The Value-Driven Laboratory

Reticulocyte Hemoglobin Content (RET-He): A Parameter with Well-Established Clinical Value

Sysmex America
White Paper
The Complete Blood Count (CBC) with reticulocytes, one of the most frequently ordered lab tests, may be underutilized by physicians for diagnostic purposes. In the United States, 3.4 million people have anemia and 2 billion people are affected globally. Many of these people have iron deficiency and/or iron deficiency anemia (ID/IDA). New technologies are providing a more in-depth evaluation of immature cell forms, expanding the number of reportable parameters included in reticulocyte results. This paper suggests ways in which the use of comprehensive reticulocyte parameters, and especially RET-He, can help provide physicians with new information. This information may assist them with the earlier detection, differential diagnosis, and management of ID/IDA across the continuum of wellness, prevention and chronic disease.

Reticulocyte Hemoglobin Content (RET-He): A Parameter with Well-Established Clinical Value

The Complete Blood Count (CBC) with reticulocytes, one of the most frequently ordered lab tests, may be underutilized by physicians for diagnostic purposes. In the United States, 3.4 million people have anemia and 2 billion people are affected globally. Many of these people have iron deficiency and/or iron deficiency anemia (ID/IDA). New technologies are providing a more in-depth evaluation of immature cell forms, expanding the number of reportable parameters included in reticulocyte results. This paper suggests ways in which the use of comprehensive reticulocyte parameters, and especially RET-He, can help provide physicians with new information. This information may assist them with the earlier detection, differential diagnosis, and management of ID/IDA across the continuum of wellness, prevention and chronic disease.

A Quick Overview of Reticulocyte Parameters

1. **Reticulocyte counts** are the quantity (# and %) of the youngest erythrocytes normally released from the bone marrow into circulating blood.

2. **Immature Reticulocyte Fraction (IRF)** is the rate of production of reticulocytes which is largely dependent on the bone marrow response to erythropoietin.

3. **Reticulocyte hemoglobin content (RET-He)** measures the amount of hemoglobin in the reticulocytes. The reticulocyte hemoglobin content is also known as CHr. RET-He indicates cell hemoglobinization, reflecting the quality of the newly produced reticulocytes. Ongoing reticulocyte production in the absence of sufficient iron eventually yields microcytic, hypochromic RBCs. Therefore, RET-He is an earlier measure of diminished hemoglobin production compared to hemoglobin and hematocrit.

4. **RET-He** is a direct measurement of hemoglobinization of the developing reticulocyte in contrast to indirect assessment using biochemical assays.

5. **RET-He** is faster, easier, more standardized and less expensive than the assessment of stainable marrow iron.

Reticulocyte hemoglobin content (RET-He /CHr) is now available to most clinical laboratories and can be automatically reported as part of the comprehensive reticulocyte result. In the clinical setting, this parameter can:

1. Be easily implemented by the clinical lab and performed at little, if any, incremental cost.

2. Be used by clinicians in conjunction with other indicators for the early identification and treatment of ID/IDA.

3. Be incorporated, along with other current indicators, by coding departments into the HIS (Hospital Information Systems) that support patient diagnosis code assignment.

4. Become a routine, integral part of quality and cost initiatives in healthcare systems.

RET-He and CHr Equivalency

Two different hematology systems now report reticulocyte hemoglobin content using different nomenclature: RET-He and CHr. At least two studies have been published to show their equivalence. In the study by Brugnara of both pediatric and adult samples, a “very good level of agreement” was found between the RET-He and CHr in pediatric patients (Y = 1.04X-1.06; r^2 = 0.88) and in normal adults (Y = 1.06X-0.43; r^2 = 0.83). The normal ranges of the two methods are “superimposable”, according to this study.
Diagnosis and Assessment of ID/IDA: What’s In and What’s Out?

