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Potential utility of the new Sysmex XE 5000 red blood cell extended parameters in the study of disorders of iron metabolism

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Abstract

Background: New erythrocyte parameters are reported by the Sysmex XE 5000 analyzer. This instrument measures the hemoglobin (Hb) content of individual red cells, calculates the percentage of hypochromic red cells (%Hypo He) and the percentage of hyperchromic red cells (%Hyper He) and quantifies the proportion of marginally sized erythrocytes (%Micro R and %Macro R). The goals of the study were to establish the reference range for erythrocyte extended parameters, their value in different types of anemia and to investigate their reliability in the study of disorders of iron metabolism.
Materials: Three hundred and ninety samples were analyzed. The Kolmogorov-Smirnoff test, independent samples t-test and Pearson correlation were calculated; receiver operating characteristic (ROC) curve analysis was used to determine their diagnostic performance.
Results: The values of the four parameters studied were normally distributed and statistically different ($p<0.0001$) in the different groups of patients; the only exception was %Hypo He in cases of iron deficiency and thalassemia ($p=0.3758$). Results of ROC curve analysis for %Hypo He in the diagnosis of restricted erythropoiesis reticulocyte Hb content (reticulocyte hemoglobin content, Ret He <29 pg) were: area under the curve 0.963; cut-off 1.8%; sensitivity 98.3%; specificity 91.1%.
Conclusions: The new parameters appear to be sensitive for detecting small changes in the number of red cells with inadequate hemoglobinization and volume.
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Keywords: hypochromia; mature erythrocyte; microcytosis; restricted erythropoiesis.

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Introduction

Iron metabolism is a dynamic process, which cannot be defined by one biochemical or hematological parameter only. Iron status is the result of the balance between the rate of erythropoiesis and the size of iron stores. Diagnostic tests for the evaluation of iron metabolism status include indicators of disrupted hemoglobin (Hb) synthesis, mature erythrocyte indices and biochemical markers of iron uptake, availability and stores (1).

Red blood cells are continuously produced in the bone marrow. When iron deficiency manifests and iron stores decrease, mean cell volume (MCV), mean cell hemoglobin (MCH) and red blood cell count (RBC) tend to decline.

The indices, MCV, MCH and mean cell hemoglobin concentration (MCHC) represent the mean values obtained for the total red cell population (2).

In contrast, modern hematology analyzers that utilize flow cytometric analysis provide information about individual cell characteristics, identifying small subpopulations of erythrocytes within the total red cell population.

To date, these measurements have been restricted to analyzers from a single manufacturer, ADVIA series (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). From simultaneous analysis of volume and Hb concentration, this system provides quantitative analysis of the RBC subpopulations and calculates percentages for micro-, normo-, macrocytic cells, and for the hypo-, normo- and hyperchromic RBCs (3).

Measurement of the proportion of hypochromic and microcytic red cells is a useful way for detecting small changes in the number of red cells with inadequate hemoglobinization (4–8). However, because of the long circulating life span of mature erythrocytes, the percentage of hypochromic cells is related to iron status over the previous 2–3 months, and this parameter has been recognized as a reliable indicator of functional iron deficiency.

Automated measurement of RBC microcytosis and hypochromia have also proven their clinical usefulness in the differential diagnosis of iron deficiency and β thalassemia trait (9–12).

The Sysmex XE 5000 instrument (Sysmex Corporation, Kobe, Japan) is a fully automated hematology analyzer that provides complete blood cell and leukocyte differential counts (13). Derived from flow fluorescence cytometry technology, the analyzer incorporates four new RBC parameters including:

- %Hypo He which indicates the percentage of hypochromic red cells with an Hb content <17 pg.

- %Hyper He which indicates the percentage of hyperchromic red cells with an Hb content >49 pg.
- %Micro R indicates the percentage of microcytic red cells with a volume <60 fL.
- %Macro R indicates the percentage of macrocytic red cells with a volume >120 fL.

Using these new parameters allows hemoglobinization of marginally sized RBC to be monitored, while measurement of the proportion of the small and large RBC (based on their marginal volumes) completes the analysis of RBC morphological details.

The goals of this study were to establish the reference range for RBC extended parameters in a normal population, to establish their mean values in different types of anemia and to investigate their correlation with the other erythrocyte indices and reticulocyte hemoglobin content (Ret He).

