



Establishment of reference intervals for reticulocytes in adolescent females using Sysmex XN-1000 hematology analyzer

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Abstract

Background: Iron Deficiency Anemia (IDA) remains a significant health concern among adolescent females. Reliable and effective parameters are required for the monitoring of anemia. Reticulocyte, reported as absolute reticulocyte count or reticulocyte percentage, is a valuable parameter for assessing the degree of effective erythropoiesis in anemia cases. Establishing appropriate reference values is essential for accurate interpretation of reticulocyte measurements.

Objective: This study aimed to establish reference intervals for reticulocyte parameters specifically in adolescent females using the Sysmex XN-1000 hematology analyzer.

Method: A peripheral blood sample was collected from female high school students aged 14-19 years who were not menstruating at the time of sampling. Blood collection was done via venipuncture into K3-EDTA tubes and further analyzed using the Sysmex XN-1000 hematology analyzer.

Results: The established reference intervals were as follows: reticulocyte percentage (Ret%) 0.665–2.099%; absolute reticulocyte count (Ret#) $0.0312\text{--}0.0977 \times 10^6/\mu\text{L}$; and reticulocyte hemoglobin (Ret-He) 26.524–32.574 pg. Data were normally distributed ($p > 0.05$). Reference intervals were calculated using the formula $\text{mean} \pm 1.96$ standard deviation, with a 95% confidence level.

Conclusion: This study successfully established specific reference intervals for reticulocyte parameters in adolescent females using the Sysmex XN-1000 hematology analyzer.

Keywords: Reticulocyte, Sysmex XN-1000, adolescent females, anemia

1. Introduction

Iron deficiency anemia (IDA) represents one of the most prevalent nutritional disorders among adolescent females and remains a significant public health issue in Indonesia. According to the 2018 Basic Health Research Survey (Riset Kesehatan Dasar, 2019), the prevalence of anemia was reported to be high, ranging from 26.8% among individuals aged 5–14 years to 32% among those aged 15–24 years (Indonesian Ministry of Health, 2019). Optimal management of this condition involves addressing the underlying cause, providing iron supplementation, and monitoring iron intake to ensure adequate replenishment (Halterman & Segel, 2023). Hence, reliable and effective parameters are required for the monitoring of anemia.

Reticulocyte count is a widely accepted hematological parameter for monitoring anemia and assessing the response to its treatment. Reticulocytes are described as immature, anucleate



red blood cells that serve as indicators of erythropoiesis. Measurement of reticulocytes provides an indirect assessment of bone marrow activity and effective erythropoiesis. Results can be expressed as reticulocyte count, either the absolute reticulocyte count or the reticulocyte percentage (Sedick *et al.*, 2021). An earlier study reported that reticulocyte count begins to rise within approximately three days after initiating iron supplementation in children with IDA (Russo *et al.*, 2020). Furthermore, reticulocyte measurement is considered more practical and cost-effective compared to other iron status tests such as serum ferritin, transferrin saturation, or soluble transferrin receptor (Ringoringo *et al.*, 2023). This highlights the potential utility of reticulocyte count as a key parameter for monitoring patients undergoing IDA treatment.

The clinical interpretation of reticulocyte measurements requires reference intervals that are specific to the population being evaluated. Currently, the commonly used reference interval for reticulocyte percentage is 0.5–1.5%, which is primarily derived from healthy adult populations. The establishment of reference intervals is done by using statistical methods based on data from healthy individuals. These values are heavily affected by other factors such as geographic region, population characteristics, methodology, and instrumentation (Posada-Franco *et al.*, 2024). Accordingly, the International Federation of Clinical Chemistry (IFCC), the Clinical and Laboratory Standards Institute (CLSI), and hematology analyzer manufacturers recommend that each laboratory establish its own reference intervals tailored to its specific population (CLSI, 2010).

In the Indonesian population, females in adolescence, age around 10-19 years old, are at risk of developing iron deficiency anemia (IDA) (Sari *et al.*, 2022). This study aimed to establish reference intervals for reticulocyte parameters in a specific population of adolescent females using the Sysmex XN-1000 hematology analyzer. This study provides the first set of reticulocyte reference values for the adolescent female population in Indonesia, which will serve as an important and valuable resource to support clinical decision-making.

