Using the Hemoglobin Content of Reticulocytes (RET-He) to Evaluate Anemia in Patients With Cancer

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ABSTRACT

Objectives: Evaluation of anemia, particularly iron deficiency, in patients with cancer is difficult. This study examined using the hemoglobin content of reticulocytes (RET-He) to rule out iron deficiency, as defined by serum iron studies (transferrin saturation <20%, serum iron <40 µg/dL, and ferritin <100 ng/mL), in an unselected cancer patient population.

Methods: Patients were entered into the study based on the existence of concurrent laboratory test requests for CBC and serum iron studies.

Results: Using a threshold of 32 pg/cell, RET-He ruled out iron deficiency with a negative predictive value (NPV) of 98.5% and 100%, respectively, in the study population (n = 209) and in a subpopulation of patients with low reticulocyte counts (n = 19). In comparison, the NPV of traditional CBC parameters (hemoglobin, <11 g/dL; mean corpuscular volume, <80 fL) was only 88.5%.

Conclusions: These results support the use of RET-He in the evaluation of iron deficiency in a cancer care setting.

Advanced reticulocyte indices, such as the cellular hemoglobin content of reticulocytes, designated CHr and RET-He, are reportable parameters on Siemens ADVIA 2120 (Siemens, Tarrytown, NY) and Sysmex XE and XN series automated hematology analyzers (Sysmex, Lincolnshire, IL), respectively, without additional blood requirements or technical intervention. Good agreement between CHr and RET-He measurements has been reported.1-4 These indices correlate with iron-deficient erythropoiesis and are useful markers of iron deficiency in infants and children,5 adult blood donors,6 geriatric patients,7,8 pregnant women,9 and patients with chronic kidney disease undergoing hemodialysis.3,4,8,11 In addition, the hemoglobin content of reticulocytes is a useful tool for monitoring patients’ response to iron replacement therapy,12,13 and detecting iron-restricted erythropoiesis in patients receiving erythropoietin therapy.14-18 However, little is known about the utility of RET-He in the evaluation of complex anemias in malignancy.

Although iron deficiency is the leading cause of anemia worldwide, the anemia of chronic disease is more common among patients with malignancies. In contrast to iron deficiency anemia caused by inadequate iron stores, the anemia of chronic disease is associated with decreased iron availability despite abundant stores.19-21 The hemoglobin content of reticulocytes may assist in differentiating between these forms of anemia22,23 and shows a lower degree of within-patient variability than ferritin or other serum indicators of iron status.24

In the current outpatient cancer care setting, assessing iron deficiency requires additional biochemical tests, results of which are not available in real time. Iron deficiency anemia may be inferred from limited information provided...
by automated CBC and reticulocyte analysis, including hemoglobin (Hb), hematocrit, and RBC indices. However, measures of mature erythrocyte indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin, and RBC distribution width) cannot detect early iron-deficient erythropoiesis due to the slow turnover of erythrocytes (~120 days) in circulation.25

In contrast, the hemoglobin content of reticulocytes reflects the recent functional availability of iron for erythropoiesis, and results are available in real time as part of automated reticulocyte analysis. The measurement is not affected by physiologic interferences, except in cases of thalassemia30 and macrocytosis/megaloblastosis.31 The present study, therefore, investigated RET-He, reported by the Sysmex XE 2100, as a tool to rapidly rule out iron deficiency, defined by results of serum iron studies in an outpatient oncology setting. Iron deficiency is associated with significant morbidity in oncology patients and is readily treated. Thus, the ability to assess iron deficiency, as part of automated CBC/reticulocyte analysis, may significantly enhance patient management, particularly in an outpatient setting. Since the etiology of anemia in patients with cancer is complex, involving cytopenias secondary to chemotherapy, bone marrow failure, chronic disease/inflammation, and/or iron deficiency, the goal of the present study was to establish an RET-He cutoff that could rapidly rule out iron deficiency. The data suggest that RET-He, at a threshold of 32 pg/cell, may be an important discriminator to rule out iron deficiency anemia in patients with cancer.

Materials and Methods

Patients and Blood Samples

Remnant peripheral blood samples (n = 255), collected in EDTA anticoagulant, were retrieved from the Hematology Laboratory between May 25, 2012, and November 7, 2012, based on the existence of concurrent laboratory test requests for CBC analysis and ferritin, as well as sample availability within 6 hours of blood collection. In 209 cases, orders for ferritin were accompanied by orders for serum iron and transferrin saturation. RET-He was quantified using the Sysmex XE 2100. Chart review was undertaken to obtain laboratory test results and clinical diagnosis. The study population consisted of 114 males (aged 17 months to 94 years) and 95 females (aged 11-88 years). Patients were entered into the study without regard for diagnosis or treatment and consisted of individuals with solid tumors or hematologic malignancies who were undergoing and/or recovering from diverse chemotherapy and/or radiation treatment regimens. This study was approved by the institutional review board of Memorial Sloan Kettering Cancer Center for the protection of human subjects.

