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ORIGINAL ARTICLE

Usefulness of Reticulocyte Parameters for Diagnosis of Hereditary Spherocytosis in Children

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Abstract Innovations in laboratory equipment have enabled a widening of the spectrum of hematological parameters obtained from single measurements of peripheral blood samples, including reticulocyte parameters. The usefulness of reticulocytes indices to confirm the diagnosis of pediatric anemia was analyzed in this study. The study group consisted of 163 children, aged 1 month–17 years, with anemia. Complete blood count extended with an analysis of reticulocyte parameters were measured using a Beckman Coulter LH 750. The mean sphered corpuscular volume (MSCV) in the group of children with hereditary spherocytosis (HS) was 66.71 ± 8.45 fL, whereas in other anemic patients MSCV was 87.76 ± 11.22 fL, $p < 0.0001$. In HS children the average mean corpuscular volume of red blood cells was higher than the MSCV value, while an inverse correlation was observed in the group of children with other anemias, $p < 0.0001$. A significant difference was found between the ratio of absolute reticulocyte count and IRF fraction (Ret#/IRF)—0.6 ± 0.28 in the HS group and 0.23 ± 0.16 in the non-HS group, respectively. Our results suggest that analysis of reticulocyte parameters is useful in the diagnosis of anemia and should be included in the routine CBC analysis in anemic children.

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Keywords Anemia · Hereditary spherocytosis · Mean sphered corpuscular volume · Reticulocyte

Introduction

Innovation in automated hematological analyzers has enabled the spectrum of hematological parameters obtained from single measurements of peripheral blood samples to be widened [1]. Significant improvements have been made in reticulocyte analysis. Enumeration of reticulocytes has enhanced measurement of reticulocyte volume, content of hemoglobin, and differentiation into reticulocyte subpopulations at different stages of maturation, etc. Extended red blood cell analysis has been found to be a useful tool in detecting functional iron deficiency or in the diagnostics of other types of anemias [2–17]. A significant increase in absolute reticulocyte count has been found to be a reliable marker of early response to iron deficiency treatment [12]. Mean hemoglobin content in reticulocytes (CHr), also known as Ret-He, is a sensitive marker of iron deficiency [3, 4, 9, 11, 14], including iron deficiency in chronic kidney disease [7], as well as an indicator of successful response to iron supplementation therapy [12, 18]. Moreover, CHr has been included into the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Guidelines for the monitoring of recombinant erythropoietin therapy in patients with renal disorders [11]. The limitation of usefulness of CHr is in thalassemia trait subjects, in whom the hemoglobin content in reticulocytes is decreased regardless of iron accumulation status [11]. Moreover CHr depends on the instrument used. Mean reticulocyte volume (MRV) and the red blood cell size factor counted as $\sqrt{MCV \times MRV}$, where MCV is the mean corpuscular red

blood cell volume, has been proved to be a sensitive marker of inefficient erythropoiesis in β-thalassemia and iron deficiency anemia (IDA) [13, 15]. Modern hematology analyzers combine classical hematological methods of peripheral blood analysis (i.e. impedance method) with more advanced flow cytometric techniques using fluorescent dyes for differentiation of single cells. Based on RNA staining in reticulocytes, automated hematology analyzers are able to separate reticulocytes with different nucleic acid contents. Immature reticulocyte fractions (IRF) are cells with high RNA levels, which are recognized as less mature reticulocytes. Depletion of iron stores induces immature reticulocyte release from bone marrow [16]. The parameter which is suggested to be the most reliable of all red blood cell indices in identifying hereditary spherocytosis (HS) samples is a result of the measurement of erythrocyte volume in hypo-osmotic solution [2, 19]. The obtained mean sphered corpuscular volume (MSCV) in regular red blood cells is higher than mean corpuscular volume (MCV) measured under iso-osmotic conditions. Contrary, MSCV of spherocytes is lower than MCV, due to the decreased ratio of cell surface area to cell volume, which causes that spherocytes in the hypo-osmotic environment hemolyse and their MSCV value decreases [2].

Hereditary spherocytosis is an inherited disorder of red blood cells which results in cytoskeleton protein deficiency [2, 6, 8, 20]. The consequence of a lack of α- and β-spectrin, ankyrin, band 3 protein, protein 4.2 and/or other erythrocyte plasma membrane proteins is a characteristic red blood cells shape [8, 20, 21]. Spherocytes are small, globulous cells which are typical for HS subjects; however, their presence can also be detected in other cases of hemolytic anemias; thus, their occurrence cannot be the basis of hereditary spherocytosis diagnostics. Recently, guidelines for HS diagnosis were published [20]. The key recommendations include spherocyte presence in peripheral blood smears, increased mean corpuscular hemoglobin content (MCHC) and increased reticulocyte count as being sufficient in the diagnostics of patients with a family history of HS and typical symptoms of the disease. If the diagnosis is unclear, diagnostics should be widened by screening tests (maleimide-5′-binding assay or cryohemolysis) [2, 20]. In atypical cases, gel electrophoresis of the RBC membrane should be performed [20]. Here, we are trying to establish the usefulness of reticulocyte parameters in the diagnosis of hereditary spherocytosis in children.

