Research Article

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Determination of reference ranges for automated erythrocyte and reticulocyte parameters in healthy adults

Abstract

Objectives: Recent advances in hematology analyzers have enabled to improve the reliability in the results and also provided additional hematological parameters. In the present study, we aimed to determine the reference ranges for automated erythrocyte and reticulocyte parameters in healthy individuals on Sysmex XN 1000 hematology analyzer.
Methods: One hundred and thirty-three subjects with normal physical examination and complete blood count results within the reference ranges were included in the study. Venous blood samples collected in tubes containing K2-EDTA were analyzed on Sysmex XN-1000. The references intervals for IRF, RBC He, Ret He, LFR, MFR, HFR, Delta He, Micro R, Macro R, Hypo He and Hyper He were determined according to CLSI EP28-A3c.

Results: The reference ranges of the parameters were estimated with 90% confidence intervals. The reference ranges were 3.4–17% for IRF, 26.9–32.8 pg for Ret-He, 25.2–30.5 pg for RBC-He and 0.5–3.7 pg for delta-He. Gender specific reference ranges were calculated for of Ret-He (male (M): 26.8–32.9 pg, female (F): 23.9–33.6 pg), RBC-He (M: 26.3–30.8 pg, F: 25.3–30.5 pg) and delta-He (M: 0.5–3.7 pg, F: 0.3–3.7 pg).
Conclusions: The new reticulocyte and erythrocyte parameters may be conveniently used in clinical diagnosis and follow-up of patients, as they offer reliable, automated and cheap results. Each laboratory is recommended to determine its own reference intervals considering the differences like the instrument used and population studied.

Keywords: delta-He; hematology; immature reticulocyte fraction; reference range; reticulocyte hemoglobin.

Öz

Amaç: Hematoloji analizörlerindeki son gelişmeler, sonuçların güvenilirliğini artırmakla birlikte, ek hematolojik parametreler de sağlamıştır. Bu çalışmada sağlıklı bireylerde otomatize eritrosit ve retikülosit parametreleri için Sysmex XN 1000 hematoloji analizörü kullanılarak referans aralıklarının belirlenmesi amaçlanmıştır.
Gereç ve yöntem: Fizik muayenesi normal ve tam kan sayım sonuçları referans aralık içerisinde olan 133 kişi çalışmaya dahil edilmiştir. K2-EDTA'lı tüplere toplanan venöz kan örnekleri Sysmex XN-1000 analizöründe analiz edilmiştir. IRF, RBC He, Ret He, LFR, MFR, HFR, Delta He, Micro R, Macro R, Hypo He ve Hyper He için referans aralıkları CLSI EP28-A3c'ye göre belirlenmiştir.
Bulgular: Parametrelerin referans aralıkları %90 güven aralığı ile hesaplanmıştır. Referans aralıkları IRF için % 3,4–17, Ret-He için 26,9–32,8 pg, RBC-He için 25,2–30,5 pg ve delta-He için 0,5–3,7 pg olarak belirlenmiştir. Cinsiyete özel referans aralıkları Ret-He (erkek (E): 26.8–32.9 pg, kadın (K): 23.9–33.6 pg), RBC-He (E: 26.3–30.8 pg, K: 25.3–30.5 pg) ve delta-He (E: 0.5–3.7 pg, K: 0.3–3.7 pg) için hesaplanmıştır.

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Sonuçlar: Retikülosit ve eritrosit parametreleri güvenilir, otomatize ve zahmetsiz sonuçlar sağladıkları için hastaların klinik tanı ve takibinde rahatlıkla kullanılabilir. Her laboratuvarın kullanılan cihaz ve hizmet sunulan popülasyon gibi farklılıklar göz önünde bulundurularak kendi referans aralıklarını belirlemesi önerilir.

Anahtar Kelimeler: delta-He; hematoloji; immatür retikülosit fraksiyonu; referans aralığı; retikülosit hemoglobin.

Introduction

Recent advances in hematology analyzers have enabled to improve the reliability in the results of complete blood count (CBC) and also provided additional hematological parameters for diagnosis and/or treatment of various pathologies [1].