Materials and methods

Analytical methods

The Sysmex XE 5000 is a fully automated blood cell counter (13).

The flow fluorescence cytometry technology enables independent measurements of the volume and Hb content of individual red cells to be performed.

Measurement of RBCs and platelets employs a Sheath Flow DC detection method. The sample flow is focused on the center of the aperture. Changing the generated electrical resistance into pulses allows precise size distributions of RBCs and platelets to be obtained. The percentage of red cells subpopulations can be calculated and the new parameters %Micro R and %Macro R determined.

In the reticulocyte channel, blood cells are stained by a polymethine dye that is specific for RNA/DNA, and then analyzed with flow cytometry using a semiconductor laser. A bi-dimensional distribution of forward scattered light and fluorescence is presented as a scattergram, indicating mature red cells and reticulocytes. Forward scatter correlates with erythrocyte Hb content and Ret He. The percentage of red cells subpopulations can be calculated and the new parameters %Hypo He and %Hyper He can be determined.

Patients

During a 3-month period, samples from 90 healthy individuals (reference group), 124 patients with iron deficiency anemia (IDA), 78 with chronic kidney disease (CKD) and 98 β thalassemia carriers were collected in EDTA anti-coagulant tubes (Vacutainer™ Becton-Dickinson, Rutherford, NJ, USA). Samples were obtained from the routine workload and prospectively analyzed on the Sysmex XE 5000 (Sysmex Corporation, Kobe, Japan) within 6 h of collection.

Reference ranges for the new RBC extended parameters, %Hypo He, %Hyper He, %Micro R and %Macro R, were obtained from 45 male and 45 female healthy adult subjects, with no clinical symptoms of disease and with results of the complete blood count and biochemical markers of iron metabolism within the normal reference range. Reference ranges were calculated as the 95 central percentiles of the distribution.

IDA patients fulfilled traditional diagnostic criteria for IDA with serum iron <7.5 μmol/L, transferrin saturation <20%, ferritin <100 μg/L, and Hb <110 g/L prior to treatment with iron (14, 15).

CKD patients were managed according to the recommendations of the NKF-K/DOQI (National Kidney Foundation, Kidney Disease Outcomes Quality Initiative) anemia guidelines (16). All patients were treated with various doses of erythropoietin given three times a week at the time of hemodialysis. In addition, the majority of patients were treated with a maintenance dose of intravenous iron (100–200 mg of iron gluconate) weekly or every other week in order to maintain Hb at the recommended concentration of 110–120 g/L.

The thalassemia group included patients with a previous diagnosis of the disease. β thalassemia screening is routinely performed in our laboratory by analysis of RBC indices and the concentration of HbA_2. Molecular characterization of mutations is performed with allele specific oligonucleotide-polymerase chain reaction (ASO-PCR) techniques (17, 18).

Samples with erythrocytosis (RBC>5.5×10^{12}/L) and microcytosis (MCV<70 fL) were selected for HbA_2 quantitation (HPLC HA 8160, Menarini Diagnostics, Florence, Italy). Increased HbA_2 (>3.5%) is considered to be confirmatory for β thalassemia trait. Molecular analysis is performed if genetic counseling is required. β Thalassemia carriers with ferrokinetic parameters within reference range were included, only. Biochemical and hematological data of the patients are summarized on Table 1.

Statistical evaluation of analytical results

The statistical software package SPSS version 16.0 for Windows (SPSS; Chicago, IL, USA) was used for analysis of results. The Kolmogorov-Smirnoff test was used to verify a Gaussian distribution of RBC extended parameters values in our normal population. Correlation coefficients between erythrocyte indices and new RBC extended parameters were

Table 1 Hematological and biochemical data for our study patients comprised of 90 healthy individuals, 124 with iron deficiency anemia (IDA), 98 β thalassemia carriers and 78 patients with chronic kidney disease (CKD).