The purpose of the present study is an evaluation of the usefulness of reticulocyte parameters in anemia diagnostics in children.

Materials and Methods

Patients

As many as 163 peripheral blood hematology results from children aged 1 month–17 years, either boys or girls, with anemia were included in the retrospective analysis. Complete blood counts with reticulocyte parameter analysis were performed in the Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Clinical Pediatric Hospital of the Medical University of Warsaw (Poland). Peripheral blood samples were collected into tubes containing EDTA, and were analyzed within 2 h after blood collection. All data were acquired with a Beckman Coulter LH750 (Beckmann Coulter, Miami, USA).

The study group consisted of 7 main groups of anemic patients, for whom different diagnoses had been established. The patient groups were hereditary spherocytosis (n = 17), iron deficiency anemia (n = 16), first quarter of life anemia (n = 54), hemolytic anemia (excluding HS) (n = 17), β-thalassemia (n = 12), chronic kidney disease (n = 16) and other (n = 31). In the "other" group we had children with different diagnosis and concomitant anemia: methemoglobinemia (n = 1), transient erythroblastopenia (n = 2), aplastic anemia (n = 5), neutropenia of infancy (n = 4), acute lymphoblastic leukemia (n = 2), juvenile myelomonocytic leukemia (n = 1), acute kidney injury (n = 1), sickle cell anemia (n = 1), lymphadenitis (n = 1), thrombocytosis (n = 1), bone marrow dysfunction (n = 2), vitamin B12 deficiency (n = 2), chronic posthemorrhagic anemia (n = 1), and anemia of undefined etiology (n = 7). Diagnostic tests used to confirm the type of anemia are presented in Table 1.

In the present study, we separated a group of children with anemia of the first quarter of life. Mainly, these were children with iron deficiency and hemoglobin concentrations less than 9 g/dL aged 1–3 months of life. These individuals suffered from anemia as a result of preterm delivery (insufficient iron stores due to the shortened pregnancy). Other causes of first quarter anemia is an anemia occurred in term infants with serological incompatibility, infections and bleeding at birth. The most often cause of insufficient erythropoiesis, besides iron deficiency, is an unsatisfactory erythropoietin production [22].

Children specified as IQA suffered from iron deficiency anemia, however they were analyzed separately from older IDA subjects due to their specific red blood cells indices, which physiology differs from adults red blood cell, especially regarding their volume.

Table 1 Diagnostic tests used for confirmation of anemia type in enrolled subjects

Disease type	Laboratory and clinical test performed for disease confirmation
Hereditary spherocytosis (n = 17)	Peripheral blood smear, presence of spherocytes, EMA binding test, osmotic fragility test, family history
Iron deficiency anemia (n = 16)	Complete blood count, serum iron concentration, serum total iron-binding capacity, serum ferritin concentration
First quarter of life anemia (n = 54)	Complete blood count
Autoimmune hemolytic anemia (n = 17)	Complete blood count, the peripheral blood smear, reticulocyte count, direct antiglobulin test, antiRBC-antibody screening
β-thalassemia (n = 12)	Complete blood count, the peripheral blood smear, hemoglobin electrophoresis, family history
Methemoglobinemia (n = 1)	Complete blood count, the peripheral blood smear, methemoglobin concentration, family history
Erythroblastopenia (n = 2)	Complete blood count, reticulocyte count, bone marrow examination
Aplastic anemia (n = 5)	Complete blood count, reticulocyte count, bone marrow examination
Neutropenia of infancy (n = 4)	Complete blood count, bone marrow examination
Acute lymphoblastic leukemia (n = 2)	Complete blood count, bone marrow examination, bone marrow immunophenotyping
Myelomonocytic leukemia (n = 1)	Complete blood count, bone marrow examination, bone marrow immunophenotyping
Acute kidney injury (n = 1)	Urine analysis, eGFR, electrolytes serum concentration, BUN, creatinine concentration, protein, complete blood count
Chronic kidney disease (n = 16)	Urine analysis, eGFR, electrolytes serum concentration, BUN, creatinine concentration, protein, complete blood count
Sickle cell anemia (n = 1)	Complete blood count, the peripheral blood smear, hemoglobin electrophoresis
Lymphadenitis (n = 1)	Complete blood count, the peripheral blood smear, chest radiography, chest ultrasonography
Thrombocytosis (n = 1)	Complete blood count, bone marrow examination
Bone marrow dysfunction (n = 2)	Complete blood count, reticulocyte count, bone marrow examination, the peripheral blood smear
Vitamin B12 deficiency (n = 2)	Complete blood count, the peripheral blood smear, vit.B12 concentration, increased urine concentration of methylmalonic acid
Chronic posthemorrhagic anemia (n = 1)	Complete blood count, reticulocyte count, the peripheral blood smear
Anemia of undefined etiology (n = 7)	Complete blood count, reticulocyte count, the peripheral smear