What’s Out

The time-honored hemoglobin and hematocrit as sole indicators of ID are out. They can provide a valuable snapshot, but they are relatively static parameters. The H+H may change too slowly to be used as the only indicators of the patient’s rate of erythropoiesis or cellular iron status. In a study by Muusze, changes in patient therapy resulted in a significant change in hemoglobin after 2 – 3 weeks whereas Ret-He indicated a response after only 2 days.

The time-honored biochemical tests for changes of iron status as sole indicators are out. In a study published in 2010, Van Wyck et al. looked at the imprecision of iron parameters in 30 dialysis patients for 12 consecutive dialysis days over four weeks—a total of 360 samples. The authors concluded that the low biological variation demonstrated by RET-He, Hb level and HCT (table 1) made them suitable for trend analysis. TSAT and ferritin levels would “have limited value in evaluating changes in iron status within individual hemodialysis patients”.

The empirical approach as a way to efficiently treat ID/IDA is out. Taking a “wait and see” attitude toward evaluating iron or ESA (Erythropoiesis Stimulating Agent) therapy is no longer needed. RET-He can give physicians information on a patient’s response to therapy in days, not weeks.

What’s In?

A comprehensive reticulocyte evaluation: Three parameters can now be reported with every reticulocyte order. They can provide the comprehensive information needed by physicians to assess the rate of red cell production and hemoglobinization. Reticulocyte count (RET #, %) indicates the quantity of circulating reticulocytes. The Immature Reticulocyte Fraction (IRF) indicates the rate of production of reticulocytes and RET-He indicates cell hemoglobinization, the reflecting quality of the newly produced reticulocytes. These three parameters enable “dissociation” of iron-dependent hemoglobinization from erythropoiesis and the tests are rapid and inexpensive to perform.

Causes of ID/IDA

1. Limited dietary intake of iron
2. Increased demand for iron due to growth (e.g. infants and toddlers)
3. Gastrointestinal bleeding
4. Blood loss from menstruation
5. Frequent blood donations
6. Surgical blood loss
7. Malabsorption of iron (e.g. caused by medications or underlying medical conditions)
8. Shortened RBC survival times or mechanical destruction of RBC (e.g. hemodialysis, burns, cardiopulmonary bypass)
9. Chronic disease (e.g. chronic kidney disease, congestive heart failure)
10. Effect of medications (e.g. chemotherapy, anti-retro viral therapy)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb</td>
</tr>
<tr>
<td>Analytical</td>
<td>2.0</td>
</tr>
<tr>
<td>Biological</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 1: Hb, Hct and RET-He show low biological and total variation, making them valuable tests for physician use. From Van Wyck.
Focusing on Wellness, Prevention and Chronic Disease Management

We maintain our wellness through the use of blood tests that can identify the early onset of diseases or disorders at a time when they can be reversed or treated more effectively. The Health Care Cost Institute reports that 78 percent of total healthcare costs in the U.S. are attributable to chronic disease. The inclusion of RET-Hr in the screening of at-risk populations for anemia, should contribute to wellness, disease prevention and long term cost savings in healthcare. In this paper we explore each of these scenarios: wellness, prevention, and chronic disease.

Wellness

RET-Hr: Keeping Well Babies Well

Primary care is becoming more focused on wellness and prevention. Children, especially toddlers between the ages of one and three years, are particularly vulnerable to becoming iron deficient because iron stores may be exhausted during this period of rapid growth. Iron deficiency has adverse effects on long-term neurodevelopment and behavior in children. Therefore, early and accurate identification of ID before the onset of anemia is an important public health issue. In a study by Baker7 of 24,894 individuals above the age of one year, 9% of toddlers aged one to two years were iron deficient; of these, iron deficiency anemia was found in 3% percent. This prevalence corresponds to approximately 700,000 toddlers in the US with iron deficiency; of these, approximately 240,000 toddlers have iron deficiency anemia. Baker concludes that in patients with Hb <11.0 mg/dL or at significant risk of ID or IDA, serum ferritin (SF) and CRP or reticulocyte hemoglobin levels should also be measured to increase the sensitivity and specificity of the diagnosis.

