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Reticulocyte Indices in Diagnosis and Control of Effectiveness of Treatment of Iron-Deficiency Conditions in Children

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High prevalence of iron-deficiency conditions (IDC) in children dictates the need in searching for sensitive, accurate and simple methods of identifying such diseases on early stages of development. New methods of laboratory screening diagnosis are especially needed. The article presents an analysis of new methods of IDC laboratory diagnosis – hematological and biochemical. It presents a discussion of role of a new parameter – mean reticulocyte hemoglobin content (CHr) – for IDC diagnosis and treatment effectiveness control. The article presents data on CHr sensitivity and specificity as compared with the other hematological parameters frequently used for IDC diagnosis.
Keywords: *children, iron deficiency, hematological parameters, mean reticulocyte hemoglobin content, iron preparations.*

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Terminological issues

The terms used in Russian and foreign literature to define iron deficiency-related conditions differ. Russian literature employs term “iron-deficiency conditions” (IDC), i.e. latent iron deficiency (LID) and iron deficiency anemia (IDA). Foreign literature employs term “iron deficiency” (ID), which is divided into ID without anemia (analogous to LID in Russia) and ID with anemia (analogous to IDA in Russia). LID is not a disease; most authors see it as a functional disorder, which is why it is usually classified as E61.1 in accordance with the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) [1]. LID may conclude with recovery and normalization or further progression of ID and development of IDA. Unlike LID, IDA is a separate nosological form classified as D50 in ICD-10 [1].

Epidemiology of iron-deficiency conditions

IDC – the most widespread pathology among children negatively affecting health. According to experts of the World Health Organization, there are 600 mn preschool- and school-aged children diagnosed with anemia around the world; presumably, at least 50% of these cases of anemia are

iron-deficient [2]. Experts have determined that if the IDA incidence rate is 5%, then the prevalence of ID is 15%; if the IDA incidence rate is 20%, then the prevalence of ID is 50%; if the IDA incidence rate is 40%, then the prevalence of ID is close to 100% [3].

Relevance of the issue

Identification and treatment of IDC patients on early stages may prevent development of adverse consequences [3, 4]. That is why it remains relevant to seek methods of timely and effective diagnosis of IDC among children, as well as of adequate treatment of such conditions and control of its effectiveness.

The primary clinical sign of both LID and IDA is Plummer-Vinson syndrome, which involves dystrophic alterations of skin and its appendages, mucosal atrophy, taste perversion, parosphresia, myalgia, hypomyotonia and enuresis. IDA differs from LID with anemic syndrome: skin and mucosal pallor, anorexia, physical and mental fatigability, performance decrement, vertigo, tinnitus, muffled heart tones, systolic murmur on auscultation of the hear [13].

The studies conducted by Russian scientists show that it is impossible to diagnose IDC in children using only clinical signs of the disease as most symptoms of both anemia and sideropenia are characterized by extremely low sensitivity (Se) [4]. That is why clinical practitioners use laboratory methods, that are being constantly improved thanks to the development of new devices and methods, for IDC diagnosis. The methods that allow not only diagnosing IDC, but also controlling iron preparation treatment effectiveness attract special interest.

Laboratory diagnosis of iron-deficiency conditions

IDA diagnosis is based primarily on identification of microcytic (anisocytosis, microcytosis), hypochromic (blood color index [BCI] ≤ 0.85), normal and hyporegenerative (reticulocyte count 1-2%) anemia by means of complete blood count.

Complete blood count is the most widely used laboratory screening method of IDC diagnosis in children. Wide use of automatic hematology analyzers in clinical laboratory diagnosis resulted in the development of new possibilities of IDC diagnosis – determination of red blood cell and reticulocyte indices. The following hematological indicators are used especially often for IDC diagnosis, especially in ambulatory practice:

- hemoglobin concentration;
- hematocrit;
- blood color index;
- red blood cell (RBC) count;
- red blood cell indices – mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW).