Diagnosis were made with accordance to the newest literature guidelines [22–26]

Laboratory Tests

Analysis of complete blood count and reticulocyte parameters were performed using a Beckman Coulter LH750. The parameters of our main interests were red blood cell count (RBC), hemoglobin concentration (Hgb), mean corpuscular volume of red blood cells (MCV), reticulocyte count (Ret), immature reticulocyte fraction (IRF), mean corpuscular volume of reticulocytes (MRV), mean sphered corpuscular volume (MSCV) and high light-scatter reticulocyte count (HLR). For extended analysis a Red Blood Cell size factor (Rsf) was counted as a square root of (MCV × MRV). The LH750 uses an impedance method to measure cell volume and complexity. To measure reticulocyte fractions, a non-fluorescent dye which stains residual RNA within red blood cells was used. Volume, conductivity, scatter (VCS) technology was applied for the measurement of MSCV parameters. As a piece of laboratory equipment used routinely in laboratory practice, daily internal Quality Control (IQC) was run and the lab participated in external Quality Control (QC) for the parameters studies.

Additional biochemical analysis were performed using a Vitros 5600 (Ortho Clinical Diagnostics, Rochester, USA). Measured and reported data were as follows: iron concentration (Fe), ferritin, total iron binding capacity (TIBC), and total bilirubin concentration (TBil).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Prism, San Diego, USA) and Microsoft Office Excel (Microsoft Corporation, Redmond, USA). Data were presented as mean ± standard deviations or means (minimum; maximum). A Mann–Whitney U test was used to evaluate statistical significance for the

parameters with non-Gaussian distribution. A p value of <0.05 was considered significant. A receiver-operator characteristics (ROC) curve was used to determine the cut off values for Ret, delta (MCV–MSCV), Ret/IRF and MRV, to distinguish between HS-patients and non-HS anemic individuals.

Results

Data from basic complete blood count (RBC, Hgb, MCV) as well from reticulocyte parameters analysis (Ret#, IRF, MRV, MSCV, HLR) for 8 selected groups are presented in Table 2. Additional results of biochemistry analysis are presented in Table 3.

Hereditary spherocytosis patients showed increased reticulocyte counts, delta (MCV–MSCV) values and Ret#/IRF ratios when compared with other studied subjects. A contrasting relationship was found with regard to MSCV and MRV values (Fig. 1). The mean value of delta (MCV–MSCV) for HS subjects was 13.68 ± 4.49 fL, whereas non-HS anemia associated patients had a value of -0.58 ± 5.65 fL, $p < 0.0001$. According to the ROC curve analysis, the optimum value of delta (MCV–MSCV) to distinguish between HS and non-HS anemia was 6.9, giving a 91.5 % sensitivity and 94.1 % specificity. One hundred sensitivity would be obtained for the value 13.4; however, the specificity of the test would be significantly decreased to 58.82 %. A specificity of 100 % was achieved when the cut off value was 6.65, although the sensitivity of the test was 90.2 %. Of all the studied parameters, delta (MCV–MSCV) gave a ROC curve with the greatest area under curve, and thus was the most reliable marker for distinguishing between anemic HS and non-HS subjects (Fig. 2). Delta (MCV–MSCV) >10.00 fL was obtained in five children with other hemolytic anemia, three of whom suffered from AIHA (autoimmune hemolytic anemia) and two with undefined hemolytic anemia. In three patients with chronic kidney disease, the delta (MCV–MSCV) values were higher than 8 fL.