Although bone marrow examination is the conventional gold standard for assessing iron stores, it is not performed routinely at our center for the sole purpose of identifying iron deficiency because of its invasive nature. Thus, in the present study, iron deficiency anemia was defined biochemically using serum iron (<40 µg/dL), transferrin saturation (<20%), and Hb (<11 g/dL), with or without ferritin (<100 ng/mL), as described previously,22,20,21 consistent with current clinical practice recommendations. Transferrin saturation is a particularly useful biochemical marker of iron deficiency, since it reflects the interrelationship between bone marrow iron stores, iron absorption, and the availability of iron in circulating blood.32 In contrast, ferritin is less useful in oncology patients due to its elevation in patients with diverse solid tumors and its association with more progressive disease and shorter survival when elevated.33 Indeed, World Health Organization guidelines indicate that reliable assessment of iron status is not possible using ferritin in the presence of inflammation and infection.34 Thus, the present study evaluated RET-He performance characteristics against biochemical markers of iron deficiency, including serum iron and transferrin saturation with and without ferritin.

Statistical Analysis

The ability of RET-He to identify iron deficiency in a diverse patient population with cancer was evaluated by calculating sensitivity, specificity, and positive and negative predictive values35-37 against routinely ordered serum iron studies as the diagnostic standard. Differences between selected data sets were evaluated by performing a t test for independent samples using Excel Windows 2010 (Microsoft, Redmond, WA). Statistical differences between data sets were defined by a P value less than .05. Results were not stratified by type of malignancy or evidence of marrow infiltration because of limited sample size.

Results

Table 1 summarizes major characteristics of the study population. Patients consisted of approximately equal numbers of males and females. The mean age of the female population was slightly younger than that of the male cohort (P = .006), and as expected, the mean Hb was slightly lower in females than in males (P = .013). However, a similar proportion of males and females were anemic based on thresholds of Hb of 12 g/dL and 11 g/dL, respectively. No differences in MCV, serum iron, transferrin saturation, or ferritin were noted.

The distribution of RET-He values (pg) in the study population is illustrated in Figure 1. No differences were
observed between males and females \( (P = .831) \) (Table 1).

Values ranged from 19.7 to 47.6 pg. Although reference intervals vary among published series, RET-He values ranging from 28 to 36 pg are generally considered normal. \(^{28,31}\)

The distribution of RET-He values for the current patient population was shifted to the right because of an increase in the number of patients with macrocytic RBCs \((95\% \text{ confidence interval for } \text{MCV} , 73-105 \text{ fL})\). Macrocytosis has been correlated with an increased RET-He. \(^{31}\)

RET-He is a measure of the amount of Hb in reticulocytes and as such has been identified as a convenient indicator of iron-deficient and iron-restricted erythropoiesis. In the current study, statistically significant differences \((P < .05)\) in Hb, MCV, serum iron, and transferrin saturation were noted when comparing patients with RET-He levels above and below the published normal population reference range of 28 pg. \( \text{Table 2} \).

Consistent with observations that ferritin is of limited value as an indicator of iron status in patients with cancer, \(\text{serum ferritin did not correlate significantly with RET-He or Hb } (P = .20)\) and even showed an inverse relationship with RET-He, suggesting inflammation and chronic disease and/or the presence of tumor-derived ferritin. \(^{27,33}\)

In the current study of 209 patients, 93 were anemic with Hb levels less than 11 g/dL, the threshold identified by Steinmetz et al. \(^{38}\) for consideration of iron deficiency in oncology patients. Iron deficiency anemia was identified in 23 of these patients, based on biochemical studies, including serum iron and transferrin saturation. In contrast, only nine patients were identified with iron deficiency anemia based on the combination of decreased serum iron, transferrin saturation, and ferritin. Iron deficiency without consideration for anemia was identified in 34 patients based on decreased serum iron and transferrin saturation alone and in 18 patients based on the combination of decreased serum iron, transferrin saturation, and ferritin. \( \text{Table 3} \).

\( \text{Figure 2} \) illustrates the relationship between the sensitivity and specificity of RET-He for identifying iron deficiency, defined using biochemical standards, including serum iron less than 40 µg/dL and transferrin saturation less than 20%. An RET-He threshold of 31 to 32 pg/cell was identified, representing the 34th percentile of RET-He values in the study population. The same RET-He threshold was identified when the definition of iron deficiency was expanded to include low ferritin (<100 ng/mL) and/or low Hb less than 11 g/dL.

\( \text{Table 3} \) summarizes the ability of RET-He at thresholds of 31 and 32 pg to rule out iron deficiency in the study patient population. Uniformly good negative
predictive values were noted. No difference in RET-He performance was found when applied to the evaluation of male and female patients. Indeed, RET-He performance was better (negative predictive value of 98.5%) than that of traditional CBC parameters (negative predictive value of 88.5%), consisting of MCV less than 80 fL, and Hb less than 11 g/dL for women or less than 12 g/dL for men. However, the positive predictive value of an abnormal RET-He result was low because of the high selected cutoff and the high rate of inflammation and chronic disease, leading to iron-restricted hematopoiesis in the study population.

In a subanalysis of patients with iron deficiency, defined by serum iron less than 40 µg/dL and transferrin saturation less than 20%, iron deficiency was missed in three of 23 patients using an RET-He cutoff of less than 32 pg. Interestingly, the three observed false-negative cases would not have been characterized as iron deficient using in-house reference ranges for serum iron (34 µg/dL). When serum ferritin (<100 ng/mL) was included in the definition of iron deficiency, RET-He missed two of nine patients with biochemical iron deficiency. These two false-negative cases did not meet criteria for anemia (Hb of 15.7 and 11.7 g/dL), and ferritin was normal by in-house reference ranges (<22 ng/mL for males and <6 ng/mL for females). Similarly, when monitoring patients (n = 15) receiving oral iron supplementation, RET-He (<32 pg) identified two of two patients with residual iron deficiency. However, three of 13 patients, who were deemed iron replete based on biochemical studies, were classified as iron deficient by the high RET-He cutoff. Finally, RET-He (<32 pg) ruled out iron deficiency (serum iron <40 µg/dL and transferrin saturation <20%, with or without ferritin <100 ng/mL) with a negative predictive value of 100% in patients (n = 19) with low reticulocyte counts (<20 × 10⁹/L). Given the small sample size, additional prospective studies are required to confirm RET-He performance in these settings.

**Table 3**

Performance of RET-He or the Combination of Hb and MCV in the Evaluation of Iron Deficiency/Iron Deficiency Anemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>RET-He</th>
<th>MCV and Hb</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Negative Predictive Value, %</th>
<th>Positive Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td></td>
<td></td>
<td>&lt;/11 g/dL with transferrin saturation &lt;20%, serum iron &lt;40 µg/dL, and ferritin &lt;100 ng/mL</td>
<td></td>
<td>1.00</td>
<td>0.697</td>
</tr>
<tr>
<td>Transferin saturation &lt;20% and serum iron &lt;40 µg/dL and ferritin &lt;100 ng/mL</td>
<td></td>
<td></td>
<td>Hb &lt;11 g/dL (F) or &lt;12 g/dL (M) and MCV &lt;80 fL</td>
<td></td>
<td>0.24</td>
<td>0.90</td>
</tr>
<tr>
<td>Hb &lt;11 g/dL and MCV &lt;80 fL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Hb, hemoglobin; MCV, mean corpuscular volume; RET-He, hemoglobin content of reticulocytes.
Discussion

Anemia is a major cause of morbidity in patients with cancer. There are multiple causative factors, including absolute iron deficiency due to blood loss and/or nutritional deficiencies, anemia of chronic disease, and myelosuppressive effects of chemotherapy, as well as metastatic infiltration of the bone marrow. The identification of iron deficiency in patients with cancer is particularly important in patients being considered for therapy with erythropoiesis-stimulating agents. Approximately 30% to 50% of patients with cancer with chemotherapy-related anemia have been reported to experience poor to absent responses to erythropoiesis-stimulating agents, and iron therapy has been shown to improve the response in some of these patients. In addition, iron therapy has been associated with a reduction in the RBC transfusion requirements in women with gynecologic cancers treated with chemoradiotherapy. Since the identification of iron deficiency has important therapeutic implications in oncology patients, current recommendations are to investigate the cause of anemia if the Hb falls below 11 g/dL.

The present study supports the use of RET-He to rapidly rule out iron deficiency, defined by traditional serum biochemical markers, in a complex patient population with cancer, during routine automated CBC and reticulocyte analysis. To evaluate RET-He in the broadest possible oncology population, we did not stratify patients by malignancy, staging, treatment, or transfusion therapy. In this unselected patient population with cancer, a high negative predictive value for ruling out iron deficiency was achieved using an RET-He cutoff of 31 to 32 pg, when iron deficiency was defined by serum iron and transferrin saturation, with or without ferritin. In this setting, RET-He outperformed the combination of Hb and MCV. The MCV (mean RBC volume) is of limited utility in oncology patients, because it is observed in the later stages of iron deficiency and is influenced by reticulocytosis, liver disease, myelodysplastic syndrome, aplasia, myelofibrosis, and impaired DNA synthesis and cell division due to chemotherapy.

Despite the excellent negative predictive value of RET-He for ruling out iron deficiency in the present study, the corresponding specificity was low. This was expected based on the high selected RET-He threshold relative to established reference ranges and the high incidence of anemia of chronic disease in the oncology population. All false-positive cases identified by an RET-He threshold of 32 pg (n = 50) were examined by chart review. Indeed, in 40 cases, the anemia was characterized as anemia of chronic disease in the patient record. Since anemia of chronic disease is characterized by iron-restricted hematopoiesis, RET-He levels are expected to be low and to overlap with those found in iron deficiency.

In the remaining 10 cases with falsely positive RET-He values and normal biochemical iron studies, one case of a hemoglobinopathy combined with β-thalassemia trait (heterozygous Hb Mississippi/β-thalassemia) was identified with expected low RET-He. Two patients were found to be iron deficient by biochemical tests on a follow-up visit, and seven patients had borderline serum iron (<45 μg/dL) and transferrin saturation (<25%). Further studies are required to determine how well RET-He distinguishes iron-deficient or iron-restricted hematopoiesis from bone marrow suppression. Data from a small subanalysis of patients with low reticulocyte counts in the present study appear promising.

The hemoglobin content of erythrocytes, measured either as ADVIA 2120 CHr or Sysmex RET-He, has been reported to be a reliable indicator of iron-deficient erythropoiesis in diverse patient populations, including pediatrics, geriatrics, pregnancy, and particularly chronic kidney disease. Its performance characteristics in evaluating anemia in patients with cancer, however, are not well understood. This study provides insight into the utility of measuring RET-He in an oncology setting, in which patients are routinely monitored for cytopenias, typically in an outpatient setting. Biochemical iron studies are often ordered in conjunction with a CBC and other laboratory tests as part of automated order sets designed to expedite the interpretation of anemia in patients at risk. Thus, the ability to obtain information regarding the availability of iron for erythropoiesis, as part of routine peripheral blood CBC and reticulocyte analysis, performed in an outpatient setting before the patient is evaluated by a specialist, would significantly benefit patient management.

To focus on patients at risk for iron deficiency, we evaluated RET-He only in patients with orders for CBC and serum iron studies. This method may have introduced an unintended bias into the analysis, since only those patients who had further laboratory studies ordered were included in the current study. This limitation precludes understanding the role of RET-He in a broader patient population with cancer. Other limitations of the study include the small number of patients who actually had iron deficiency anemia, in the context of a significant population with iron-restricted erythrocytes secondary to inflammation and chronic disease. In addition, the diagnosis of iron deficiency was inferred from biochemical studies and medical record review, in the absence of a gold standard for iron deficiency such as bone marrow iron studies. Standard biochemical tests of iron metabolism, such as serum iron, ferritin, and transferrin, are affected by the acute phase response. During the acute phase, serum iron and ferritin are increased, whereas transferrin is decreased. Therefore biochemical markers of iron status are less than ideal standards for diagnosing iron deficiency. In current clinical practice, however, bone marrow evaluation to assess iron status is not performed in most patients with cancer who have anemia, and iron deficiency is diagnosed based on results of serum iron studies.
In summary, the data support the use of RET-He to rapidly rule out iron-deficient erythropoiesis, reduce unnecessary iron studies, and provide rapid diagnostic information when the automated CBC and reticulocyte counts are reported. Based on data from the present study, using RET-He to rule out iron deficiency would reduce the number of required iron studies by 66% (from 209 to 70) at our center. If a combination of RET-He (<32 pg) and anemia (Hb <11 g/dL) were used to rule out iron deficiency anemia, the number of required iron studies would decrease by approximately 80% (from 209 to 43). Since RET-He and its CHr equivalent are routinely available with automated reticulocyte analysis on Sysmex XE/XN series and Siemens ADVIA hematology analyzers, respectively, this parameter can be integrated readily into the diagnostic algorithm of anemia evaluations. RET-He and CHr cutoff values reported for the identification of iron deficiency vary widely in the literature, from 26 to 33 pg, depending on the study patient population. Thus, customizing the RET-He threshold to rule out iron deficiency in specific patient populations is recommended. Of note, the RET-He threshold of 32 pg/cell, selected in the present study to rule out iron deficiency, was applicable also to an independent study cohort described by Canals et al. to correctly rule out iron deficiency anemia and iron-restricted hematopoiesis.

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