	RBC, 10^{12}/L	Hb, g/L	MCV, fL	MCH, pg	MCHC, g/L	Ret He, pg	Iron, μmol/L	Transf, g/L	Ferritin, μg/L	Sat, %
Healthy, mean	4.95	151	90.9	30.5	335	33.8	17.1	2.46	103	28
(SD)	(0.37)	(9)	(2.9)	(0.9)	(9)	(1.4)	(2.3)	(0.3)	(54)	(7.5)
IDA, mean	4.12	97	74.2	23.2	304	25.2	4.8	3.00	25	7
(SD)	(0.74)	(17)	(7.6)	(3.1)	(15)	(4.8)	(2.8)	(0.7)	(34)	(4.3)
β Thal, mean	6.2	117	65.1	20.9	321	22.6	16.5	2.36	109	27
(SD)	(1.3)	(11)	(3.5)	(1.2)	(66)	(1.9)	(6.1)	(0.4)	(99)	(12)
CKD, mean	3.65	108	91.8	29.8	322	32.4	10.7	1.92	286	24
(SD)	(0.5)	(8)	(5.9)	(2.3)	(15)	(3.6)	(5.7)	(0.47)	(188)	(15)

SD, standard deviation; RBC, red blood cells; Hb, hemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; Ret He, reticulocyte hemoglobin content; Transf, transferrin; % Sat, % transferrin saturation; β Thal, β thalassemia.

calculated using the method of Pearson. Independent t-tests were performed to detect statistical deviation between the groups of patients; $p<0.05$ were considered to be statistically significant. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of the new parameters. The κ index was used to determine concordance between new and traditional parameters.

Results

The values obtained for the new four parameters were normally distributed.

(%Hypo He, $p=0.626$; %Hyper He, $p=0.288$; %Micro R, $p=0.751$ and %Macro R, $p=0.093$).

Reference ranges (95th central percentile) were calculated from results obtained in the healthy subjects group. The values of the RBC extended parameters in the four groups of patients are summarized on Table 2.

Figure 1 shows the box and whiskers plot of the values for %Hypo He in the different groups, while Figure 2 shows the box and whiskers plot of the values for %Micro R in the different groups.

The correlation coefficients for the various parameters were: %Hypo He vs. MCH ($r=-0.75$); %Hyper He vs. MCH ($r=0.75$); %Micro R vs. MCV ($r=-0.91$); %Macro R vs. MCV ($r=0.81$). The values obtained for the four new RBC extended parameters were statistically different ($p<0.0001$) between the different groups; the only exception was %Hypo He in cases of IDA and thalassemia ($p=0.3758$). The IDA and β thalassemia groups showed restricted erythropoiesis, due to lack of iron or globin as demonstrated by the low Ret He values. Healthy subjects and patients with CKD undergoing therapy had Ret He concentrations greater than the cut-off value of 29 pg.

Significant differences in %Hypo He values ($p<0.001$) were detected when groups with restricted erythropoiesis, such as those with IDA and β thalassemia were compared with patients with CKD and normal individuals.

ROC curve analysis for %Hypo He in the diagnosis of restricted erythropoiesis (Ret He <29 pg) showed an area under the curve (AUC) of 0.963 [95% confidence interval (CI), 0.928–0.985] at a cut-off threshold of 1.8%; sensitivity 98.3%; specificity 91.1%.

The diagnostic performance of MCH and MCV for the detection of restricted erythropoiesis (Ret He <29 pg) was also evaluated. ROC analysis for MHC showed an AUC of 0.924 (95% CI, 0.89–0.94) at a cut-

Table 2 RBC extended parameters values, mean (standard deviation, SD), from 90 healthy individuals, 124 patients with iron deficiency anemia (IDA), 98 β thalassemia carriers and 78 patients with chronic kidney disease (CKD).

	Healthy	Thalassemia	IDA	CKD	Reference range
%Hypo He	0.3 (0.16)	14.2 (9.4)	16.2 (15.8)	1.7 (1.8)	0.0–0.6
%Hyper He	0.8 (0.16)	0.08 (0.07)	0.24 (0.2)	0.6 (0.18)	0.5–1.1
%Micro R	1.1 (0.44)	37.6 (11.5)	16.5 (13.1)	2.5 (2.4)	0.2–1.9
%Macro R	8.5 (1.4)	3.2 (0.9)	4.6 (1.1)	10.0 (4.4)	5.0–12.0

%Hypo He, percentage of hypochromic red cells with an Hb content equivalent to <17 pg; %Hyper He, percentage of hyperchromic red cells with an Hb content equivalent to >49 pg; %Micro R, percentage of microcytic red cells with a volume <60 fL; %Macro R, percentage of macrocytic red cells with a volume >120 fL.