Other studies show that ID/IDA can affect any child, anywhere in the world, and is likely to be underdiagnosed and underreported. A study on the prevalence of ID done in the toddler population in the military health system utilized RET-Hr as a primary screening test1. These children did not have common risk factors such as lack of primary care, and have healthful nutrition. When ID is defined solely by low reticulocyte hemoglobin, the prevalence of ID in the low risk toddler population of this study was 18.8%, which was roughly twice the level of 9% previously reported in national surveys.

In 2009, Shaker et al9 and colleagues compared the cost-benefit profile of reticulocyte hemoglobin content (CHr) with hemoglobin as a component of the CBC for detection and treatment of iron deficiency in 9- to 12-month-old infants. “Under current market conditions, the incremental cost to diagnose and treat iron deficiency (with CHr), compared with diagnosing and treating anemia by Hb, was only $22 per patient screened with $440 per case of anemia prevented. With a 10-year time horizon incorporating risks and costs of neurocognitive delays associated with untreated iron deficiency, the cost of the CHr strategy was $280 per case of anemia prevented.” They concluded that “CHr is an affordable strategy to prevent anemia in infants with possible iron deficiency.”

Additional emerging data show evidence that RET-Hr utility is not limited to children and toddlers but may also be helpful to physicians in monitoring and treating Very Low Birth Weight (VLBW) infants, adolescents, pregnant women and geriatric patients.6,7,8

Prevention

Presurgical Screening

Anemia in the presurgical patient is common. In fact, a study by Goodnough10 showed the prevalence of anemia may be as high as 75% in the elective surgery patient. The authors of a supplemental article for Critical Care11 found that the prevalence of anemia in orthopedic surgery may be as high as 35%, and increases with the age of the patient. These authors also found that veterans undergoing non-cardiac surgery had an anemia prevalence of 34%; colorectal surgery 46%, and radical mastectomy for breast cancer 38% before the first chemotherapy treatment and 59% after surgery and chemotherapy. It is well understood that patients with anemia have poorer outcomes and it stands to reason that appropriate screening and treatment for iron deficiency and iron deficiency anemia in the presurgical patient is warranted.

In 2009, Muusze et al.4 reported on a recovery plan for hip and knee surgery patients with a goal to reduce recovery time by ensuring adequate pre-operative hemoglobin levels. Anemia, defined as Hb <13g/dL prior to elective major orthopedic surgery, can be treated presurgically or postsurgically with erythropoiesis stimulating agents (ESA) and/or IV iron. The outcome of the recovery plan relied, in part, on the identification of non-responders to ESA therapy. In this study two thirds of the patients were “responders” and the other third were “non-responders.” Even for institutions where ESAs are not widely given to orthopedic patients, results from this study may have applicability to other patient groups who have sudden demand for increased erythropoiesis, similar to the demands of blood loss or ESA administration.
The authors were interested in determining how early ESA non-responders could be identified so that an alternate intervention could be considered. They concluded that early detection of non-responders is not possible based solely on hemoglobin measurements as shown in Fig 1A. However, early detection of non-responders is possible using RET-He measurements as shown by the large differences in the hemoglobinization of the two groups in Fig 1B. Another significant finding was the complete reduction in the administration of packed red blood cells (PRBC) as a result of a comprehensive anemia management program.

Additional emerging data show evidence that RET-He utility is not limited to presurgical screening but may also be helpful to physicians in monitoring blood loss replacement postsurgically and with blood donors as well as in the care of women in the third trimester of pregnancy.8,10

**Chronic Disease Management**

Although chronic diseases are more common among older adults, they affect people of all ages and are now recognized as a leading health concern. Chronic diseases that affect the kidney are of special concern in the consideration of ID/IDA.

