Reticulocyte Hemoglobin Equivalent (RET-He) as Measurement of Bone Marrow Iron Storage

Shinta Oktya WARDHANI[1], Amaylia OEHADIAN[2]

[1] Brawijaya University, Faculty of Medicine, Department of Internal Medicine Division of Medical Hematology-Oncology, Dr. Saiful Anwar General Hospital Malang, East Java

[2] Padjajaran University, Department of Internal Medicine Division of Medical Hematology-Oncology, Dr. Hasan Sadikin Central General Hospital Bandung, West Java, INDONESIA

ABSTRACT

Prussian blue staining examination is the current gold standard for evaluation of bone marrow iron storage. This procedure requires bone marrow aspiration examination, which is physically uncomfortable, expensive, and burdensome for patients. Reticulocyte hemoglobin equivalent (RET-He) determination is a method used to detect hemoglobin in reticulocytes, an indicator of potential iron deficiency and erythropoietic disorders. The purpose of this study is to assess RET-He as a method of measuring bone marrow iron storage. From August–December 2017, secondary data were collected from 50 patients (24 females and 26 males) without history of blood transfusion four days prior to RET-He. Data was analyzed using chi-squared and unpaired t-test. Between positive and negative bone marrow iron storage groups, there were significant differences in mean age (44 vs. 53 y, respectively, p= 0.024) and RET-He (29.7 vs. 26.9, respectively, p= 0.041). Based on ROC analyses, the AUC score was 0.672 (p= 0.030), which indicates that RET-He > 28.8 represents positive bone marrow iron storage with 72.0% sensitivity and 68.0% specificity. RET-He can be used as a method of measuring bone marrow iron storage.

Keywords: Bone marrow iron storage, Prussian blue staining, RET-He

INTRODUCTION

Prussian blue staining is the current gold standard for evaluating bone marrow iron storage and diagnosing iron-deficiency anemia. In this method, which assesses iron storage in the tissue reticuloendothelial system, ferric iron reacts with soluble ferrocyanide in the stain to form a complex of hydrated ferric ferrocyanide substance that is visualized microscopically as blue or purple deposits within cells.[1] Measuring iron storage using bone marrow aspiration is sensitive and specific, yet some patients find it uncomfortable, expensive, and burdensome.[1,2]

Methods currently used to support the diagnosis of iron deficiency anemia have limitations. Mean corpuscular volume or peripheral blood smear measurements demonstrate low sensitivity. The total iron-binding capacity and transferrin saturation measurements demonstrate inadequate specificity. The best indicator of iron reserves is the serum ferritin level. Still, as serum ferritin is an acute-phase reactant, it often increases during inflammation and can lead to unspecific results. Combinations of the above tests could not improve the examination efficiency.[3]

Clinicians need a simple, affordable, and comfortable test that can provide a definitive clinical value in diagnosing iron deficiency.

The development of flow cytometry techniques is the latest hematology tool to measure the content of hemoglobin within reticulocytes (reticulocyte hemoglobin equivalent /RET-He). This measurement could indicate the availability of iron during erythropoiesis within recent bone marrow counts and detect iron deficiency at an early stage.[1,4,5] RET-He is measured using automated fluorescent flow cytometry which in the reticulocyte channel, using a polymethine dye, also measures the mean value of the forward light scatter intensity of mature red blood cells and reticulocytes. These values correspond with reticulocyte hemoglobin content.[6] According to Laurenco, the clinical utility of RET-He is to differentiate between functional iron deficiency anemia and true iron deficiency anemia or anemia due to other causes or chronic diseases.[7] Lian et al.[8] concluded that RET-He and red blood cell distribution width are two convenient indexes capable of differentiating thalassemia from the other two microcytic anemias, congenital sideroblastic anemia and iron deficiency anemia. Therefore, the purpose of this research is to assess RET-He as a method of measuring bone marrow iron reserves.

MATERIALS AND METHODS

Research Design

The study was approved by the Ethics Committee of The Saiful Anwar General Hospital (Ethical Clearance No.400/067/K.3/302/2019) in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki and its contemporary amendments). Remnant peripheral blood samples (n= 50), collected in EDTA anticoagulant, were retrieved from the Hematology Laboratory between 1 June 2017 - 31 May 2018, based on the existence of concurrent laboratory test requests for CBC analysis as well as sample availability within six hours of blood collection. Orders for CBC analysis was accompanied by orders for bone marrow aspiration and Prussian blue staining to diagnose bone marrow iron stores. RET-He was quantified using the Sysmex XE 2100. Samples were collected from hematology-oncology patients having no blood transfusion history four days prior to the test. Patients under the age of 18, as well as those who had a chronic disease, were pregnant, or had received iron treatment within the previous three months, were excluded. Patient charts were reviewed to obtain laboratory test results and clinical diagnoses.

