© Ю. О. Еремина¹,², К. Магалеш¹

Содержание гемоглобина в ретикулоцитах (Hb-ret) является эффективным индикатором состояния синтеза гемоглобина в реальном времени, который позволяет диагностировать и контролировать дефицит железа и железодефицитную анемию во всех возрастных группах с сопутствующими заболеваниями или без них, включая бета-талассемию. Анализ Hb-ret – менее инвазивный метод, чем исследование желез костного мозга, менее дорогой, чем биохимический анализ показателей обмена железа, и может быть доступен даже в местных лабораториях. Этот обзор посвящен материалам, опубликованным, в основном в 2020 г., и некоторым исследованиям клинического применения Hb-ret измеренного на гематологических анализаторах Sysmex.

Ключевые слова: содержание ретикулоцитарного гемоглобина; дефицит железа; железодефицитная анемия; талассемия.

---

Reticulocytes are immature red blood cells, that released into the blood at 2–3 days following hematopoiesis and become mature red blood cells 1–2 days later. During hematopoiesis in the bone marrow, iron is taken up from the blood and then binds to heme protein to form hemoglobin (Hb). The hemoglobin content of reticulocytes (Hb-ret) is considered to reflect Hb synthesis potential, as well as the iron levels used in hematopoiesis [1].

In the beginning of 2020, an excellent review about Hb-ret was published by Chie Ogawa, et al. [2]. The review covered iron metabolism in the body, hematopoiesis in the erythroid system, and the measurement principle and characteristics of Hb-ret as...
well as its application in clinical studies mainly dedicated to use of commercial H*3 and ADVIA hematology analyzers. In this paper we aimed to analyze more recent studies about Hb-ret and some not previously included reports, such as clinical application of Hb-ret parameter measured by Sysmex hematology analyzers.

**Diagnosis of iron deficiency and iron deficiency anemia**

Anemia is a significant worldwide health problem. Approximately one third of the world's population suffers from anemia, half of which is due to iron deficiency (ID) [3,4]. Traditional indices of body iron such as serum ferritin (SF), serum iron, total iron-binding capacity, and transferrin saturation (TSAT) are affected by other factors (inflammatory state, diurnal variation, diet/malnutrition, decreased liver function) and iron metabolism. Differently, Hb-ret is not affected by any factors other than those participating in iron metabolism, as long as there is no abnormality in Hb synthesis [2]. Although, a study of 405 healthy adolescents (subjects with a hematological or systemic disease, present or previous infectious or febrile disease or C-reactive protein $>$0.5 mg/dL excluded) found that greater body weight was associated with slightly but significantly lower Hb-ret with no ID associated [5].

Hb-ret level was found to be significantly lower in non-ID anemia than in healthy controls, but significantly and substantially higher than in ID [6]. The areas under the curve (AUC) resulting from receiver operating characteristic curve analysis for the diagnosis of absolute iron deficiency anemia (IDA) were reported to be 0.69-0.89 and 0.64-0.84 for SF and TSAT, respectively, in contrast to 0.74-0.93 for Hb-ret [2,3]. Variability of AUC for Hb-ret explained also by a different cut-off value applied: 27.2 pg to 30.5 pg [2,7].

Hb-ret is proposed for IDA detection in infants, who have difficulties with blood draws [8]. American retrospective records review of 190 paired ferritin and Hb-ret measurements in neonates showed that the Hb-ret gives the more accurate ID diagnosis than ferritin [9]. A study of 274 samples from infants hospitalized in intensive care units confirmed Hb-ret levels at birth are higher than any other day of healthy life, in accordance with a relatively high level of erythropoietin before birth. In term infants (more than 37 weeks of gestation) the later Hb-ret decreased was milder in comparison to preterm infants (before 37 weeks of gestation), which is partly explained by the minor incidence of the conditions related to hospitalization [10].

Hb-ret permits differentiation between IDA, ID without anemia and normal state in children and adolescents [2]. In a study conducted on 207 Indonesian children Hb-ret was significantly different between ID without anemia (normal Hb according to age and one out of two criteria: TSAT$<$15% and/or SF$<$15 mg/L) and normal state, but not between iron depletion (IDep) (normal Hb according to age, normal TSAT, and SF$<$15 mg/L) and normal state [11]. All venous blood samples in this study were analyzed at two different laboratories using ADVIA 2120 and Sysmex ST 2000i. It is not clear whether the results between these equipment were compared, as comparing results produced by different analytical systems all together with the small sample number could explain the absence of difference between normal state and IDep.

Recent Spanish study reported that Hb-ret addition to the serum transferrin receptor (sTfR, conventional analytic parameter not influenced by proinflammatory states) increased the sensitivity of ID assessment from 9.1 to 12.1% in adolescents [4].