- Chronic diseases cause 7 in 10 deaths each year in the United States.15
- Chronic kidney disease (CKD), diabetes, and congestive heart failure, affect less than half of Medicare beneficiaries; however, these three related diseases consume well over 70% of current national CMS spending. The prevalence, complications, and costs of treating these disorders are increasing at an alarming rate.

**Iron Therapy Targets**

- Medicare spending on advanced end stage renal disease (ESRD) alone was $22.7 billion in 2006, and is on track to reach $53.6 billion by 2020.

CKD and ESRD, with or without complications from diabetes or heart disease, present with a severe form of ID/IDA. Since the kidney is the sole source of naturally occurring serum erythropoietin, disruption of the ability to produce the hormone will hamper erythroid precursor production. The successful use of RET-He in kidney disease and the incorporation of its use in ESRD is described in the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines13 (Fig 2). These guidelines propose CHr at 29 pg/cell as a treatment threshold with a reference range of approximately 30-35 pg/cell. In addition, reticulocyte hemoglobin is included as part of the CMS clinical performance measures4 (CPM) for assessment of iron stores in the management of ESRD patients on hemodialysis.

**Figure 1:** Early detection of patients who did not respond to therapy was possible using RET He but not Hemoglobin, from Muusze.4

istemocytin

The course of two clinical chemical parameters during preoperative epoetin treatment (injections done 3, 2 and 1 week prior to an orthopaedic operation; Protocol 1A) in 14 patients with preoperative Hb < 8.1 mmol/L. After three epoetin injections, 10 patients had ∆Hb ≥ 0.8 mmol/L (responders) and 4 patients ∆Hb < 0.8 mmol/L (non-responders).

A: Average Hb course. The difference between future responders (green dots) and non-responders (blue squares) is not yet evident after one week.
B: Average Ret-He course. There is a clear difference between responders (green dots) and non-responders (blue squares). The haemoglobinisation level of reticulocytes is an early detector of functional iron deficiency due to epoetin injections.

**Figure 2:** NKF Evaluation of Anemia KDOQI Guidelines (2006)

**Initial Evaluation in CKD**

**Cellular Assessment**
- Hgb < 12 g/dL
- RBC indices (MCH, MCHC)
- Absolute Retic
- WBC & Diff
- Platelet

**Iron Assessment**
- Serum ferritin to assess iron stores
- Serum Tsat or CHr to assess adequacy of iron for erythropoiesis

**Iron Therapy Targets**

- Tsat > 20% or CHr > 29 pg/cell
- AND
- Serum ferritin > 200 ng/ml

**HD-CKD**
- Tsat > 20% or CHr > 29 pg/cell
- AND
- Serum ferritin > 200 ng/ml

**ND-CKD and PD-CKD**
- Tsat > 20%
- AND
- Serum ferritin > 200 ng/ml

---

**Chronic Disease Management**

Although chronic diseases are more common among older adults, they affect people of all ages and are now recognized as a leading health concern. Chronic diseases that affect the kidney are of special concern in the consideration of ID/IDA.

- Chronic diseases cause 7 in 10 deaths each year in the United States.15
- Chronic kidney disease (CKD), diabetes, and congestive heart failure, affect less than half of Medicare beneficiaries; however, these three related diseases consume well over 70% of current national CMS spending. The prevalence, complications, and costs of treating these disorders are increasing at an alarming rate.

The successful use of RET-He in kidney disease and the incorporation of its use in ESRD is described in the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines13 (Fig 2). These guidelines propose CHr at 29 pg/cell as a treatment threshold with a reference range of approximately 30-35 pg/cell. In addition, reticulocyte hemoglobin is included as part of the CMS clinical performance measures4 (CPM) for assessment of iron stores in the management of ESRD patients on hemodialysis.