Examination of Prussian Blue Staining and RET-He

The results of bone marrow Prussian blue staining were grouped into positive and negative results. Positive results based on a positive Prussian-blue reaction (Perl's reaction). Hemosiderin, which acts as an iron reserve, especially in bone marrow macrophages, will react with potassium ferrocyanide to form blue ferric ferrocyanide. The hemosiderin painting method carried out at the Central Laboratory of Saiful Anwar General Hospital Malang was based on methods in Dacie and Lewis. Then, data is compared with the results of RET-He. RET-He collected in EDTA- containing vacutainers and quantified using the Sysmex XE 2100 following the manufacturer's instructions.

Statistical Analysis

The ability of RET-He to identify iron deficiency in a diverse patient population with hematologic disease and malignancy was evaluated by calculating sensitivity and specificity using a 2 x 2 table. The results were analyzed using chi-squared and unpaired t-tests. Data were processed using SPSS (v. 21). The RET-He diagnostic test utilizes numeric-scaled data with an upper limit of 28.8 and an area under curve (AUC) using the receiver operating characteristic (ROC) method.

RESULTS

Of the 50 subjects meeting the inclusion criteria, 25 (50%) had positive marrow iron stores, and 25 (50%) had negative iron stores. Characteristics of the study subjects are shown in Table 1.

Twenty-four of the patients (48%) were female, 14 of which had positive bone marrow storage by Prussian blue staining (59%), and 10 which had negative storage by Prussian blue staining (41%).

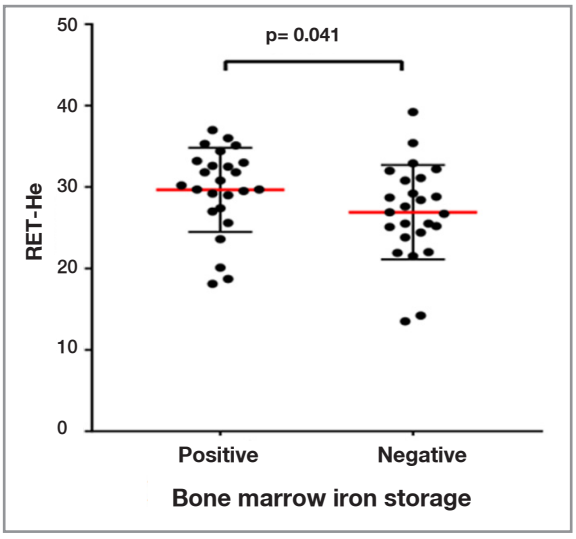

Figure 1. Ratio of RET-He and Prussian blue staining bone marrow iron storage results

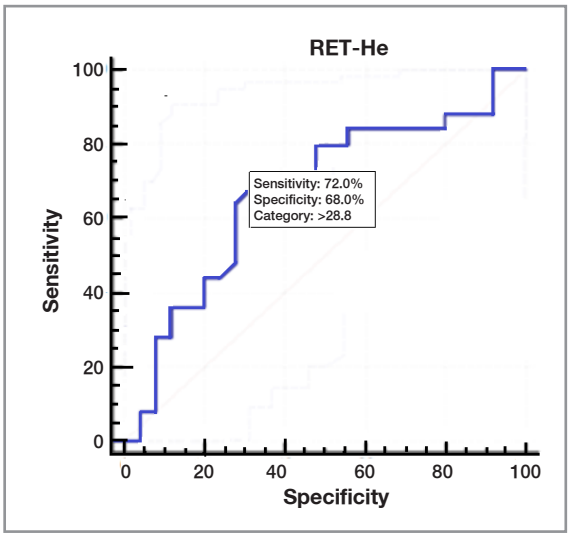

Figure 2. ROC RET-He Curve

Twenty-six of the patients (52%) were male, 11 of which had positive Prussian blue staining results (42%), and 15 which had negative Prussian blue staining results (58%). The average age of the positive (44 y) and negative (53 y) bone marrow iron groups was significantly different (p= 0.024).

RET-He values in positive and negative bone marrow iron storage groups (Table 1, Figure 1) were significantly different (p= 0.041). In the positive bone marrow iron storage group, mean RET-He is slightly higher (29.7) than the negative group (26.9). Patient diagnoses and corresponding bone marrow iron storage statuses are shown in Table 2. There were 15 (63%) hematologic malignancy cases with positive bone marrow iron storage and 17 (65%) with negative storage. Positive and negative bone marrow iron storage for nonmalignant hematologic cases was 9 (37%) and 8 (35%) patients, respectively.

