-H\(\bar{e}\))
Conditions Affecting Serum Iron, Transferrin, and Ferritin

<table>
<thead>
<tr>
<th>Test</th>
<th>Elevated Test Results</th>
<th>Decreased Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron</td>
<td>Sample late in the day&lt;br&gt;Meal iron intake&lt;br&gt;Supplement iron intake&lt;br&gt;Hemolysis</td>
<td>Infection&lt;br&gt;Inflammation</td>
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<tr>
<td>Serum Transferrin</td>
<td>Oral Contraceptives</td>
<td>Inflammation/Infection</td>
</tr>
<tr>
<td>Serum Ferritin</td>
<td>Malignancy&lt;br&gt;Inflammation/infection&lt;br&gt;Hyperthyroidism, Aging&lt;br&gt;Liver disease (HCV)&lt;br&gt;Alcohol consumption&lt;br&gt;Oral contraceptives</td>
<td>Vitamin C deficiency&lt;br&gt;Hypothyroidism&lt;br&gt;Exercise</td>
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Laboratory Anemia Work-up Hematology Parameters

Hematological Parameters

- Based on entire RBC population
  - Hb, MCV, RDW
  - Microcytic/Hypochromic cells
  - Erythrocyte zinc protoporphyrin
- Based on reticulocyte population
  - Reticulocyte Hemoglobin (CHr or RET-He)
What is Reticulocyte Hemoglobin (RET-He / CHr)?

• The measurement of Reticulocyte Hemoglobin (RET-He /CHr) is a direct assessment of the incorporation of iron into erythrocyte hemoglobin and thus a direct estimate of the recent functional availability of iron into the red cell.

• RET-He /CHr are not affected by inflammation/infection, malignancy, or anemia of chronic disease and, thus is a preferable biomarker of iron status.

Clinical Utility of RET-He / CHr

• To monitor erythropoiesis following chemotherapy

• To monitor the response of ESRD patients Erythropoietin therapy

Screening Test for Iron Deficiency Anemia
Clinical Utility of RET-He / CHr

• RET-He > 28 pg the patient is iron replete. Iron is available for incorporation into the red cell

• RET-He < 28 pg patient may not have enough iron available to produce healthy RBC’s

• Reference Range 28.2 –36.6 pg

Iron Deficiency in Infants and Toddlers

• Adverse consequences of iron deficiency in pediatrics:
  ▪ Increased lead absorption
  ▪ Impaired immunity
  ▪ Anemia
  ▪ Impaired neuro-cognitive development

• 2.1% of infants and toddlers in the US have iron deficiency anemia; 10% have iron deficiency without anemia
Study Objective and Design

- Develop an effective approach to diagnose iron deficiency in children.
- Comparison of hematological and biochemical parameters in 210 children (mean age 2.9 + 2 years).
- Iron deficiency defined as TSAT < 20%.
- Anemia defined as Hb < 11 g/dL.

CHr and Probability of Iron Deficiency

- Each pg increase in CHr lowers the risk of ID by 30%.
- The estimated probability of iron deficiency is 90% when CHr < 20 pg.
- Children with CHr > 29 pg have virtually zero probability of being iron deficient.
Iron deficiency and CHr: ROC Curve

For Iron Deficiency
- CHr cutoff of 26 pg
  - 70% sensitivity
  - 78% specificity

For Iron Deficiency Anemia
- CHr cutoff of 26 pg
  - 83% sensitivity
  - 75% specificity


Diagnosis of Iron Deficiency in Young Children

Article Summary
CHr is a strong predictor of iron deficiency and iron deficiency anemia in children.

- CHr is always significantly lower in the iron deficient group, regardless of how ID defined.
- Promising sensitivity and specificity in diagnosing iron deficiency.
- Optimal CHr cutoff level for predicting iron deficiency 24-26 pg.