**Figure 1:** Effect preoperative epoetin

The course of two clinical chemical parameters during preoperative epoetin treatment (injections done 3, 2 and 1 week prior to an orthopaedic operation; Protocol 1A) in 14 patients with preoperative Hb < 8.1 mmol/L. After three epoetin injections, 10 patients had ∆Hb ≥ 0.8 mmol/L (responders) and 4 patients ∆Hb < 0.8 mmol/L (non-responders).

A: Average Hb course. The difference between future responders (green dots) and non-responders (blue squares) is not yet evident after one week.
B: Average Ret-He course. There is a clear difference between responders (green dots) and non-responders (blue squares). The haemoglobinisation level of reticulocytes is an early detector of functional iron deficiency due to epoetin injections.

**Figure 2:** NKF Evaluation of Anemia KDOQI Guidelines (2006)

**Initial Evaluation in CKD**

**Cellular Assessment**
- Hgb < 12 g/dL
- RBC indices (MCH, MCHC)
- Absolute Retic
- WBC & Diff
- Platelet

**Iron Assessment**
- Serum ferritin to assess iron stores
- Serum Tsat or CHr to assess adequacy of iron for erythropoiesis

**Iron Therapy Targets**

- Tsat > 20% or CHr > 29 pg/cell
- AND
- Serum ferritin > 200 ng/ml

**HD-CKD**
- Tsat > 20% or CHr > 29 pg/cell
- AND
- Serum ferritin > 200 ng/ml

**ND-CKD and PD-CKD**
- Tsat > 20%
- AND
- Serum ferritin > 200 ng/ml

---

**Chronic Disease Management**

Although chronic diseases are more common among older adults, they affect people of all ages and are now recognized as a leading health concern. Chronic diseases that affect the kidney are of special concern in the consideration of ID/IDA.

- Chronic diseases cause 7 in 10 deaths each year in the United States.15
- Chronic kidney disease (CKD), diabetes, and congestive heart failure, affect less than half of Medicare beneficiaries; however, these three related diseases consume well over 70% of current national CMS spending. The prevalence, complications, and costs of treating these disorders are increasing at an alarming rate.

The successful use of RET-He in kidney disease and the incorporation of its use in ESRD is described in the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines13 (Fig 2). These guidelines propose CHr at 29 pg/cell as a treatment threshold with a reference range of approximately 30-35 pg/cell. In addition, reticulocyte hemoglobin is included as part of the CMS clinical performance measures4 (CPM) for assessment of iron stores in the management of ESRD patients on hemodialysis.

**Figure 1:** Effect preoperative epoetin

The course of two clinical chemical parameters during preoperative epoetin treatment (injections done 3, 2 and 1 week prior to an orthopaedic operation; Protocol 1A) in 14 patients with preoperative Hb < 8.1 mmol/L. After three epoetin injections, 10 patients had ∆Hb ≥ 0.8 mmol/L (responders) and 4 patients ∆Hb < 0.8 mmol/L (non-responders).

A: Average Hb course. The difference between future responders (green dots) and non-responders (blue squares) is not yet evident after one week.
B: Average Ret-He course. There is a clear difference between responders (green dots) and non-responders (blue squares). The haemoglobinisation level of reticulocytes is an early detector of functional iron deficiency due to epoetin injections.

**Figure 2:** NKF Evaluation of Anemia KDOQI Guidelines (2006)
Clarify the current strategy for anemia management:
1. When is it clinically important for our clinicians to identify ID/IDA as early as possible?
2. Which of our well patient populations are at greatest risk for ID/IDA?
3. Which of our acute and chronic patient populations are at greatest risk for readmission? How is this impacted by ID/IDA?

Identify the clinical lab tests that currently trigger action by the physicians:
4. Which tests are our clinicians currently using to identify ID/IDA?
5. How early in the medical condition do our physicians want to see indicators of decreased iron stores in bone marrow?
6. Given that chemistry tests are indirect measures and have limitations due to infection and/or inflammation, how comfortable are we that we are accurately identifying ID/IDA in these patients?
7. Given that ESA and iron therapy regimens require careful monitoring and dose adjustment, how comfortable are we that our clinicians are using the most appropriate test to make these decisions?