It is noteworthy that AUC for MRV as a parameter which could differentiate HS from non-HS patients was 0.9635 with a sensitivity of 87.66 % and specificity of 94.12 %, at a cut off value of 91.15 fL. The specificity of

Table 2 Hematological parameters of different patient groups presented as mean ± SD

	RBC ($\times 10^6$/μL)	Hgb (g/dL)	MCV (fL)	Ret ($\times 10^6$/μL)	IRF	MRV (fL)	MSCV (fL)	HLR ($\times 10^6$/μL)
HS	3.42 ± 0.95	8.88 ± 1.69	80.4 ± 7.78	0.27 ± 0.12	0.41 ± 0.09	81.79 ± 6.58	66.71 ± 8.45	0.09 ± 0.09
IDA	4.61 ± 0.38	10.46 ± 1.41	73.53 ± 7.96	0.05 ± 0.03	0.28 ± 0.07	97.97 ± 6.48	76.74 ± 6.48	0.03 ± 0.04
IQA	3.15 ± 0.58	9.41 ± 1.13	92.47 ± 7.94	0.10 ± 0.04	0.44 ± 0.08	111.73 ± 8.14	94.01 ± 8.50	0.04 ± 0.03
HA	3.35 ± 0.64	10.16 ± 1.69	93.25 ± 11.57	0.17 ± 0.17	0.43 ± 0.12	114.31 ± 21.14	91.80 ± 13.31	0.06 ± 0.11
TM	5.49 ± 0.43	11.87 ± 0.97	69.24 ± 4.46	0.07 ± 0.03	0.27 ± 0.06	90.2 ± 4.57	72.95 ± 4.33	0.03 ± 0.03
CKD	3.90 ± 0.61	11.29 ± 1.30	88.84 ± 4.78	0.07 ± 0.03	0.32 ± 0.06	108.56 ± 9.80	85.64 ± 5.48	0.04 ± 0.03
Other	3.72 ± 0.71	10.56 ± 1.70	88.13 ± 9.86	0.06 ± 0.05	0.33 ± 0.10	114.57 ± 18.49	88.02 ± 10.69	0.05 ± 0.09
IDA and IQA	3.48 ± 0.82	9.65 ± 1.27	88.14 ± 11.24	0.09 ± 0.04	0.40 ± 0.10	108.58 ± 9.69	90.07 ± 10.86	0.04 ± 0.03

HS hereditary spherocytosis, *IDA* iron deficiency anemia, *IQA* anemia of first quarter of life, *HA* hemolytic anemia, *TM* thalassemia minor, *CKD* chronic kidney disease

Table 3 Biochemical parameters of different patient groups presented as mean (min; max)

	Fe (μg/dL)	Ferritin (μg/L)	TIBC (μg/dL)	T Bil (mg/dL)
HS	92.75 (69; 130)	95.88 (37.4; 166.9)	n/a	2.18 (0.6; 3.1)
IDA	41.80 (14; 98)	8.15 (2.6; 20.9)	472.11 (424; 535)	0.33 (0.3; 0.4)
IQA	72.19 (30; 119)	150.36 (7.2; 674)	n/a	4.99 (0.4; 18.3)
HA	95.50 (26; 210)	656.3 (45.1; 4316.3)	n/a	2.32 (0.6; 4.3)
TM	45.67 (16; 65)	20.62 (4.9; 40.7)	349.4 (295; 444)	0.63 (0.2; 1.5)
CKD	86.86 (37; 176)	203 (17.5; 756.1)	274.17 (200; 432)	0.6 (0.3; 0.8)
Other	110.41 (29; 322)	698.08 (6.3; 6147.9)	355.25 (238; 589)	1.14 (0.8; 2)
IDA and IQA	64.95 (14; 119)	102.95 (2.6; 674)	472.11 (424; 535)	4.16 (0.3; 18.3)

HS hereditary spherocytosis, *IDA* iron deficiency anemia, *IQA* anemia of first quarter of life, *HA* hemolytic anemia, *TM* thalassemia minor, *CKD* chronic kidney disease, *n/a* not applicable