The possibility of quick ID/IDA diagnosis and the test availability in a local laboratory are crucial, especially for children, pregnant women and elderly people [12-14], which is another important benefit of Hb-ret use. This biomarker is currently available on many hematology analyzers, being determined simultaneously with the other hematological parameters and decreasing economical burden of ID/IDA diagnosis and monitoring [14,15]. All these reasons also made Hb-ret be suggested as a preoperative marker of
latent anemia within Patient Blood Management program. Identifying and treating patients with ID proactively in the sense of enhanced recovery after surgery concept, help to prevent complications and extended hospital stays [16]. Hb-ret can be used as a routine screening test to detect latent ID in blood donors, providing an opportunity to make appropriate and timely interventions like dietary changes or drug supplementation to prevent development of an overt IDA [17].

**Thalassemia**

Microcytosis and hypochromia can be falsely interpreted as ID/IDA in individuals with thalassemia. Beta thalassemia trait (BTT) is an autosomal dominant disorder that results in defective production of beta globulin chains, which are Hb-constitutive proteins. BTT is very common in African populations, it also shows a significant prevalence in the Mediterranean, Middle East, Transcaucasus, Central Asia, Indian subcontinent, and Far East, the incidences of 14 and 12% were reported in Cyprus and Sardinia respectively. However, BTT due to population migration is now also common in Northern Europe, North and South America, in the Caribbean, and in Australia. Since this disease has a very strong socio-economic impact and, diagnosis is usually based on specific expensive tests, there is a need for rapid, simple and low-cost screening tests [18].

Based on the study of 293 samples from Italian children a discrimination index (DI) for BTT screening (DI-BTT) was developed, calculable using the following formula:

\[
(RBC \times MCHC \times 50/MCV)/CHr,
\]

where: RBC – red blood cell, MCHC – mean cellular hemoglobin concentration, MCV – mean cellular volume and CHr – mean reticulocyte hemoglobin. The evaluation of this index permitted identification of patients with BTT: DI-BTT was significantly higher in BTT patients (6.93±1.64) and clearly separated them from normal controls (3.43 +/-0.39) and IDA patients (3.71±0.39) with p<0.001. The authors state the utility of this promising and low-cost method requires further confirmation on a larger number of cases [18].

**ID screening in Thalassemia patients**

As it is explained above, efforts have been made to differentiate IDA from thalassemia, and still, a bigger challenge is to find a simple indicator for identifying ID in geographic areas in which thalassemia is prevalent [19]. Hb-ret combined with red blood cell distribution width (RDW) standard deviation (RDW-SD) was found to be powerful in differentiation of thalassemia from IDA [20]. A recent study of 304 female university students with an age range of 18-30 years, all of whom resided in northeast Thailand, revealed 25% had ID and 50% carried the thalassemia gene. Additional formulas derived from the Hb-ret and other parameters were also established and tested for predicting ID. Performance with an AUC of 0.874 was shown by the following formula:

\[
(Hb-ret/RDW-SD) \times 10,
\]

was the best predictor for identifying ID among participants: with an optimal cutoff value less than 6.1, at least 80% of individuals with and without ID were correctly identified (80% sensitivity and 86.5% specificity) [21].

**Monitoring of ID/IDA therapy**

Hb-ret is useful for early measurement of response to iron therapy, increasing on day 2nd after the initiation of iron therapy [22]. It helps monitoring of intravenous and oral iron therapy in adults [6,23], children [24] and infants [25,26].

In a recent study dedicated to evaluation of a low monthly dose of intravenous iron sucrose in peritoneal dialysis patients no difference was found between Hb-ret levels determined in baseline, 1 month after and 3 months after treatment time-points [27]. The study included very small sample number of 18 patients, leading to high SD values. It is unclear whether it was possible to stratify patients and be able to see the difference (as the Hb level raised up significantly) or the Hb-ret levels stability was due to measuring long periods after iron supplementation.

During oral iron therapy only small proportion of ingested iron is absorbed, necessitating higher intakes, which may result in adverse side effects, reduced compliance, and inefficient repletion of iron stores through hepcidin
activation. Increasing the absorption of iron could be a strategy for solving these problems.

A study in female iron-deficient athletes showed that group with daily intake of $10^{10}$ colony forming units of probiotic strain Lactobacillus plantarum 299v (Lp299v, LP299V<sup>®</sup>) in addition to iron (LpFe) displayed a tendency towards higher ferritin levels after the first 4 weeks compared to iron alone intake. Over the 12-week study, both groups increased their iron status, demonstrated by a 70% increase in plasma ferritin after intake of Lp299v with 20 mg of iron as compared to 42% after 20 mg iron alone, but the difference between groups did not reach statistical significance. LpFe use led to the same Hb-ret rise as iron alone after the first 4 weeks, but showed tendency towards higher Hb-ret levels over the 8 and 12-week study with $p=0.275$ and $p=0.083$ respectively [28].

