

EDITORIAL

Reticulocyte Hemoglobin Content (CHr): The Gold Standard for Diagnosing Iron Deficiency

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Reticulocytes are newly produced, relatively immature red blood cells (RBCs). Reticulocyte count is not a part of standard CBC. It has to be ordered and used along with CBC. Reticulocyte count in the blood reflects bone marrow (BM) function or activity. These cells are 25% higher in volume in comparison with mature RBCs. Mature RBCs have no nuclei, but reticulocytes still have some remnant genetic material i.e. RNA. As reticulocytes mature, they lose the last residual RNA and fully develop into RBCs. Reticulocyte count or percentage is an indicator of ability of person's bone marrow to produce adequate RBCs (Erythropoiesis).

Reticulocytosis reflects responsive marrow. It is seen in acute or chronic bleeding, hemolysis and following treatment of deficiency anaemias. Reticulocytopenia suggests non-functional bone marrow i.e. Aplastic anaemia etc.

Reticulocyte count is, traditionally, performed manually. With refinement of technology and the principles of fluorescence, modern cell analyzers incorporate automated reticulocyte counting.¹ Automated reticulocyte counts have greater precision, accuracy, and reproducibility than manual counts.¹ The reference range for reticulocyte count for adults is 0.5%-1.5%.

Corrected reticulocyte count (CRC) or Reticulocyte Index (RI) are calculated (formula underneath) and these give a more accurate assessment of marrow function. The reference range for CRC in adults is 0.5%-1.5%.

$RI = \text{Reticulocyte count (\%)} \times (\text{measured hematocrit} / \text{normal hematocrit}).^1$

Reticulocyte Production Index (RPI) is another calculation (Formula underneath) & it corrects for the degree of reticulocyte immaturity. An increased RPI (>3) is seen with hemorrhage, hemolysis and response to hematinics.

$RPI = RI \times (1/\text{maturation time}).^2$

Immature Reticulocyte Fraction (IRF) is a quantitative measurement of the RNA content of reticulocytes. It is a ratio of immature reticulocytes to the total number of reticulocytes. Younger reticulocyte contains a higher RNA. IRF is automatically reported by modern blood cell analyzers capable of doing reticulocyte count testing. It is a much better indicator of responsive marrow than total reticulocyte cell count.¹ Increased IRF reflects early marrow recovery and it precedes increase in absolute reticulocyte count.

Reticulocyte hemoglobin content (CHr) is a measurement of hemoglobin inside the reticulocyte. It correlates directly with the functional availability of iron in the marrow. Today, it is called as the gold standard for diagnosing iron deficiency.

Any laboratory test to be called a perfect test, it should be accurate, simple & inexpensive. It then acquires clinical utility. Many tests are described to assess iron deficiency (ID), however, no single test fulfills all these criteria. Usually, simultaneous measurements of a group of tests

is needed. These include: Hb, reticulocyte count, RBC indices, S. iron (SI), total iron binding capacity (TIBC), S. Ferritin (SF), soluble transferrin receptor (sTfR) assay etc.

Modern automated particle cell counters utilize flow-cytometry technique and measure reticulocyte cellular characteristic i.e. IRF, CHr (or Ret-He).^{3,4} These are proposed as a surrogate marker to predict early response to iron therapy.⁵ Flow cytometry assesses the maturity of reticulocytes separating them into 3 areas according to the degree of fluorescence i.e. low-fluorescence reticulocytes (LFR), middle-fluorescence reticulocytes (MFR) & high-fluorescence reticulocytes (HFR); LFR being the most mature one.^{3,4} It is the HFR having more RNA that corresponds to the earliest of reticulocytes and hence indicates erythropoietic activity following treatment of anaemia. In various studies, HFR or CHr have been applied to predict early response to hematinics.

IRF increases in response to treatment with Erythropoiesis Stimulating Agents (ESAs) much before an increase of reticulocyte count and hence can be used in clinical practice for quick assessment during treatment of renal anaemia with ESA.⁵

The IRF has been also proposed as an early marker of engraftment in bone marrow or hematopoietic stem cell transplantation and bone marrow regeneration following chemotherapy.⁶

Also, in patients of myelodysplastic syndrome and

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dyserythropoietic anaemias, IRF is increased without reticulocytosis.⁷

IRF has also been used to diagnose hereditary spherocytosis (HS). In HS, there is high reticulocyte count without equally elevated IRF.⁸

CHr constitutes the most valuable screening tool for identifying iron deficiency (ID) with or without anaemia. Decreased CHr (cutoff value of 25 pg) stands for accurate diagnosis of functional iron deficiency with sensitivity of 94% & specificity of 80%.^{9,10}

Due to increased hepcidin production, in patients with inflammatory disorders, systemic infections and malignancies, iron is trapped in reticuloendothelial system (RES) and also poorly absorbed from GI tract leading to functional iron deficiency resulting in anaemia of systemic disease (ASD). This is also a hypochromic microcytic anaemia and it mimics iron deficiency anaemia (IDA). Also, in the presence of ASD, it is difficult to diagnose underlying IDA. Such patients have high or normal S. Ferritin despite iron deficiency (acute phase response). In this situation, CHr differentiates ID from ASD. The discriminatory power of CHr, both with respect to sensitivity and specificity, is better than MCV and Ferritin.³

While treating anaemia, one is curious to know the response to treatment at the earliest. This confirms that the treatment is on correct line, and also avoids in advertent hazards of over treatment. It takes weeks to observe a significant response to hematinics (oral or I.V, Iron, vitamin B12 or folic acid) by looking at hemoglobin (Hb), Packed cell volume (PCV) or hematocrit and erythrocyte indices. The main reason for late response is the long lifespan of mature RBC. Hence there is a need to seek an earlier, more sensitive and reliable marker for assessing the response. Over last decade or more, there is a growing body of evidence suggesting that reticulocyte, the newly produce erythrocyte may be the solution. Its cellular characteristics can be measured by modern automated particle cell counters. These parameters

provide early information which is sensitive, accurate and reproducible both for diagnosis and assessing therapeutic response.

Both IRF & CHr have been used as early response to treatment following Intravenous (IV) iron therapy in diverse patient populations, including pediatrics, geriatrics, pregnancy & chronic kidney disease (CKD).¹¹⁻¹⁶

Mehta et al in their study published in this issue of JAPI have shown that CHr is superior to conventional erythrocyte and iron metabolism indices including IRF. CHr serves as an earliest predictor of response to treatment & hence it is of great clinical utility.

No test is free from flaws. Mean cellular volume (MCV) is used for calculating CHr. That is its biggest diagnostic limitation. CHr is low in subject with thalassaemia and hemoglobinopathies without iron deficiency. Similarly, it is elevated in iron deficiency subjects with confounding megaloblastic anaemia because of high MCV. Therefore, it is important that CHr is interpreted in the context of patient's overall erythrocyte physiology including co-existing megaloblastic anaemia, presence of thalassaemia/hemoglobinopathies or blood transfusion.

In conclusion, reticulocyte hemoglobin content (CHr) is an extremely valuable recent addition to an expanding list of biomarkers that can be used to differentiate iron deficiency from other causes of anaemia. In olden days, stainable marrow iron was used as a gold standard for diagnosing iron deficiency. After that, soluble transferrin receptor (sTfR)-Ferritin index was used for this purpose. Today, CHr can be called as the gold standard replacing both of these.

It is a pity that despite its simplicity and utility, it is rarely used in clinical practice.

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