2. Method

2.1. Sample preparation

This study employed an analytical observational approach with a cross-sectional design. A total of 461 female high school students (SMA/SMK) in Prambanan, who met the inclusion criteria, namely being aged 14-19 years and not menstruating at the time of sampling, were

recruited for this study. Sampling was conducted from August to September 2018. Participation was confirmed through the completion of informed consent forms and a questionnaire.

Among 461 subjects, 412 were excluded because they fit into the exclusion criteria as follows: frozen samples, mean corpuscular volume (MCV) < 80 fL, mean corpuscular hemoglobin (MCH) < 26 pg, hemoglobin (Hb) < 12 g/dL, body mass index (BMI) > 30 kg/m², ferritin < 20 ng/mL, transferrin saturation < 20%, and the absence of iron status testing. Each participant provided 3 mL of peripheral blood in one EDTA tube (QTUBE, Asamedika, Indonesia) for hematological and reticulocyte analysis and one serum tube for ferritin and iron status testing. Hematological measurements were performed using the Sysmex XN-1000 analyser (Sysmex, Kobe, Japan). Out of 461 subjects, 49 were included in this study.

Samples were analyzed in the Laboratory of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Nursing, and Public Health, Universitas Gadjah Mada.

2.2. Method and result analysis

The establishment of reference intervals was conducted in accordance with the guidelines of the CLSI EP28-A3c. To establish the reference intervals, a robust method was applied in this study because the total number of participants (n=49) did not meet the minimum recommended sample size of 120 (CLSI, 2010). Data normality was assessed using the Kolmogorov–Smirnov test, with a p-value > 0.05 indicating a normal distribution. Parametric data were presented as mean ± standard deviation. Reference intervals were determined using a two-sided 95% confidence interval approach, followed by Tukey's method to exclude outliers. Data analysis was performed using MedCalc software version 13.0.6.0.

3. Results and discussion

Of the total 49 study participants, the majority were between 16 and 17 years of age. Menarche occurred between the ages of 12 and 14 years in 29 participants (59.1%). The majority of participants reported a menstrual duration of 3–7 days with menstrual cycles ranging from 23 to 35 days. Only 12.2% experienced prolonged menstrual bleeding lasting more than seven days, and only 16.3% had shorter menstrual cycles, as shown in **Table 1**. Only a small proportion of participants were at risk of anemia due to heavy menstrual bleeding (Munro *et al.*, 2023). Therefore, this population is suitable for establishing a specific reference interval.

The results of the examination showed that basic hematological parameters, including hemoglobin, erythrocytes, hematocrit, leukocytes, and platelets, demonstrated a normal data distribution within this homogeneous group of subjects, with no significant differences observed across all hematological parameters (**Table 2**). When compared with other references using the same methodology (Bildirici *et al.*, 2023), basic hematological parameters in this study were found to fall within the established normal range, although the different population characteristics (age and pubertal status) in this study and those presented in the study by Bildirici *et al.* may limit the applicability of this finding to the specific population of Indonesian female adolescents.

Table 1. Subject characteristics

Parameters	N (%)
Age:	
14-15 years old	13 (26.5)
16-17 years old	34 (69.4)
≥ 18 years old	2 (4.1)
Parent's occupation:	
Manual labourer	18 (36.7)
Private sector employees	12 (24.6)
Entrepreneur	9 (18.4)
Government employees	3 (6.1)
Retired	3 (6.1)
Army	2 (4.1)
Farmer	1 (2)
Unemployed	1 (2)
Parent's educational background:	
Undergraduate	6 (12.2)
Academy	4 (8.2)
High-school	24 (49.1)
Junior high-school	6 (12.2)
Elementary school	8 (16.3)
No education	1 (2)
Menarche:	
10-12 years old	16 (32.7)
12-14 years old	29 (59.1)
> 14 years old	4 (8.2)
Menstruation duration:	
3-7 days	43 (87.8)
> 7 days	6 (12.2)
Menstruation cycle:	
15-23 days	8 (16.3)
23-35 days	34 (69.4)
> 35 days	7 (14.3)

Reticulocyte count is commonly used to estimate the rate of effective erythropoiesis and reported as either the absolute reticulocyte count (Ret#) or the reticulocyte percentage (Ret%)