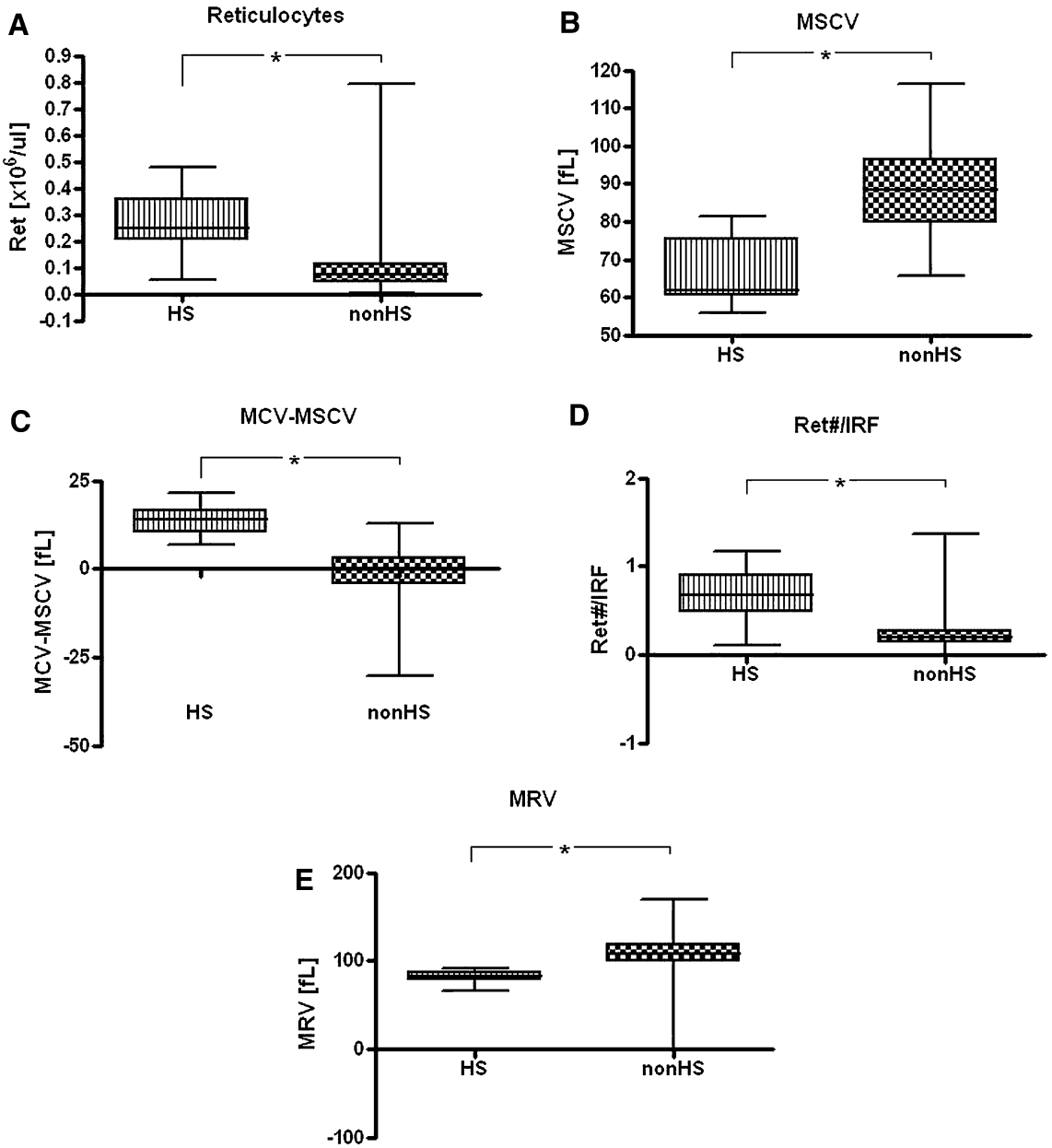

Fig. 1 Comparison of reticulocyte parameters analysis between anemic patients suffering from hereditary spherocytosis (HS) and children with non-HS associated anemia. Statistical significance $*p < 0.0001$. **a** Reticulocyte count, **b** MSCV value, **c** MCV–MSCV value, **d** Ret#/IRF ratio and **e** MRV value

the test increased to 100 % at a cut off value of 92.55 fL; however, sensitivity decreased to 86.36 %.

As they were similar in terms of basic laboratory test results, other hemolytic anemias were compared with hereditary spherocytosis with regard to reticulocyte parameters. We showed that the difference in reticulocyte count between HS and non-HS hemolytic anemia is relevant ($p = 0.015$). Similarly, a significant difference in Ret#/IRF and MRV was shown, although the p values were lower than those obtained when comparing HS and non-HS anemias ($p = 0.013$, and $p = 0.0003$, respectively). Instead, MSCV and delta (MCV–MSCV) were the most sensitive parameters for discriminating between HS subjects and all individuals with hemolytic anemias ($p < 0.0001$ for both analysis).