One of these parameters is immature reticulocyte fraction (IRF), helps to evaluate the maturation of the reticulocyte based on the stained RNA content. Reticulocytes are classified according to the fluorescent intensity as high-fluorescent reticulocytes (HFR), medium-fluorescent reticulocytes (MFR) and low-fluorescent reticulocytes (LFR). IRF is the sum of high-fluorescent reticulocytes (HFR) and medium-fluorescent reticulocytes (MFR) [1, 2]. IRF is considered to be a good marker reflecting reticulocyte maturation and bone marrow recovery after transplantation [1, 3–5]. Also an increase in IRF shows the presence of circulating CD34+ cells for stem cell collection and helps monitoring after treatment with erythropoiesis-stimulating agents [6]. Reticulocyte hemoglobin content (Ret-He) represents the reticulocyte hemoglobin (Hb) content and allows the evaluation of available functional iron for erythropoiesis and bone marrow response to stimulating agents [1]. Erythrocyte hemoglobin content (RBC-He) is the marker of erythrocyte Hb content and shows the incorporation of iron into erythrocyte hemoglobin. Delta-He, the difference between reticulocyte and erythrocyte hemoglobin content, is a marker reflecting the availability of iron and is also suggested as an inflammation marker in relation with hepcidin [7]. Micro-R (Microcytic erythrocytes) represents red blood cells (RBC) with volume <60 fL, microcytic, and Macro-R (Macrocytic erythrocytes) represents RBC with volume >120 fL, macrocytic. Micro-R or Macro-R may be helpful in the diagnosis of anemia besides the MCV (mean corpuscular volume) value. As in the presence of dimorphic red cell populations, normal MCV values could be seen [8]. Hypo-He indicates the RBCs with hemoglobin content of <17 pg and Hyper-He indicates the RBCs with hemoglobin content of >49 pg [9].

These novel automated hematological parameters assessed in addition to complete blood count results may have potential contributions to clinical decision making. In clinical practice, reference intervals attributed to the values for healthy status are commonly used in the interpretation of test results. Therefore, we aimed to determine the reference ranges for automated erythrocyte and reticulocyte parameters, including IRF, RBC-He, Ret-He, Delta-He, MicroR, MacroR, Hypo-He and Hyper-He in healthy individuals on Sysmex XN 1000 hematology analyzer.

Materials and methods

Subjects

The study was conducted prospectively. Subjects admitted to the Hematology Department of a training and research hospital were included in the study. Complete blood count was performed for the participants following the physical examination done by a skilled hematologist. Having any acute or chronic diseases was an exclusion criterion. One hundred and thirty-three subjects with normal physical examination and complete blood count results within reference ranges were included in the study. Informed consent was obtained from all individual participants.

Methods

All study procedures were approved by the local ethics committee (Ref number 2534/2019). Venous blood samples were collected in evacuated tubes containing K2-EDTA (BD vacutainer, Franklin Lakes, USA) and analyzed on a Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan) analyzer. All samples were analyzed within 4 h after collection and kept at room temperature (22–26 ℃) until analysis. Reticulocyte, IRF (%), LFR (%), MFR (%), HFR (%), Micro-R (%), Macro-R (%), Ret-He (pg), RBC-He (pg), Delta-He (pg), Hypo-He (%), Hyper-He (%) parameters were taken into account.

In the RET (reticulocyte) channel of XN-1000, red blood cells, white blood cells and platelets are lysed and so that the fluorescence marker can penetrate the cells. The fluorescence marker labels the intracellular nucleic acids and the fluorescence signal is directly proportional to the nucleic acid content. According to their fluorescence intensity, reticulocytes are fractionated into three categories, representing different stages of maturity: LFR, MFR, HFR. The IRF is calculated from the sum of MFR plus HFR. Hypo-He and Hyper-He are parameters analyzed in the RET channel. They are derived from the hemoglobin content of all mature RBC (RBC-He), which can be calculated based on the high-angle forward scatter (FSC). Ret-He is a measure of the forward scatter of stained reticulocytes. MicroR and MacroR parameters are obtained from end of the RBC histogram. A microcytic and a macrocytic population of red blood cells can be determined at the lower and upper areas of the histogram.

Statistical analysis

The analysis of data was performed using SPSS Statistical Packages. Outliers were detected according to Tukey's method. The distribution of each parameter was analyzed for normality using the Shapiro–Wilk test. According to the findings from the Shapiro–Wilk test, the reference intervals (RIs) of the parameters were estimated by nonparametric methods according to the IFCC recommendations [10]. The confidence intervals of the limits of the nonparametric reference interval were determined using a bootstrap method. When evaluating in terms of genders, the reference range was calculated according to the robust method for statistically significant analytes.

Results

One hundred and thirty-three healthy adults were evaluated in the study. The mean age of the study population was 36 years (range: 18–77 years). There were 57 (42.8%) male and 76 (57.2%) female subjects. The median values of the parameters are presented in Table 1. The reference ranges and 90% confidence intervals were estimated within 90% CIs for IRF, RBC He, Ret He, LFR, MFR, HFR, Delta He, Micro R, Macro R, Hypo He and Hyper He (Table 1). There were differences between genders the reference ranges within

90% confidence intervals for RBC-He, Ret-He and Delta-He levels respectively as shown in Tables 2 and 3.