ROC analysis results (Table 3) show the AUC was 0.672 (p= 0.030). This result indicates that RET-He can be used to predict bone marrow iron storage > 28.8 with 72.0% sensitivity and 68.0% specificity (Figure 2).

DISCUSSION

No significant difference in RET-He test results was found between male and female patients, similar to Scherer.[9] A significant age difference was ob-

Table 1. Study subject characteristics

	Bone marrow iron storage		p
	Positive (n= 25)	Negative (n= 25)	
Sex*			
Male	11 (44.0)	15 (60.0)	0.258
Female	14 (56.0)	10 (40.0)	
Age (years)**	44 ± 18	53 ± 15	0.024#
RET-He**	29.7 ± 5.2	26.9 ± 5.8	0.041#

*Notes: Analysis using *chi-square with significance p< 0.05*
***Unpaired t-test with significance p< 0.05, #= statistically significant*

Table 2. Bone marrow iron storage in patients diagnosed with hematologic disorders

Hematology disorder diagnosis	Number of cases with positive bone marrow iron storage	Number of cases with negative bone marrow iron storage
Hematologic Malignancy		
Acute myeloblastic leukemia	8	5
Lymphoma	1	–
Chronic lymphocytic leukemia	2	–
Multiple Myeloma	2	1
Myeloproliferative Neoplasm:		
Chronic granulocytic leukemia	–	8
Chronic neutrophilic leukemia	1	–
Polycythemia	–	1
Essential Thrombocytosis	1	2
TOTAL	**15 (63%)**	**17 (65%)**
Non-Hematologic Malignancy:		
Immune thrombocytopenic purpura	–	1
Evans syndrome	1	–
Autoimmune hemolytic anemia	2	–
Aplastic anemia	2	–
Iron-deficiency anemia	–	6
Myelodysplastic syndrome	1	
Chronic myelomonocytic leukemia	1	–
Megaloblastic anemia	2	–
TOTAL	**9 (37%)**	**8 (35%)**

served between the positive bone marrow reserve group and the negative (p= 0.024), supporting data that indicates iron deficiency anemia is often observed in older patients, with implications for the quality of life and the survival rate of the patient. In older patients, iron deficiency is caused by measurable factors including low nutritional intake, obstruction of iron absorption, occult bleeding and medication usage used RET-He to screen iron deficiency anemia in older patients and found higher sensitivity (93% vs. 72%) and similar specificity (69% vs. 68%) to that found in the current study.[9-11]

No significant correlation was observed between bone marrow iron reserve status with hematology malignancy or non-malignancy diagnosis. Unfortunately, this research could not determine if RET-He test could be utilized to eliminate iron deficiency in patients with hematology malignancies. Peerschke et al.[13] found that RET-He can help eliminate iron deficiency in cancer patients with a sensitivity rate of 98.5% and specificity of 100%.[13] This difference in results can occur due to differences in comparison. Peerschke's research used serum ion (SI), total iron binding capacity (TIBC) and ferritin iron deficiency markers, while study used Prussian blue staining of bone marrow aspirations, considered the gold standard for determining iron stores.

Serum ferritin levels are being applied in several clinical assessment. It has been used in some study which were designed to investigate the impact of hyperferritinemia irrespective of iron status.[14-16] Regarding studies about diseases related to iron level, measuring ferritin is only used. However, ferritin is inadequate surrogate for iron level. There are many circumstances associated with ferritin enhancement such as infection, liver injury, inflammation and macrophage activation.[14]

Mean RET-He values in positive and negative bone marrow iron storage groups were significantly dif-

Table 3. ROC RET-He analysis of bone marrow iron storage

Variable	AUC (95% CI)	p	Cut off	
RET-He	0.672 (0.525 - 0.798)	0.030	> 28.8	Sensitivity: 72.0% Specificity: 68.0%

Note: CI= confidence interval

ferent (p= 0.041), similar to a study by Mehta.[5] The ability of RET-He to determine the iron status within bone marrow is generated as RET-He is a test to detect the contents of hemoglobin within reticulocyte. Reticulocytes circulate for only 24-48 hours, having RET-He portraying the real conditions of iron within the bone marrow. When iron declines, so too will RET-H.[11,17] These results can provide additional benefits to doctors and patients while examining iron deficiency, as RET-He is performed using an automated blood cell count and does not require an additional blood tube, reported as part of reticulocyte count.[11,13,18,19]

Conclusion

The average difference of RET-He between a positive and negative bone marrow iron reserves groups. The positive bone marrow iron group had a higher RET-He average (29.7) than the negative group (26.9). RET-He test is a good predictor of the availability of the bone marrow iron reserve. This research can be continued with the case-control method to increase sensitivity and specificity.

Acknowledgement

This study was supported by the Malang and Bandung Indonesian Society of Medical Hematology and Oncology. Clinical Pathology Saiful Anwar Hospital. The authors also thank to all patients, health care workers and teachers.