It appeared that hematological indicators have certain disadvantages. First of all, red blood cell indices characterize mature erythrocytes, i.e. an IDC must persist for a sufficiently long time to alter them as peripheral blood erythrocyte lifespan is ca. 120 days. Hemoglobin concentration and hematocrit volume also change only at later stages of ID when IDA develops.

IDC are confirmed by means of biochemical indicators

- serum iron (SI) concentration decrease (< 12.5 mcmol/l);
- increase in total iron binding capacity (TIBC) of serum (> 69 mcmol/l);
- serum ferritin (SF) concentration decrease (< 30 mcg/l).

Biochemical indicators may be used as diagnostic (confirmatory) tests, although not as screening tests for IDC identification, as they do not feature sufficiently high sensitivity [4]. Moreover, diagnostic value of most biochemical indicators (especially of SF) decreases considerably at inflammatory diseases; biochemical indicators are prone to diurnal variations (SI), depend on food intake, require venous blood collection [4, 5]. Other significant disadvantages of biochemical tests are complexity and relatively high cost [5].
Several new biochemical indicators have been proposed for ID diagnosis in recent years. One of the discussed parameters is the concentration of soluble transferrin receptors (sTFR), which increases in case of IDC and is used to identify erythropoiesis [6]. Absence of sTFR concentration changes in the setting of an infection or an inflammation is advantageous in comparison with determining SF concentration [6]. However, this method is not always available [4] and therefore cannot be used in ambulatory practice.
The zinc-protoporphyrin (ZPP) level is a relatively new indicator; it is the first biochemical sign of erythrocyte changes when body iron stores start to dwindle [7], which is why ZPP is used as a screening test for IDC diagnosis. Rapidity and ease of use, low cost and high sensitivity (80.6%) facilitate the use of this method [8-10]. However, this method has not gained wide spread yet.
In difficult cases, differential diagnosis of anemias requires determining serum blood hepcidin concentration. Hepcidin is a hepatocyte-synthesized protein, the primary iron homeostasis regulator, as it inhibits iron absorption in the small intestine and release from macrophages and hepatocytes [6, 11, 12].

Treatment effectiveness control: iron-deficiency conditions

Along with early IDC diagnosis, it is highly important to control effectiveness IDC treatment with iron preparations. Early (up to 1 month of treatment with iron preparations) and late (after 1 month and up to 6 months of treatment) criteria are used for this purpose.
Early effectiveness criteria of therapy with iron preparations include [13, 14]:
• mean reticulocyte hemoglobin content (CHr) increase;
• increase in the CHr/MCH difference;
• appearance of an immature reticulocyte fraction (IRF);
• treatment with iron preparations (reticulocyte reaction);
• increase in the concentration of hemoglobin by 10 g/l and of hematocrit by 3% by the end of treatment week 4.

Reticulocyte indices are the earliest criteria of response to therapy with iron preparations; the use thereof for evaluating treatment effectiveness allows not waiting for the hemoglobin content increase for 1 month.
Late effectiveness criteria of therapy with iron preparations include [13, 14]:
• disappearance of clinical symptoms of the disease in 1-3 months of treatment;
• termination of tissue sideropenia in 3-6 months of treatment (depending on anemia severity) determined by SF concentration normalization (> 30 mcg/l).

Reticulocyte indices in diagnosis and control of effectiveness of treatment of iron-deficiency conditions in children

Hypochromic erythrocytes develop in bone marrow at ID; erythrocyte progenitors – reticulocytes – are also hypochromic. Modern hematology analyzers allow not only determining the total number, but also evaluating quality parameters of reticulocytes – the so called reticulocyte indices [15]. It is widely accepted that CHr adequately reflects the condition of erythropoiesis as reticulocytes transform into erythrocytes in peripheral blood in 1.5-2 days and contain the amount of hemoglobin synthesized within them in the previous 60 hours [16, 17].