We analyzed Rsf indices (red blood cell size factor) from iron deficiency anemia subjects and thalassemia minor patients. AUC for IDA individuals was 0.79

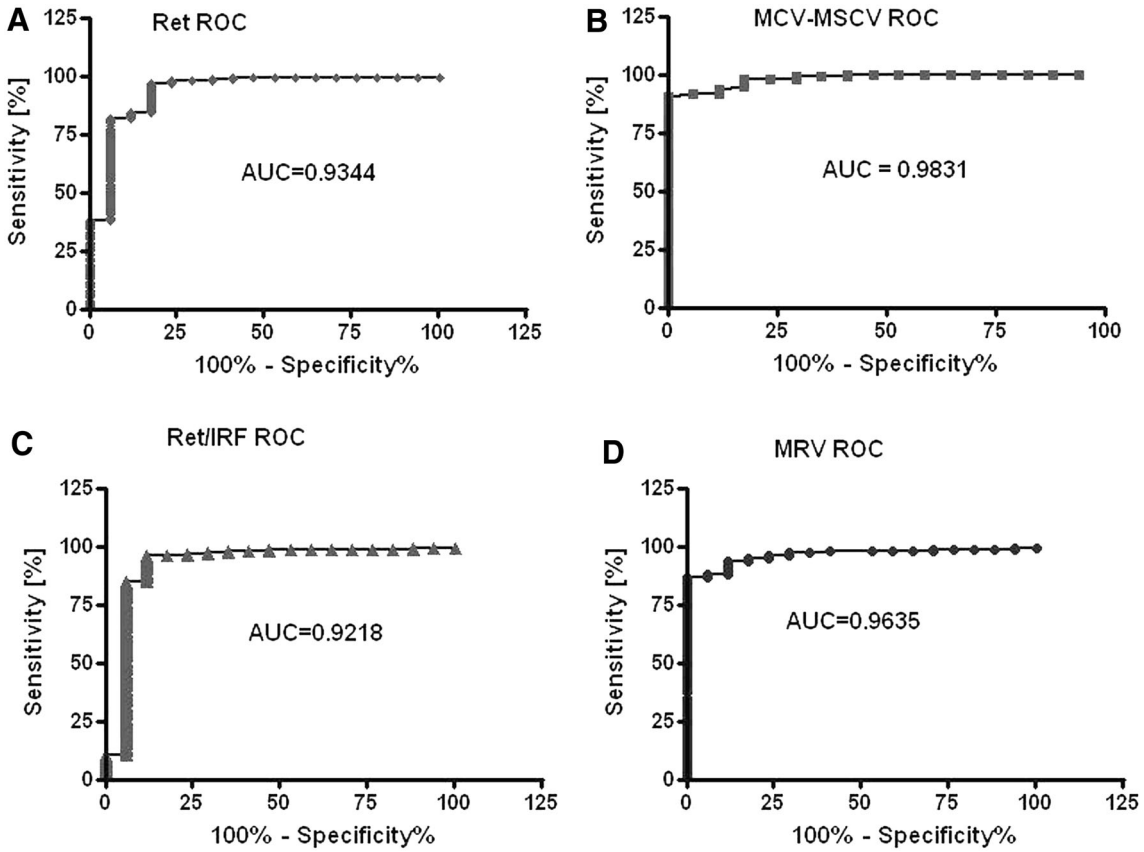

Fig. 2 Receiver-operator characteristic (ROC) curves for **a** reticulocyte count, **b** MCV–MSCV values, **c** Ret#/IRF ratio, and **d** MRV values for discrimination between hereditary spherocytosis and non-HS anemias

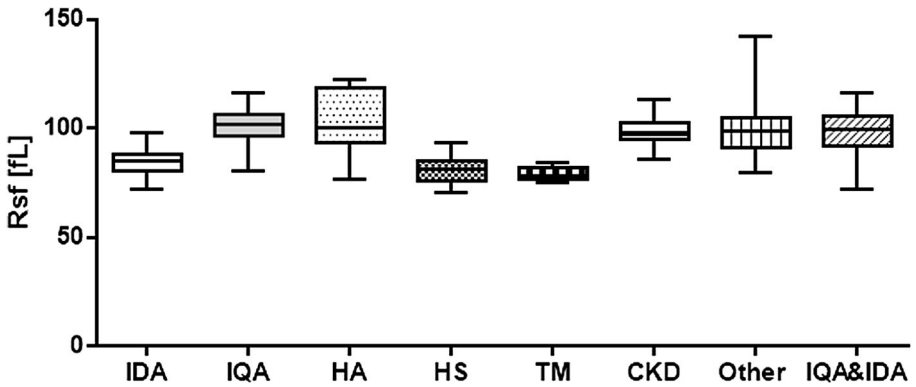

Fig. 3 *Box* and *whisker* plot showing red blood cell size factor (Rsf) distribution in studied groups of anemia patients. The *horizontal line* in the *center* of the *box* shows the median value, and the whiskers show the minimum and maximum values for each group. *IDA* iron deficiency anemia, *IQA* anemia of the first quarter of life, *HA* hemolytic anemia, *HS* hereditary spherocytosis, *TM* thalassemia minor, *CKD* chronic kidney disease

($p = 0.0002$), and for TM 0.92 ($p < 0.0001$), when compared with all other anemic patients. Rsf values for TM subjects were significantly lower than those obtained from IDA subjects ($p = 0.0031$); however, they did not differ from values obtained in hereditary spherocytosis patients ($p > 0.05$) (Fig. 3).