Discussion

Traditional reticulocyte counts provide limited information about bone marrow activity [1, 11]. Modern hematology analyzers provide new erythrocyte and reticulocyte indices and so valuable data for clinicians in order to use in the diagnosis and treatment strategy of the patients. Reference intervals are the most commonly used tools in the interpretation of an individual's laboratory results. Therefore, accurate determination of these intervals with respect to the factors like age and gender is quite important. Although most laboratories use the product insert values provided by the manufacturer, the recommended practice is to define its values for each laboratory.

Under normal circumstances, 90% of the circulating reticulocytes are in the most mature stage [12]. Modern automated analyzers allow watching the maturity of the reticulocytes according to their RNA content. IRF is calculated from the sum of MFR and HFR. It is indicated that IRF is an early marker for follow-up after hematopoietic stem cell transplantation and evaluation of bone marrow response to anemia [13, 14]. Also, in hereditary spherocytosis

Table 1: Reference intervals for the erythrocyte and reticulocyte parameters.

(n=133)	Outlier exclusion Median (min–max)	Reference interval	Lower limit (90% CI)	Upper limit (90% CI)
IRF, %	9.6 (2.8–17.8)	3.4–17	2.8–4.4	15.5–17.8
LFR, %	90.4 (82.2–97.2)	82.9–96.8	82.2–84.6	95.7–97.2
MFR, %	8.6 (2.5–16.3)	3.2–16.3	2.5–4.1	14.1–16.3
HFR, %	1.0 (0.1–3.2)	0.1–2.7	0.1–0.2	2.4–3.2
Ret-He, pg	30.7 (25.4–33.8)	26.9–32.8	25.4–27.3	32.5–33.8
RBC-He, pg	28.3 (25.1–30.8)	25.2–30.5	25.1–25.6	30.2–30.8
Delta-He, pg	2.3 (0.3–4.1)	0.5–3.7	0.3–0.9	3.3–4.1
Micro-R, %	1.7 (0.4–6.1)	0.5–5.8	0.4–0.7	4.5–6.1
Macro-R, %	4.1 (3.3–5.9)	3.3–5.1	3.3–3.5	4.9–5.9
Hypo-He, %	0.2 (0.1–0.7)	0.1–0.7	0.1–0.1	0.4–0.7
Hyper-He, %	0.5 (0.2–0.8)	0.2–0.7	0.2–0.3	0.7–0.8

Data was presented as median (min–max). IRF, immature reticulocyte fraction; RBC-He, erythrocyte hemoglobin content; Ret-He, reticulocyte hemoglobin content; LFR, low-fluorescent reticulocytes; MFR, medium-fluorescent reticulocytes; HFR, high-fluorescent reticulocytes; Delta-He, difference between reticulocyte and erythrocyte hemoglobin content; Micro-R, microcytic erythrocytes; Macro-R, macrocytic erythrocytes; Hypo-He, erythrocytes with hemoglobin content of <17 pg; Hyper-He, erythrocytes with hemoglobin content of >49 pg.

Table 2: Reference intervals for males.

Male (n=57)	Median (min–max)	Reference interval	Lower limit (90% CI)	Upper limit (90% CI)
RBC-He, pg	28.5 (25.6–30.8)	26.3–30.8	25.8–26.8	30.4–31.4
Ret-He, pg	30.7 (25.4–33.8)	26.8–32.9	25.4–27.3	32.5–33.8
Delta-He, pg	2.3 (0.3–4.1)	0.5–3.7	0.3–0.9	3.3–4.1

Data was presented as median (min–max); RBC-He, erythrocyte hemoglobin content; Ret-He, reticulocyte hemoglobin content; Delta-He, difference between reticulocyte and erythrocyte hemoglobin content.

Table 3: Reference intervals for females.

Female (n=76)	Median (min–max)	Reference interval	Lower limit (90% CI)	Upper limit (90% CI)
RBC-He, pg	28.1 (25.1–30.6)	25.3–30.5	24.8–25.9	30.3–30.6
Ret-He, pg	29.7 (21.8–33.8)	23.9–33.6	19.1–24.3	33–34.1
Delta-He, pg	2 (0.3–3.7)	0.3–3.7	0.3–0.6	3.5–3.7

Data was presented as median (min–max); RBC-He, erythrocyte hemoglobin content; Ret-He, reticulocyte hemoglobin content; Delta-He, difference between reticulocyte and erythrocyte hemoglobin content.

and pyruvate kinase deficiency, a disproportionate increase in IRF level was observed when comparing with reticulocyte count [2, 9]. Also, it was indicated that IRF could be a choice comparing with absolute neutrophil count to observe the bone marrow recovery after chemotherapy in pediatric malignancies [4].