Identify potential improvements that the clinical laboratory can support:
8. Given that RET-He responds to changes much earlier than H&H, are we offering the most appropriate tests to our clinicians?
9. What are our system’s initiatives to help physicians order the correct lab tests at the correct time?
10. How can the lab support the education of physicians, nurses, pharmacists, coding, and other ancillary departments to help identify ID/IDA sooner?

To look more closely at how the RET-He parameter might impact laboratory costs as well as total cost of care in your institution, a sample worksheet has been provided. This worksheet considers the various factors discussed in this article.

Although the laboratory represents a small part of the overall hospital operating spend, the value-driven laboratory – a laboratory that meets market demands by providing new types of diagnostic information to support the delivery of high-quality, cost-effective patient care - can help physicians provide incremental improvement in patient outcomes and an incremental reduction in the overall cost of care.

Conclusion
There is little doubt that laboratories offering RET-He results can contribute to physicians’ identification and monitoring of therapy in iron deficiency and iron deficiency anemia. The RET-He value has been shown to be simple, reliable and available at no additional cost and is automatically reported as part of the comprehensive reticulocyte. It has been shown to be the earliest indicator of either decreasing or increasing iron availability in the bone marrow. Physicians may be able to gauge a patient’s response to therapy in days, instead of weeks, and respond accordingly.

RET-He, when used with other red cell indices and the clinical picture, can contribute to the improvement of anemia management by physicians treating children and teens, women of child bearing age, surgical patients and patients with chronic disease.

Benchmarking Your Progress
When considering the potential return from investing in the adoption of Ret-He, the following ten considerations can provide valuable benchmark information.
Factors to consider when assessing the potential benefit of improving anemia management to improve patient outcomes, more effectively manage blood product utilization, and reduce overall cost of care:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Example</th>
<th>Your Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Enter the average number of annual PRBC transfusions for your institution*</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>B. Enter the fully burdened cost** of 1 PRBC</td>
<td>$3,000</td>
<td></td>
</tr>
<tr>
<td>C. Multiply A times B. This is an approximation of the average cost of transfusing PRBCs in your institution</td>
<td>1,000 X $3,000 = $3,000,000</td>
<td></td>
</tr>
<tr>
<td>D. Enter % of PRBC transfusions you think you may avoid next year</td>
<td>25%</td>
<td></td>
</tr>
</tbody>
</table>
| E. Multiply C times D. This is an approximation of annual savings from additional screening with RET-H
  | $3,000,000 X 0.25 = $750,000                                           |
| F. Enter the annual $ for ESA                                          | $120,000                                                                |
| G. Enter the % of ESA cost you think you can avoid by identifying nonresponders annually | 20%                                                                    |
| H. Multiply F by G for total amount saved in ESA doses                 | $120,000 X .20 = $24,000                                                |
| I. Subtract “risks and costs of neurocognitive delays associated with untreated iron deficiency” for total savings of implementing RET-H strategy in pediatrics. Consider cost of ID/IDA as a comorbidity for other patients. | This probably cannot be estimated                                          |
| J. Enter the $ amount of incremental reagent spend by adding RET-H into your clinical regimen*** | $2,500                                                                 |
| K. Subtract J from E. This is an approximation of your net annual savings from additional screening with RET-H | $750,000 - $2,500 = $747,500                                             |

Potential Sources of Data:
*Lab Administrator or Director of Transfusion Services
**Fully burdened cost (includes: Product, lab testing, nursing time, supplies, pharmaceuticals, etc.) Lab Director, Director of Transfusion Services, or CFO.
***Sysmex Sales representative can provide you with incremental cost per test multiplied by your new incremental Retic volume, assuming the volume of Retics increases. (Example: $0.25 X 10,000 tests).
Bibliography:


