Reticulocyte haemoglobin content – adding value to anaemia diagnostics

The purpose of this newsletter is to provide an overview of the reticulocyte haemoglobin content and its role in the diagnosis of iron deficiency anaemia.

Key words:
anæmia, iron deficiency, reticulocyte haemoglobin content, Ret-He

Anaemia diagnosis and the role of the laboratory
Anaemia is an enormous problem worldwide, and consequently the complete blood count (CBC) is the most frequently requested laboratory investigation. The actual value of haemoglobin, expressed in g/dL, is used to define anaemia, but does not provide any information about its probable cause. In order to guide clinicians regarding the possible underlying pathology, the assessment of erythropoiesis, which forms an integral part of the CBC, is required. Automated analysis permits assessment of erythropoiesis at three levels.

a) Mature erythrocytes
The traditional red blood cell (RBC) indices, namely mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), have formed the cornerstone of categorising anaemias into broad classes in order to direct further investigations as to the probable underlying cause. One of the biggest challenges is to accurately differentiate between iron deficiency anaemia and anaemia of chronic disorder (both of which have microcytic red cells) and subsequently to monitor response to treatment, or more often to elucidate why there is a lack of response to conventional therapy. The major limitation of relying on the traditional red cell parameters to assess response is the fact that they only give an indication of average red cell status over the past 120 days, which is the life span of red blood cells.

b) Reticulocytes
The reticulocyte count, expressed either as a percentage or absolute count, is an extremely valuable parameter that is unfortunately significantly underutilised. It provides an indication of erythropoietic activity with respect to the absolute number of cells generated over the past one to two days.

c) Functional red blood cell information
"Reticulocyte haemoglobin content" provides a measure of the haemoglobinisation of reticulocytes as reported in pg.

What is reticulocyte haemoglobin?
Reticulocyte haemoglobin is the measure of the average haemoglobin content within the reticulocyte fraction of the red blood cells in the peripheral circulation. It is a reflection of the haemoglobinisation of red blood cells that have been produced by the bone marrow in the past one to two days. As such, it provides a real-time snapshot of the quality of developing red blood cells.

How is reticulocyte haemoglobin measured?
Information on reticulocyte haemoglobin content can be obtained as part of a CBC measurement when the sample is run in the "reticulocyte mode" on a Sysmex X-Class analyser that has a reticulocyte channel and is fitted with the appropriate software. The analyser utilises fluorescence flow cytometry in conjunction with a nucleic acid staining
dye to measure the amount of haemoglobin obtained within the reticulocytes. In the reticulocyte scattergram, forward scatter, a measure of individual cell size, on the y axis is plotted against fluorescence intensity, a measure of RNA content, on the x axis. The Ret-Y and RBC-Y represent the mean forward scatter of the reticulocyte and mature red blood cell clusters respectively. They are dimensionless channel numbers from which two parameters are derived; The haemoglobin content of the reticulocytes is expressed as the parameter “reticulocyte haemoglobin equivalent” (Ret-He) and the haemoglobin content of the mature red blood cells (RBC-He) [this value is equivalent to the calculated index MCH in the standard CBC].

**Why is it important to differentiate iron deficiency anaemia from anaemia of chronic disease?**

Iron is a vital element that is essential for the production of haemoglobin. It forms the core of the haem group, which facilitates oxygen transport and transfer to and from tissues. As the iron stores in the bone marrow become depleted so the haemoglobin production slows down and individual red cell haemoglobinisation becomes progressively poorer. Consequently, individuals with iron deficiency develop anaemia characterised by the presence of hypocromic microcytic red cells, the degree of abnormality being directly correlated to the duration of depletion of iron stores. The treatment of iron deficiency anaemia is therefore quite simply iron supplementation.

Anaemia of chronic disease may present with a similar blood picture as that of iron deficiency, which is a hypocromic microcytic anaemia. In contrast to classical iron deficiency, individuals with anaemia of chronic disease have increased iron stores, primarily as a consequence of an underlying inflammatory state. However, the iron cannot be released and is therefore unavailable to be incorporated into RBC production within the bone marrow; so called functional iron deficiency. Giving iron supplements to such patients would not alleviate the anaemia and may actually cause harm by exacerbating the iron overload situation. Chronic iron overload is toxic to tissues and can cause organ damage with the heart being particularly vulnerable. It is therefore important to determine whether the hypocromic microcytic anaemia is due to true or functional iron deficiency.

**What laboratory investigations are used to assess iron status?**

Classical iron studies involve a battery of biochemical assays which typically include ferritin, serum iron, transferrin and transferrin saturation. Expected findings are shown in table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>True iron deficiency</th>
<th>Functional iron deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Serum iron</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Transferrin</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>% Transferrin saturation</td>
<td>Low</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Table 1 Typical iron study results observed in true iron deficiency and functional iron deficiency**
Whilst it might appear that these two conditions are readily identifiable based on the biochemically findings, in reality this is not the case as there is significant overlap. Patients with true iron deficiency may have concomitant inflammation, which would result in an elevation of ferritin as part of the acute phase response as well as other more subtle changes. These parameters also show significant diurnal variation; therefore, results obtained and their interpretation may differ depending on the time of day that the blood sample was taken.

**How can we differentiate between true iron deficiency and functional iron deficiency?**

The combination of the haematology parameter Ret-He and the biochemical parameters ferritin and soluble transferrin receptor can provide unequivocal distinction between these two conditions. Ferritin is an indirect measure of total body iron stores whereas soluble transferrin receptor is directly proportional to the extent of true iron deficiency. This means that the more depleted the body stores are, the greater the soluble transferrin receptor value. These two biochemical values are expressed as an index (figure 2) and then plotted against Ret-He in the so-called Thomas Plot.

The Ret-He value, plotted on the y-axis, tells us whether iron is available for incorporation into the developing red blood cells. The lower the Ret-He value, the greater the restriction on iron availability. The soluble transferrin receptor-log ferritin index (sTFR-F index) tells us whether iron stores are present or not; the greater the value, the more the stores are depleted. By applying a cut-off value for both Ret-He and sTFR-F index, four quadrants are identified as shown in figure 3. The cut-off values are determined with reference to normal populations. As inflammation impacts on the reliability of ferritin as a marker of true iron status, the cut-off values for the sTFR/log ferritin index will be different depending on whether or not the CRP values is less than or greater than 5mg/L. Although the Thomas plot has shown excellent clinical utility, it is limited by the fact that soluble transferrin receptor is a relatively expensive assay that is not in routine use.

![Figure 2: Soluble transferrin receptor - ferritin index](image)

**Figure 2** Soluble transferrin receptor - ferritin index

**Figure 3** The Thomas Plot can be used to differentiate true iron deficiency from functional iron deficiency.
Can Ret-He be used as a standalone parameter?

Even in the absence of the sTfR-F index, Ret-He is still extremely useful in the assessment of iron deficiency anaemia. Whilst a single Ret-He value that is low may either be due to classical iron deficiency or functional iron deficiency (quadrants 3 and 4 in figure 3), serial measurements are very informative. Upon starting oral iron supplementation, a person with classical iron deficiency will show a dramatic rise in Ret-He, with normalisation of levels within about three days, whereas someone with functional iron deficiency will show no change (figure 4).

The only limitation of Ret-He is that it will be low even in the absence of iron deficiency in patients that have an inherited microcytic anaemia such as the thalassaemias. However, there are other clues such as the long-standing history and the red cell distribution width (RDW) that will separate these two entities. Microcytosis due to iron deficiency is typically associated with a raised RDW, whereas the thalassaemias will have a normal or narrow RDW. Likewise Ret-He may be normal if the patient has a concomitant megaloblastic anaemia but once again there are clues such as the presence of a dimorphic population on the RBC histogram that should alert the laboratory personnel to this possibility.