Iron supplements and erythropoiesis-stimulating agents (ESAs) are used for treating anemia in the majority of patients undergoing hemodialysis (HD). The effective use of ESAs strictly demands preventing of both: ID and excess of iron. Thus, maintaining optimum iron levels is a critical challenge. The study of 181 patients, who were undergoing maintenance HD and were administered recombinant human erythropoietin, indicated Hb-ret values allow to find optimal levels of serum ferritin, as Hb-ret showed dependence upon in vivo iron levels up to a certain level, and remained unaffected at levels above these [1].

**Hb-ret in patients with other underlying pathologic conditions**

Very frequent extra-intestinal manifestation of inflammatory bowel disease (IBD) is IDA, which is associated with a poorer outcome. IDA in IBD patients linked to impaired iron absorption due to chronic inflammation, bowel resection (especially in Crohn’s disease), malnutrition and chronic blood loss. ID diagnosis and further management in these patients are difficult because of ferritin elevation related to inflammation and the possibility of anemia of chronic disease. Examining 123 anemic patients with IBD showed that among different biomarkers Hb-ret has the best performance in ID diagnosis, considering a cutoff of 30.0 pg with an AUC of 0.858, sensitivity of 76.8% and specificity of 99.8% [29].

Assessing Hb-ret in the detection of ID was recently proposed in chronic hepatitis C patients [30] and in patients with Helicobacter pylori infection [31].

Hb-ret is recommended as an easy and affordable tool for the assessment of IDA in childhood cancer during chemotherapy treatment. Cutoff level of 28 pg was suggested after a prospective study included 100 pediatric patients [32].

**Pneumonia**

Very interesting application of Hb-ret was suggested by M. Schoorl and co-authors based on Hb-ret being a sensitive indicator of monitoring short-term deteriorations in functional iron supply for erythropoiesis [33,34]. The authors remind that in anemia of inflammation a protective mechanism activates retention of iron from the blood circulation as an essential pathogens growth factor, whilst increasing the efficacy of cell mediated immunity. Results of a study of 75 subjects with community-acquired pneumonia demonstrated a temporary impairment of Hb-ret with a decline of 3-8% within the time period elapsing from hospital admission until day 4 and a significant increasing trend towards values within the reference range at day 14. These alterations were registered all together with temporary increase of cytokine-stimulated serum hepcidin-25 (studied in 25 patients) – hepatocellular peptide hormone that regulates iron redistribution during inflammation [34].

**Conclusion**

Hb-ret is an effective and widely available Hb synthesis status indicator that permits ID/IDA diagnosis and monitoring in all age groups with or without underlying diseases, including beta thalassemia. The local study of cutoff values might be helpful for accurate diagnosis resulting in perspective in raising the life quality and reducing the cost of patients care. The possibility of Hb-ret use as a biomarker, while monitoring anemia of inflammation and predicting pneumonia prognosis, has to be studied.


References


28. Axling U, Önnig G, Combs MA, et al. The Effect of Lactobacillus plantarum 299v on Iron Status and...


Дополнительная информация [Additional Info]

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, о которых необходимо сообщить в связи с публикацией данной статьи. [Conflict of interests. The authors declare no actual and potential conflict of interests which should be stated in connection with publication of the article.]

Участие авторов. Ерёмина Ю.О. – оформление, сбор и анализ материала, написание текста, Магалеш К. – концепция и редактирование. [Participation of authors. Y.O. Eremina – design of the review, collection and analysis of material, writing the text, C. Magalhães – concept of the review and editing.]

Информация об авторах [Authors Info]

*Ерёмина Юлиана Олеговна – к.м.н., специалист в области клинической патологии, ассистент отделения клинической патологии, Больница Педро Ишпану, Местное отделение здравоохранения Матозиньюш, Португалия; сотрудник исследовательской группы по экологической и лабораторной эпидемиологии, Департамент охраны окружающей среды, Институт общественного здравоохранения, Матозиньюш, Португалия; сотрудник исследовательской группы по экологической и лабораторной эпидемиологии, Департамент охраны окружающей среды, Институт общественного здравоохранения, Матозиньюш, Португалия; сотрудник исследовательской группы по экологической и лабораторной эпидемиологии, Департамент охраны окружающей среды, Институт общественного здравоохранения, Матозиньюш, Португалия; сотрудник исследовательской группы по экологической и лабораторной эпидемиологии, Департамент охраны окружающей среды, Институт общественного здравоохранения, Матозиньюш, Португалия.*


Время принятия/Accepted: 01.12.2020


Поступила/Received: 30.11.2020