Determination of reticulocyte indices became possible with the development of modern hematology analyzers and is based on fluorescent staining allowing identifying nucleic acids in cell cytoplasm. Registration of fluorescent signals of various intensity allows dividing reticulocytes into fractions of low (mature reticulocytes), medium and high fluorescence. Reticulocyte maturity index (immature reticulocyte fraction [IRF]) equals the number of cells of medium and high fluorescence. Some practitioners use IRF to monitor effectiveness of treating iron deficiency anemia. It has been confirmed that IRF increase precedes increase in the total number of reticulocytes in the range of a few days [16, 18].

B.H. Davis and N.C. Bigelow suggested calculating reticulocyte maturity index (IRF) depending on the RNA content (a parameter reflecting erythropoiesis effectiveness) as early as in 1989 [19]. IRF is used to monitor bone marrow regeneration after hemopoietic stem cell transplantation and intensive chemotherapy, to monitor IDC treatment, vitamin B_{12} or folic acid deficiency, to track the toxic effect chemotherapeutic agents have on bone morrow, to classify and identify anemia pathogenesis and to detect aplastic crises [20].

CHr is the most attention-worthy reticulocyte index; this parameter allows evaluating the current provision of bone marrow with iron, reflects hemoglobin synthesis in bone marrow erythrocyte progenitors and availability of iron for erythropoiesis [21]. CHr was first used in clinical practice for controlling effectiveness of therapy with parenteral iron preparations on early stages – as early as on therapy day 4 [22]. Later, specialists started using this parameter in nephrology as an iron deficiency erythropoiesis indicator in the setting of erythropoietin treatment of patients with the anemia developed in the setting of renal failure [23].

Further studies have showed that CHr is a sensitive method allowing revealing ID and assessing response to treatment with iron preparations on early stages of treatment. Thus, according to the study conducted by C. Ullrich et al., CHr has high sensitivity and specificity (Sp) for ID diagnosis in infants: at the optimal cut-off point of 27.5 pg the test sensitivity is 83%, specificity – 73% [5]. C. Brugnara et al. examined 1,500 blood samples of patients undergoing chronic hemodialysis and showed that CHr sensitivity as an ID marker in the optimal cut-off point of 27.2 pg is 93.3%, specificity – 83.2% [24]. CHr reduction indicates that erythropoiesis is iron-deficient and serves as a sensitive hematological indicator of LID in infants [5, 24]. Moreover, several studies showed that CHr may be used for differential diagnosis of IDA and other types of anemia (e.g. anemia at chronic diseases and their combinations) [25].

Unlike biochemical indicators, reticulocyte indices are not prone to diurnal variations, do not depend on food intake [5] and determination thereof does not require collecting additional blood besides the blood necessary for complete blood count using an automatic hematology analyzer [5]. Moreover, reticulocyte indices are determined using automatic hematology analyzers along with other parameters, so no additional expenses are required. All these advantages make use of reticulocyte indices as screening tests for IDC identification rather attractive. The fact that not all automatic hematology analyzers, but only the last generation models are capable of determining reticulocyte indices is a disadvantage of this method. Moreover, their specificity is limited by the possibility of other hematologic diseases accompanied by hypochromic anemia, such as thalassemia or sideroblastic anemia [5].

Comparison of indicators of hematological tests used to diagnose and control treatment of iron-deficiency conditions

We studied 337 11-17-year-old adolescents (median age – 15 years) studying in middle school and senior grades of Moscow general education schools. All study subjects underwent complete blood count using an automatic hematology analyzer and determination of biochemical indicators – iron transferrin saturation coefficient. ITS $\leq$ 16% was used as an ID criterion. IDA was observed in 5.3% of the examined adolescents, ID – in 17%.

Our study showed that CHr is the most accurate test out of all the hematological indicators more frequently used to diagnose ID in adolescents. This test featured the highest Youden's index (J),

which defines the aggregate estimate of sensitivity and specificity, as well as the area under the curve (AUC; table) out of all hematological indicators [26].