In the present study (Sysmex XN 1000) the median value of IRF was 9.6% (Reference range (RR): 3.4–17%), LFR, MFR and HFR levels were 90.4% (RR: 82.9–96.8%), 8.6% (RR: 3.2–16.3%) and 1% (RR: 0.1–2.7%), respectively. There are studies determining reference ranges of IRF on different platforms; 1.6–12.1% (median value: 5.3%) on Sysmex XE-5000, 1.1–11.4% (median value: of 4.7%) on Sysmex XE-2100, 0.19–0.42% (median value: 0.30%) on Beckman Coulter LH750 [15–17]. In a study evaluating the results on Abbott Cell Dyn 4,000, the reference interval of IRF was 0.14–0.35% [18]. The difference in reference intervals between the studies may be related to the technology of the automated analyzers used and another explanation for the difference may be the method used for the calculation of IRF values, as some studies define IRF value as the ratio of immature reticulocytes to all reticulocytes, while most of them define IRF value as the sum of HFR and MFR. In a study with the pediatric population, median IRF values were higher than adults (16.4% within 6.5–26.7% for 12–17 years-female, 14.7% within 6.9–23% for 12–17 years-male) [11]. It was also observed that there was a decrease in IRF levels after birth through 90-days (36.2 ± 5.6% to 10.1 ± 4.9%) [19]. Ret-He represents the hemoglobin content of reticulocytes and is indicated as a marker of available functional iron capacity [20]. RBC-He gives information of iron status about mature erythrocytes. Delta-He is the difference of these two parameters and the potential marker of available iron. It is also suggested as an inflammation marker [21]. In our study, the median value of Ret-He was 30.7 pg (RR: 26.9–32.8 pg), RBC-He was 28.3 pg (RR: 25.2–30.5 pg) and delta-He was 2.3 pg (RR: 0.5–3.7 pg). In two different studies determining the reference values on Sysmex XE-5000, median values for Ret-He were reported as 33.8 pg (30.0–37.6) and 33.8 pg (32.0–36.0); also the range of delta-He was determined as 3.1 (2.3–3.7) [7, 15]. In a study evaluating Ret-He values on Sysmex XE-2100 from 196 healthy individuals, the reference value of Ret-He was 30.2–36.7 pg [22]. In a study of the pediatric population, the median values of delta-He were 2.3 pg (1.0–3.7) for females and 2.6 pg (1.4–3.8) for males on ADVIA 2120 analyzer [11]. In our study, the female and male reference ranges of delta-He were found to be 2 pg (0.2–3.8) and 2.3 pg (0.5–3.7), respectively. Delta-He could differentiate the patients with iron deficiency anemia and anemia of chronic disease from healthy controls. Also, delta-He is indicated as a potential marker for inflammation based types of anemia [23]. In a previous study, evaluating

reticulocyte parameters in iron deficiency anemia, vitamin B12 deficiency and B-thalassemia minor patients with healthy controls, the average value of Ret-He was 28.2 ± 1.7 pg in healthy controls. Ret-He values were significantly lower in patients with iron deficiency anemia or B-thalassemia minor patients but significantly higher in vitamin B12 deficient patients. When comparing mild and severe iron deficiency anemia with the B-thalassemia trait, there was a statistically significant difference [24].

Micro R/Macro R can be useful parameters besides MCV when evaluating the microcytic or macrocytic anemia. Hypo He/Hyper He reflects the iron availability of the cells and helps to evaluate the hemoglobinization of the red cells [25]. Also, Micro R/Hypo-He ratio can be useful for defining the severity of hereditary spherocytosis (HS) [9]. HS patients had high Micro R/Hypo-He [2].

This study has some limitations, one of which was the inclusion of only adult subjects without covering the pediatric population. Another limitation was the limited number of participants, it was not possible to evaluate the reference ranges according to age or decades. In addition, one of the limitations of the study was that ferritin, folate and vitamin B12 levels were not examined for the laboratory evaluation of anemia in patients.

To our knowledge, this is the first study evaluating Sysmex XN-1000 hematology analyzer for automated erythrocyte and reticulocyte parameters in the Turkish population.

Each laboratory is recommended to determine its reference intervals considering the differences like the instrument used and the population studied. The new reticulocyte and erythrocyte parameters may be conveniently used in clinical diagnosis and follow-up of patients, as they offer reliable automated and cheap results. Further large-scale studies including the pediatric population, are needed.

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