REFERENCES

1. Rahayu ET, Ratnaningsih T. Peran reticulocyte hemoglobin equivalent (RET-He) untuk scrining deficienci besi pada anak usia 6 bulan-5 tahun. Tesis, S2 Ked. Klinik / MS-PPDS, 2014 .
2. Pujara KM, Bhalara RV, Dhruva GA. A study of bone marrow iron storage in hematological disorder. Int J Heal Allied Sci 3: 221-224, 2014.
3. Bach V, Schruckmayer G, Sam I, et al. Prevalence and possible causes of anemia in the elderly: A cross-sectional analysisof a large European university hospital cohort. Clin Interv Aging 9: 1187-1196, 2014.
4. Johnson-Wimbley TD, Graham DY. Diagnosis and management of iron deficiency anemia in the 21st century. Therap Adv Gastroenterol 4: 177-184, 2011.
5. Mehta S, Goyal LK, Kaushik D, et al. Reticulocyte hemoglobin vis-a-vis serum ferritin as a marker of bone marrow iron store in iron deficiency anemia. J Assoc Physicians India 64: 38-42, 2016.
6. Miwa N, Akiba T, Kimata N, et al. Usefulness of measuring reticulocyte hemoglobine equivalent in the management of haemodalysis patients with iron deficiency. Int J Lab Hematol 32: 248-255, 2010.
7. Laurenco EM, Thomas N, Antia V. Clinical utility of reticulocyte hemoglobine equivalent to differentiate between functional iron deficiency anemia and true anemia and different from anemia of other causes of chronic disease. Hemasphere 3: 893, 2019.
8. Lian Y, Shin J, Nie N, et al. Reticulocyte hemoglobin equivalent (Ret He) combined with red blood cell distribution width has a differentially diagnostic value for Thalassemias. Hemoglobin 43: 229-235, 2019.
9. Scherer PS, Moraes D, Munhoz TP, Sgnaolin V. New red blood cell and reticulocyte parameters and reference values for healthy individuals and in chronic kidney disease. J Bras Patol Med Lab 51: 77-84, 2015.
10. Busti F, Campostrini N, Martinelli N, Girelli D. Iron deficiency in the elderly population, revisited in the hepcidin era. Front Pharmacol 5: 1-9, 2014.
11. Joosten E. Iron deficiency anemia in older adults: A review. Geriatr Gerontol Int 18: 373-379, 2018.
12. Karlsson T. Comparative evaluation of the reticulocyte hemoglobin content assay when screening for iron deficiency in elderly anemic patients. Anemia 2011: 925907, 2011.

13. Peerschke EIB, Pessin MS, Maslak P. Using the Hemoglobin Content of Reticulocytes (RET-He) to evaluate anemia in patients with cancer. Am J Clin Pathol 142: 506-512, 2014.

14. Malkan UY, Gunes G, ELiacik E, et al. The impact of iron overload on transplant-related complications and prognosis of acute leukemias. UHOD - Int J Hematol Oncol 26: 54-60, 2016.

15. Keramati MR, Siadat Z, Mahmoudi M. The correlation between H. Pylori infection with serum ferritin concentration and iron deficiency anemia. UHOD - Int J Hematol Oncol 25: 16-20, 2015.

16. Erbaycu AE, Ucar H, Gulpek M, et al. The contribution of serum ferritin, iron, iron binding capacity and bronchoalveolar lavage ferritin levels in differential diagnosis of lung cancer from benign pulmonary diseases. UHOD - Int J Hematol Oncol 25: 129-137, 2015.

17. Primiastanti P, Sukartini N. Iron deficiency in pregnant women by haemoglobin reticulocyte. Med Clin Pathol Med Lab 19: 1-2, 2013.

18. Poffenroth C, Mabbett C, Kendrick C. The reticulocyte haemoglobin equivalent (RET-He) and laboratory screening for iron deficiency. New Zeal J Med Lab Sci 71: 120-123, 2017.

19. Amirkhani MA, Zia Aldini SH, Dashti M, et al. The prevalenceof anemia among school students in a national screening program in Iran. J Isfahan Med Sch 26: 381-386, 2009.

Correspondence:

Dr. Shinta Oktya WARDHANI
Medical Hematology-Oncology Division of
Internal Medicine Department,
Medical Faculty of Brawijaya University,
Dr. Saiful Anwar General Hospital Malang,
East Java / INDONESIA

Tel: 62 341 551611
e-mail: shinta_oktya.fk@ub.ac.id
capa.journal139@klinikjurnal.com

ORCIDs:

Shinta Wardhani	0000-0001-8889-0930
Amaylia Oehadian	0000-0002-7853-2221