Table. Overall accuracy/effectiveness of hematological tests for diagnosing iron-deficiency conditions in adolescents

Indicator	AUC (95% CI)	*p*	Cut-off point	Se, %	Sp, %	J	E, %
Hb	0.724 (0.658-0.790)	0.001	125.5 g/l	41.3	92.7	34	81.3
Ht	0.695 (0.626-0.763)	0.001	39.1%	57.3	71.4	28.7	68.2
RBC	0.583 (0.511-0.656)	0.028	4.53×10^{12}/l	44	71.8	15.8	65.6
BCI	0.725 (0.656-0.794)	0.001	0.86	58.7	74	32.7	70.6
MCV	0.648 (0.573-0.723)	0.001	82 fL	44	83.2	27.2	74.5
MCH	0.729 (0.660-0.797)	0.001	28.6 pg	62.7	71.8	34.5	69.7
MCHC	0.720 (0.653-0.788)	0.001	339.5 g/l	69.3	64.1	33.4	65.3
Reticulocytes	0.484 (0.431-0.577)	0.671	0.67%	41.6	62.7	4.3	57.9
CHr	0.733 (0.659-0.806)	0.001	32.1 pg	61.3	82.1	43.4	77.4

Effectiveness of treatment with iron preparations is usually evaluated 1 month after the beginning of such treatment on the basis of increase in the concentration of hemoglobin and hematocrit [3, 13, 14]. It is often infeasible to correct and identify such situations as erroneous diagnosis of IDA, inadequately selected iron preparation dose and iron saturation problems due to relatively late evaluation of therapy effectiveness. As reticulocyte indices and CHr in particular reflect the condition of erythropoiesis in the latest 1-2 days, they may be used for adequate evaluation of effectiveness of therapy with iron preparations as early as in the first days of such therapy. According to several studies, this parameter increases within the first 48 hours of such therapy and is ahead of the increase in the number of reticulocytes by a few days if treatment with intravenous iron preparations is effective [15]. Treatment correction on early stages allows avoiding negative consequences of iron preparation taking in case of an erroneously diagnosed IDA, timely prescribing additional examinations, as well as avoiding additional treatment expenses [14].

Conclusion

Use of new effective simpler and cost-effective IDC indicators in children, particularly, of reticulocyte indices allows revealing ID and starting treatment on the earliest stages, which may in the end help to avoid negative health consequences of anemia in children. Moreover, these indicators may be used to appraise effectiveness of treatment as early as in the first days of iron preparation taking and, if necessary, to timely correct the treatment pattern.

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Conflict of interest

The authors declared they have no competing interests to disclose.

References

1. *Mezhdunarodnaya statisticheskaya klassifikatsiya bolezneĭ i problem, svyazannykh so zdorov'em (MKB-10). Desyatyĭ peresmotr.* Vol. 1. Geneva: WHO; 1995. 698 p.
2. Guideline: Intermittent Iron Supplementation in Preschool and School Age Children. Geneva: World Health Organization. 2011. Available from:

http://www.who.int/nutrition/publications/micronutrients/guidelines guideline_iron_supplementation_children/en/(available:27.12.2015).
3. UNICEF. United Nations University. Iron deficiency anemia: assessment, prevention and control. A guide for programme managers. Geneva: World Health Organization. 2001 (WHO/NHD/01.3). 114 p. URL: http://www.who.int/nutrition/publications/micronutrients/anaemia_ iron_deficiency/WHO_NHD_01.3/en (available: 27.12.2015).
4. Tarasova IS. *Razrabotka i nauchnoe obosnovanie skrininga zhelezodefitsitnykh sostoyaniĭ u podrostkov*. Avtoref. dis ... dok. med. nauk. Moscow; 2013. 67 p.
5. Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C, Shapiro D, Bernstein H. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. *JAMA*. 2005;294(8):924–930.
6. Pavlov AD, Morshchakova EF, Rumyantsev AG. *Eritropoez, eritropoetin, zhelezo*. Moscow: GEOTAR-Media. 2011. 304 p.
7. Labbe RF, Rettmer RL. Zinc protoporphyrin: a product of iron deficient erythropoiesis. *Semin Hematol*. 1989;26(1):40–46.
8. Blumberg WE, Eisinger J, Lamola AA, Zuckerman DM. The hematofluorometer. *Clin Chem*. 1977;23(2Pt.1):270–274.
9. Labbe RF, Vreman HJ, Stevenson DK. Zinc protoporphyrin: a metabolite with a mission. *Clin Chem*. 1999;45(12):2060–2072.
10. Rettmer RL, Carlson TH, Origenes ML, Jack RM, Labbe RF. Zincprotoporphyrin/hemeratio for diagnosis of preanemic iron deficiency. *Pediatrics*. 1999;104(3):e37.
11. Mayanskiĭ NA, Semikina EL. Geptsidin: osnovnoĭ regulyator obmena zheleza i novyĭ diagnosticheskiĭ marker. *Voprosy diagnostiki v pediatrii*. 2009;1(1):18–23.
12. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113(9):1271–1276.
13. Recommendations to prevent and control iron deficiency in the United States. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 1998;47(RR-3):1–29.
14. Rumyantsev AG, Zakharova IN, Chernov VM, Tarasova IS, Zaplatnikov AL, Korovina NA, Borovik TE, Zvonkova NG, Machneva EB, Pudrikov KA. *Diagnostika i lechenie zhelezodefitsitnoĭ anemii u deteĭ i podrostkov. Posobie dlya vracheĭ.* Ed by AG Rumyantsev, IN Zakharova. Moscow: Konti Print; 2015. 76 p.
15. Buttarello M, Plebani M. Automated blood cell counts: state of the art. *Am J Clin Path*. 2008;130(1):104–116.
16. Baranov AA, Semikina EL, Mel'nichuk OS, Gordeeva OB, Namazova-Baranova LS, Morozova NA, Kozhevnikova OV, Gevorkyan AK, Mayanskiĭ NA. Pokazateli retikulotsitarnykh indeksov u zdorovykh deteĭ. *Voprosy diagnostiki v pediatrii*. 2010;2(4):17–21.
17. Papayan AV, Zhukova LYu. Anemii u deteĭ: rukovodstvo dlya vracheĭ. SPb.: Piter; 2001. 384 p.
18. Torres Gomez A, Casano J, Sanchez J, Madrigal E, Blanco F, Alvarez MA. Utility of reticulocyte maturation parameters in the differential diagnosis of macrocytic anemias. *Clin Lab Haematol*. 2003;25(5):283–288.
19. Davis BH, Bigelow NC. Flow cytometric reticulocyte quantification using thiazole orange provides clinically useful reticulocyte maturity index. *Arch Pathol Lab Med*. 1989;113(6):684–689.
20. Chang CC, Kass L. Clinical significance of immature reticulocyte fraction determined by automated reticulocyte counting. *Am J Clin Pathol*. 1997;108(1):69–73.
21. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. *Am J Hematol*. 2008;83(4):307–310.

22. Brugnara C, Laufer MR, Friedman AJ, Bridges K, Platt O. Reticulocyte hemoglobin content (CHr): early indicator of iron deficiency and response to therapy. *Blood*. 1994;83(10):3100–3101.
23. Cazzola M, Mercuriali F, Brugnara C. Use of recombinant human erythropoietin outside the setting of uremia. *Blood*. 1997; 89:4248–4267.
24. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron deficient states. *Clin Lab Haem*. 2006;28(5):303–308.
25. Baranovskaya IB, Onishchuk SA. Gemoglobin retikulotsitov v differentsial'noĭ diagnostike anemiĭ. *Vestnik Orenburgskogo gosudarstvennogo universiteta*. 2008;81:129–134.
26. Machneva EB, Zakharova IN, Tarasova IS, Chernov VM, Lazareva SI. Srednee soderzhanie gemoglobina v retikulotsite - tochnyĭ pokazatel' defitsita zheleza u podrostkov. *Pediatriya. Zhurnal im. G. N. Speranskogo.* 2015;94(6):33–38.