To evaluate the best discriminating reticulocytes indice for all groups of patients, an analysis based on ROC analysis in comparison to other anemias was made. Of all studied anemic groups and reticulocytic parameters valuable results were reached for delta (MCV–MSCV) in HS diagnosis and Rsf in thalassemia diagnosis (Table 4).

Table 4 Comparison of reticulocytes indices with the highest discriminating power for all studied types of anemia

Type of anemia	Reticulocyte indice	Cut-off value (fL)	Sensitivity (%)	Specificity (%)	AUC	*p*
HS	Delta (MCV–MSCV)	6.90	91.5	94.1	0.9831	<0.0001
IDA	Rsf	89.57	87.5	73.2	0.7941	0.0001
IQA	MSCV	88.65	81.48	73.04	0.8041	<0.0001
HA	MRV	111.1	61.11	68.26	0.6657	0.0210
TM	Rsf	82.99	91.67	87.26	0.9257	<0.0001
CKD	Delta (MCV–MSCV)	1.45	81.25	58.82	0.6599	0.0355
IDA and IQA	Delta (MCV–MSCV)	0.80	65.71	59.60	0.6807	<0.0001

HS hereditary spherocytosis, *IDA* iron deficiency anemia, *IQA* anemia of first quarter of life, *HA* hemolytic anemia, *TM* thalassemia minor, *CKD* chronic kidney disease, *AUC* area under curve, *p* statistical significance

Discussion

We conducted an assessment of the red blood cells and reticulocyte indices available on the Beckman Coulter LH750 analyzer, regarding their value in hereditary spherocytosis diagnostics. Several researchers have already proved a high sensitivity and specificity of MSCV and delta (MCV–MSCV) in HS screening [2, 6, 19], but none of these studies were exclusively performed in pediatric populations. We found that delta (MCV–MSCV) is the best marker for discriminating between HS and non-HS children, similar to the situation for adult populations. Recently, Tao et al. [27] found that sensitivity of this parameter is significantly higher than mean corpuscular hemoglobin concentration (MCHC) in discriminating between HS and non-HS subjects. The sensitivity of MCHC in HS screening depends on total hemoglobin concentration, and in anemic subjects it decreases. MSCV, as a factor dependent on the availability of cells to incorporate hypoosmotic solutions, is more reliable and stable indice than mentioned MCHC.

Chiron et al. [19] were the first to describe the usefulness of delta (MCV–MSCV) and obtained a value higher than 5 fL for 85 % HS patients, and a value higher than 10 fL for 71 % of HS subjects. Broseus et al. [2] obtained a delta (MCV–MSCV) >5 fL for 100 % HS subjects, and a value higher than 10 fL for 97 % of these. In our pediatric study, all HS children had delta (MCV–MSCV) >5 fL and 82 % had a value higher than 10 fL. Lazarova et al. [6] proposed a cut-off value for delta (MCV–MSCV) >10.4 fL with a specificity of 74 % and a sensitivity of 100 %, and >18.1 fL with a specificity of 94 % and a sensitivity of 92 %. Despite the slight difference in cut-off value, AUC values obtained in our study and those cited were comparable [6]. In the present study, we obtained a better laboratory specificity and sensitivity, which allows for a decrease in the cut off value to 6.9 fL. Interestingly, in our study almost 44 % of all non-HS included subjects had a delta (MCV–MSCV) value higher than 0 fL. This is a significantly higher number (over two times higher) than those obtained in other studies; Broseus et al. [2] found positive values for (MCV–MSCV) in 24 % of non-HS subjects, and Chiron et al. [19] in 7 %. Of all non-HS subjects children with hemolytic anemia had the highest values of delta (MCV–MSCV): 71 % a had positive value of the analyzed parameter with an average 1.45 fL. This finding remains in line with the results of other researchers [2, 19]. A surprising result of delta (MCV–MSCV) analysis is that subjects suffering from anemia resulting from chronic kidney disease had the highest average values for the studied parameter (3.2 fL); notably, 87.5 % (14/16) had positive (MCV–MSCV).