A test panel based exclusively on hematological parameters (CBC and reticulocyte parameters) provides a cost-effective alternative to the traditional biochemical panels for the diagnosis of iron deficiency of childhood.

Iron Deficiency Anemia in ESRD Patients

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

- Largely the result of insufficient production of Erythropoietin by non-functioning kidney.

- Especially true in hemodialysis, due to the coupling of accelerated erythropoiesis (rhu-EPO) and dialysis-related blood losses, including frequent laboratory testing.

Finding Balance in Anemia Management Decisions: Iron or EPO?

**Challenges**

- How do you balance dosage and timing of iron therapy?
- What is the best assessment to balance ESA and Iron therapy?
- What is the best assessment of iron stores?
- Shouldn’t we measure changes on the cellular level?
“Hb, Hct, and RET-He are useful analytes to guide dose adjustment for EPO or IV iron.”

139 hemodialysis patients from 3 dialysis centers

Patients were randomized into 2 groups:

**Group 1**: Iron management based on serum ferritin (SF) and TSAT.
Patients were dosed with IV iron if SF<100 ng/ml or TSAT <20%

**Group 2**: Iron management based on CHr, with dosing based on a CHr < 29 pg.

**Study Outcome:**

Iron utilization:
- 83.6% of Patients in Group 1 received treatment with i.v. iron
- 43.2% of Patients in Group 2 received treatment with i.v. iron

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### Results: Positive Predictive Value

<table>
<thead>
<tr>
<th>PPV (%)</th>
<th>Tsat</th>
<th>SF</th>
<th>CHr</th>
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<tbody>
<tr>
<td>0%</td>
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The presence of inflammation and uremia makes this diagnosis particularly challenging for dialysis patients.

By directly measuring the RET-He, early stages of iron deficiency may be identified, at a time that other traditional biochemical parameters are non-informative.
**RET-He Distribution in Various Patient Populations**

From: Canals C, et al., Haematologica 2005; 90: 1133-1134, Barcelona, Spain

N=504

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**Patient Evaluation by RET-He: Impact on Iron Studies**

Anemia
n=93

Anemia & RET-He < 32 pg
n=43

- 54%

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RET-Hₑ Diagnostic Performance in the Evaluation of Anemia in MSKCC Cancer Patients

Patient Screening using RET-Hₑ
n=209

- RET-Hₑ <32 pg and Hgb <11 g/dl
  n=43
  NPV- 97.84

- Rapid rule out of iron deficiency anemia
- Reduce unnecessary testing
- Cost Savings for Laboratory and Health Care System

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Implementation of RET-Hₑ

Define Anemia Panel
- CBC + RETIC including RET-Hₑ

Clinician Education
- How to interpret Anemia panel results
- Report RET-Hₑ with a clinical pathology interpretation

Allows for Clinical Consultation Opportunities
Health Care Cost Savings

Jan – Jun 2013: Performed 7701 Iron Studies
Potential Unnecessary Annual Testing based on combination of Anemia & RET-He
- 7701 ordered tests x .80 = 6165 unnecessary tests
  - Medicare reimbursement ~$66.00
  - Cost savings 6165 x $ 66 = $406,929

Reticulocyte Hemoglobin (RET-He / CHr)
Summary

- Measured at cellular level
- Monitor acute changes in hemoglobin incorporation into the red cell
  - Real-time estimate of iron availability in bone marrow
- Shown as a more sensitive tool for early detection of iron deficiency
  - Changes rapidly, more sensitive screen than Hb
  - Less variation than acute phase reactants
- Provides additional information for managing iron requirements for rHuEPO therapy
- Limitation in specificity addressed by interpreting RET-He results in conjunction with other tests and clinical picture
THANK YOU

References on Clinical Use of Reticulocyte Hemoglobin

- Baker, R., Greer, F. and The Committee on Nutrition. Pediatrics 2010;126;1040-1050. Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in and Infants and Young Children (0 - 3 Years of Age).