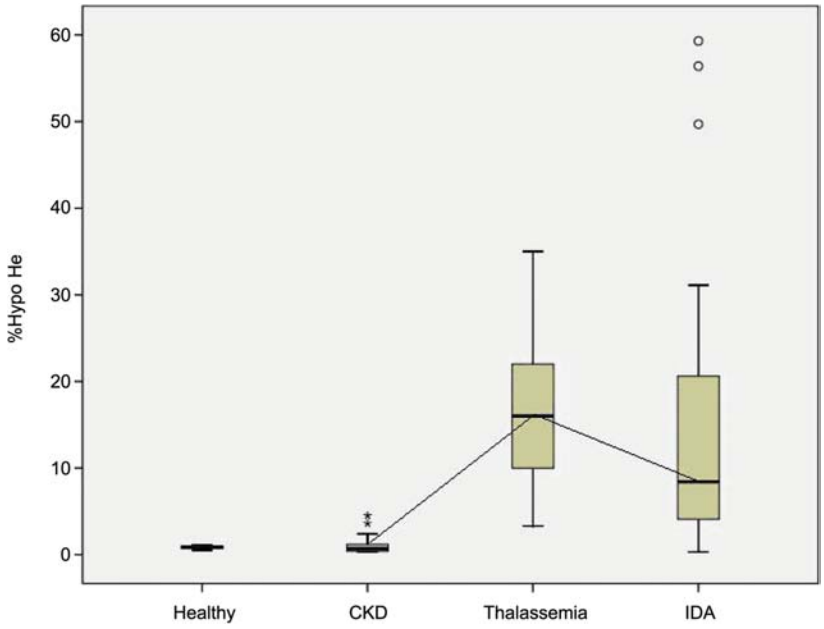

Figure 1 Comparison of %Hypo He (percentage of hypochromic red cells) between the three groups of patients with anemia: iron deficiency anemia (IDA), thalassemia carriers, chronic kidney disease (CKD) and the reference group.
The horizontal line in the center of the box shows the median value, the upper and lower limits of the box show the interquartile range, and the whiskers show the minimum and maximum values for each group.

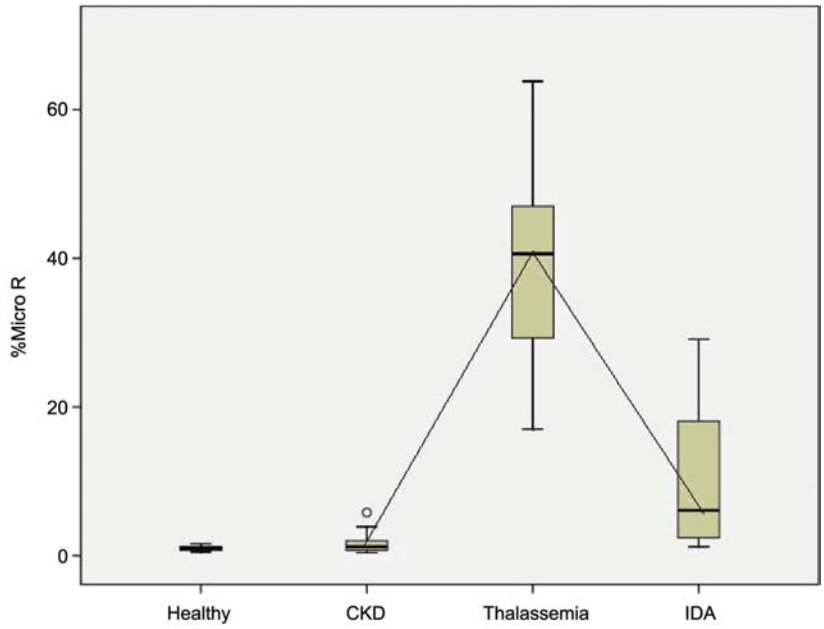

Figure 2 Comparison of %Micro R between the three groups of patients with anemia: iron deficiency anemia (IDA), thalassemia carriers, chronic kidney disease (CKD) and the reference group.
The horizontal line in the center of the box shows the median value, the upper and lower limits of the box show the interquartile range, and the whiskers show the minimum and maximum values for each group.

off threshold of 25.4 pg; sensitivity 86.5%; specificity 90.6%. ROC analysis results for MCV showed an AUC of 0.90 (95% CI, 0.86–0.93) at a cut-off threshold of 80.8 fL; sensitivity 84.2%; specificity 89.5%.