As mentioned above, MSCV is the volume of red blood cells under the hypo-osmotic condition. In hereditary spherocytosis, a loss of membrane surface area is caused by an abnormal protein content in erythrocytes membrane [6]. Similar behavior in the hypo-osmotic solution of RBCs from patients suffering from chronic kidney disease might be explained by altered protein compositions in their plasma membrane. Costa et al. [28] found that RBCs from patients with chronic renal failure show a reduction of spectrin and ankyrin content, which influences the other protein complex compositions. They explained that interactions between blood cells and artificial hemodialysis membranes affect membrane protein composition. In contrast, destabilization of a plasma membrane could depend on erythrocyte interactions with metabolites in blood plasma and reactions with free oxygen radicals released from activated leukocytes [28–31]. Taken together, chronic kidney disease is a major disease with increased anisocytosis due to the high number of reticulocytes and the presence of small spherocytic-like cells [30]. Another factor influencing red blood cell life span and decreased deformability might be an increased calcium influx to the intracellular space, due to increased parathyroid hormone (PTH) concentrations. The influence of PTH on the

osmotic fragility of red blood cells is explained by the reduction of ATP levels, which affects membrane-protein structure [32, 33].

In studies performed in relation to other parameters, MRV showed itself to be very useful in distinguishing between HS and non-HS anemia. Furthermore, as compared with other studies, obtained MRV values for β-thalassemia subjects were similar [3], but for IDA children such values were significantly higher. The highest MRV was shown for hemolytic anemia subjects (excluding HS children), especially in the course of AIHA. This is not surprising, since the hemolytic anemia subjects showed the highest number of reticulocytes and their bone marrow restoration is extremely intense. Lazarova et al. [6] showed a high level of usefulness for MRV in differentiating hereditary spherocytosis patients from other anemic subjects. In our study, AUC for MRV was 0.96 with a cut off value of less than 91.15 fL, which is similar to the value proposed as a cut-off for clinical use by other researchers [6]. Nair et al. suggest that combined (MCV–MSCV) with (MRV–MSCV) have high discriminating value for distinguish HS from AIHA subjects. They found that most of HS subjects have (MRV–MSCV) lower than 25 fL, when AIHA subjects in majority have (MRV–MSCV) >25 fL [34]. We did not observe similar correlation. Correspondingly to the mentioned study, only 2/17 (12 %) of HS subject had the parameter over 25 fL, however only 5/17 (29 %) of AIHA had (MRV–MSCV) >25 fL.

Some instruments measure red blood cell size factor (Rsf)—a parameter, where the calculation is based on the values of MCV and MRV. Rsf is related with hemoglobin status and bone marrow erythropoietic activity [9, 11, 13, 15, 17]. Urrechaga et al. [13, 17] suggest that Rsf could be useful in the identification of β-thalassemia patients, but, as we proved, subjects suffering from hereditary spherocytosis do not differ from TM subjects in Rsf indices and such a statement should be made carefully. Previous studies have shown a good utility for Rsf in iron deficiency screening [11, 15]. In contrast, our results are not so promising. The limitation of our study is that we did not compare results from anemic patients with healthy subjects; hence, comparing the above studies could be contentious. In our opinion identification of iron deficiency anemia subjects in anemic populations is more important than identification of IDA subjects in populations of children with normal parameters of red blood cell analysis.

We separated from the studied group those children with anemia of the first quartile of life. Although they could be defined as iron deficiency anemia, the cause of hemoglobin decrease was more complex than only iron deficiency. Mainly, there were preterm infants whose iron stores from pregnancy were low due to early birth. Additionally, low hemoglobin concentrations and red blood cell numbers resulted from inefficient erythropoietin release from kidneys [22]. The selected group differed from IDA patients in MCV (mainly normocytosis), red blood cell and reticulocytes numbers (lower values), IRF, MRV and MSCV (parameters significantly higher). Moreover, in biochemistry analysis IQA children presented higher total bilirubin (physiological jaundice) and ferritin concentrations. These results forced us to exclude this specific group of patients, to avoid unreliable results from our reticulocyte parameter analysis in the IDA group.

In conclusion, we tested the utility of reticulocyte parameters in anemia diagnostics in children. Mostly, the findings based on this pediatric population are in accordance with results from adults presented by others. Reticulocyte indices are now available on most hematology analyzers, but still their use in clinical practice is rare. It should be of interest to practitioners that they could employ enhanced complete blood count analysis as an excellent screening method for the diagnosis of anemia of different etiologies, since so much information can be deduced from a single analysis of a limited volume of blood.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Studies were approved by local ethical committee of Medical University of Warsaw (No. KB/71/2015).

Conflict of interest The authors declare that there is no conflict of interest.

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