Clinical applications of Ret-He

a) Chronic renal failure

Patients with renal disease commonly develop severe anaemia one of the commonest causes of which is a deficiency of erythropoietin (EPO). EPO is a hormone produced by the kidneys the function of which is to stimulate red cell production in the bone marrow. In chronic kidney disease, the kidneys are not only no longer able to adequately excrete waste products in the urine, but they also lose the normal control mechanism whereby EPO is produced in the kidneys as the oxygen carrying capacity falls; meaning that the haemoglobin value drops. Most often, it will be required that such patients be given EPO as a drug to maintain the haemoglobin level above 11g/dL (the target value required to maintain a reasonable quality of life for chronic renal failure patients). However, many patients show little or no response even after several weeks of treatment. The reason for this is not EPO resistance, but a lack of adequate iron to support the EPO stimulated erythropoiesis in the bone marrow. Patients with chronic renal failure will end up requiring haemodialysis, which is associated with iron loss through the dialysis circuit. If such EPO patients are given iron supplementation together with EPO, a dramatic response in the form of reticulocytosis and a rise in haemoglobin is observed.
EPO is a very expensive drug and is not without side effects therefore it would be important to be able to assess which patients require upfront iron supplementation. Traditionally, doctors have used conventional iron studies to do so. However, since the reticulocyte haemoglobin measurement became available as a routine parameter, several studies have shown Ret-He to be superior to ferritin and transferrin saturation. The main problem with using ferritin and transferrin saturation is that there is tremendous daily variation; hence they have very limited clinical utility in monitoring iron status and guiding decision-making with regards to whether or not a patient needs iron supplementation. Ret-He on the other hand, is a stable parameter which has been shown to superior to iron studies in this regard.2

b) Paediatrics
Iron deficiency anaemia is very common in young children. The WHO has estimated that about 50% of preschool children in Africa are affected by iron deficiency. Iron studies are however only conducted once the child has developed an overt microcytic anaemia. The problem with this is two-fold. Firstly, iron studies are often inconclusive, especially if there is concomitant infection which is common in children. Secondly, the microcytosis, which triggers the request for iron studies, may take several months to manifest during which time the cognitive development of the child is impaired due to the lack of iron. Ret-He has been shown to be extremely valuable in this situation as levels will be low within one to three days of iron deficiency first developing. It is therefore an extremely sensitive, early marker of iron deficiency that can be obtained as part of a CBC test. The great advantage of this is that no additional blood sample is required for analysis, thereby sparing the already anaemic child any further compromise. Furthermore, Ret-He has been shown to be the strongest predictor of iron deficiency in children showing superiority over biochemical iron tests.3

Because of the difficulty in obtaining sufficient blood for testing in children and relatively common inconclusiveness of iron study results, many children are simply given iron supplementation without confirming the diagnosis. This can be problematic. As it takes a while for the red cell indices to respond, it is common practice to send mothers away with a month’s supply of treatment. However, if the cause of the anaemia is not iron deficiency, then the child will remain untreated with possibly worsening anaemia for a considerable period of time. Furthermore, the stools become tarry black in colour with an unpleasant smell; mothers then sometimes stop the medication, especially if they struggle to get the child to take it (which is often the case, due to its unpleasant in taste). When using Ret-He as a screening test, only those children who have a low value should be given a trial of iron treatment and then be retested after three days. Those children in whom Ret-He has normalised must continue with treatment whereas, those who have not responded, must be investigated for other causes of anaemia.

c) Non-renal iron deficiency in adults
Similar findings have been shown in adult populations where absent bone marrow iron stores were used as the gold standard to define iron deficiency.4

d) Blood transfusion
Ret-He has also shown to be of great value in reducing blood transfusion requirements for patients undergoing major surgery that is generally associated with significant blood loss. It is common practice to stimulate endogenous red cell production to increase the haematocrit preoperatively in such patients to minimise the need for blood transfusion. EPO, together with oral iron, is administered three weeks preoperatively and patients are observed for a rise in haemoglobin and monitored using iron studies. By using this approach, about one third of patients fail to respond, because iron deficiency which is RBC production rate limiting, remains undetected and consequently they still require blood transfusion. However, if Ret-He is used to monitor these patients on a weekly basis during the pre-operative phase, and those patients who show a drop in Ret-He are supplemented with additional IV iron, then nearly all patients respond adequately and thereby
significantly reducing blood requirements. This approach has demonstrated that blood transfusion requirements for patients undergoing major surgery can be reduced by over 80%. Not only is this safer for the patient, but also ensures that blood products, which are a scarce resource, are conserved wherever possible.

**Take home message**

Ret-He is an extremely valuable parameter that has no 24-hour variability, and is easy to measure as it is obtained as part of the CBC analysis on a Sysmex X-class analyser. It is superior to traditional biochemical iron studies in the diagnosis of iron deficiency in a variety of clinical situations. A simple short trial of iron treatment and a repeat measure of Ret-He after three days, will accurately differentiate true iron deficiency from functional iron deficiency, which in turn will identify those patients that need supplementation and spare those who do not from potential harm.

**References**


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