Overall agreement between %Hypo He and Ret He was 82.%, κ index 0.656 (95% CI, 0.575–0.736).

Discussion

Disturbances in iron metabolism may occur in many patients, the challenge is to identify these patients as early as possible. However, this is not an easy task because minimal hematological changes may be present only. Careful interpretation of results is required, together with the use of these additional new parameters.

Development of new parameters may allow the complete scope of disorders of iron metabolism to be identified quickly and managed. Hematological indices and the new parameters measured with automated counters are sensitive for detecting small changes in subpopulations of red cells. They hold promise for the evaluation of iron-restricted erythropoiesis and the status of iron metabolism. However, more studies are needed.

In this study, we determined reference ranges for the new RBC extended parameters. We did not find any difference between males and females. The wide reference range found for %Macro R (5%–12%) could be attributed to the integration limits defined in the calculation algorithm for this parameter. The manufacturer may want to reconsider these limits. As a result of these limits, no patients with macrocytic anemia were included in the study.

Patients with CKD received treatment in order to maintain appropriate iron status and efficient erythropoiesis. Patients were stable with Hb concentrations near desirable limits 110 g/L (19, 16), and values for %Hyper He and %Macro R were within the reference range. The %Hypo He values obtained in this group of patients undergoing recombinant human erythropoietrin (r-HuEPO) therapy were statistically lower (1.7%) than those that were iron deficient (16.2%). All of these patients had mild anemia and %Hypo He values were above the reference range. The same findings were noted for %Micro R.

Patients with uncomplicated β thalassemia and IDA showed different results with respect to microcytic and hypochromic red cells. Iron deficient erythropoiesis is characterized by the production of RBCs with decreased Hb content resulting in a high percentage of hypochromic cells (20). As anemia progresses the number of microcytic cells increases. However, patients with β thalassemia show erythrocytosis and a high rate of microcytosis as a result of increased erythropoiesis. Red cells in patients with β thalassemia have a small volume due to impaired globin synthesis (21). The %Micro R values in thalassemia carriers were statistically different (mean 37.6%) compared with values obtained in the IDA group (mean 16.5%), $p < 0.0001$. Although our results are consistent with previous publications which showed the RBC count to be the most efficient single measurement for the differential diagnosis of these two types of microcytic anemia (22, 23), in our patients, the diagnostic performance of the RBC count was better (AUC 0.944) compared with the %Micro R (AUC 0.89).

The %Hypo He values in β thalassemia carriers were above the reference range. This is due to inef-

ficient erythropoiesis as a result of reduced synthesis of intact Hb. These results did not differ from those seen in patients with IDA ($p = 0.3785$).

Comparing patients with restricted erythropoiesis due to lack of iron (IDA) or globin (thalassemia) to those individuals with normal erythropoiesis (reference group and patients on therapy), the diagnostic performance of %Hypo He was excellent when compared to Ret He.

Ret He, reported by all Sysmex XE analyzers (Sysmex Corporation, Kobe, Japan), assess the incorporation of iron into erythrocyte Hb. This parameter is thus a direct estimate of the functional availability of iron into the erythron (24–28). ROC curve analysis revealed that a cut-off threshold of 1.8% for identifying restricted erythropoiesis (defined by Ret He <29 pg) produced a sensitivity of 98.3% and specificity of 91.1%. The AUC was 0.963 ($p < 0.0001$).

Flow cytometry provides information about individual cell characteristics and is sensitive in detecting small changes in the number of red cells with inadequate hemoglobinization and volume. The application of flow fluorescence cytometry technology from Sysmex allows for independent measurement of the volume and Hb content of individual red cells. The analysis of these new parameters could be performed simultaneously in the course of routine blood cell counts, with no incremental costs and without the need for more blood.

We report the first results obtained for the new RBC extended parameters derived from this technology, including determination of the reference range in healthy subjects and values in anemic patients with red cells abnormalities. We report our observations on the potential clinical utility of these new parameters in the monitoring of erythropoietic function.

The availability of RBC extended parameters should allow further assessment of their utility for diagnosing disturbances of iron metabolism, the response to iron or folate supplementation and the differential diagnosis of microcytic anemias. Further studies are needed in order to confirm our results.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content of the paper.

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