(Prchal, 2021). In this study, reticulocyte count is presented in 3 different parameters: reticulocyte percentage (Ret%), absolute reticulocyte count (Ret#), and reticulocyte haemoglobin (Ret-He). These data were calculated using a robust method because the number of samples was smaller than 120, as recommended by CLSI (CLSI, 2010). The reference intervals for Ret%, Ret#, and Ret-He obtained from the 49 study participants are presented in **Table 3**. The respective ranges were 0.665–2.099%; $0.0312\text{--}0.0977 \times 10^6/\mu\text{L}$; and 26.524–32.574 pg, all demonstrating a normal data distribution ($p > 0.05$). The 95% confidence intervals for the reference ranges were calculated as the mean \pm 1.96 standard deviations for data with a normal distribution.

Table 2. Hematology characteristics of the subjects

Parameters (units)	Mean \pm SD	Bildirici <i>et al.</i> , 2023
Hemoglobin (g/dL)	13.51 \pm 0.65	10.90-15.10
Erythrocyte ($\times 10^6/\text{l}$)	4.67 \pm 0.26	4.03-5.39
Hematocrit (%)	39.8 \pm 2.13	34.4-45.8
Leucocyte ($\times 10^3/\text{l}$)	8.47 \pm 1.53	4.45-10.95
Platelets ($\times 10^3/\text{l}$)	313.22 \pm 59.53	178-411

Table 3. Reticulocyte reference intervals value

Parameters (units)	N	Mean \pm SD	95%CI for mean	Range
Ret% (%)	49	1.382 \pm 0.366	1.277 – 1.487	0.665 – 2.099
Ret# ($\times 10^6/\text{l}$)	49	0.064 \pm 0.017	0.06 – 0.069	0.031 – 0.098
Ret-He (pg)	49	29.549 \pm 1.543	29.106 – 29.992	26.524 – 32.574

Table 4 presents the results of this study compared to reference intervals reported in other studies. Recent studies by Van Pelt *et al.* (2022) and Buttarello *et al.* (2017) did not present the ret% values. A study showed that it is preferable to rely on the absolute reticulocyte count (Ret#), as it provides a more accurate representation of erythropoiesis, while Ret% still requires correction for interpretation and could be misleading in case of anaemia, especially in children (Parodi *et al.*, 2020). In cases of anemia, the reticulocyte percentage may be markedly elevated and may not accurately reflect the true bone marrow response to it. Therefore, the reticulocyte percentage should be adjusted to a corrected reticulocyte percentage, which accounts for the patient’s hematocrit level (Prchal, 2021).

The number of Ret% in this study is almost similar to the previous study by Posada-Franco *et al.* (2024), who employed 175 healthy blood donor subjects in their study. However, the robust method used in their study presented a narrower interval range compared to our study (**Table 4**), while the non-parametric and Harrell-Davis bootstrap method produced a range almost similar to our study. When comparing the reference intervals of 124 healthy

females reported by Scherer *et al.* (2015) with those of adolescent females in the present study, the Ret% in this study is narrower despite the similar Ret# in both studies.

In terms of Ret#, the reference interval found in this study closely resembles previously reported studies. The difference in the hematology analyser used in the studies resulted in a similar interval range value compared to this study, as shown by Buttarello *et al.* (2017), who employed Mindray BC-600. Posada-Franco *et al.* (2024) showed a similar lower limit value compared to this study, while showing a lower upper limit value under Beckman-Coulter DxH900. Among Sysmex users, the XN series provided almost identical Ret# results to this study, whereas XN-5000 showed a slightly wider range (Buttarello *et al.*, 2017; L. Van Pelt *et al.*, 2022; Scherer *et al.*, 2015). Additionally, no prominent differences in Ret# were observed between studies utilizing exclusively female populations and those involving mixed male and female populations.

Table 4. Reference interval comparison among studies

Parameters (unit)	N	This study (Sysmex XN 1000)	(Posada-Franco <i>et al.</i> , 2024)	(L. Van Pelt <i>et al.</i> , 2022)	(Buttarello <i>et al.</i> , 2017)		Scherer <i>et al.</i> (2015) (Sysmex XE 5000)
			(Beckman Coulter DxH900)	(Sysmex XN Series)	(Mindray BC-6800)	(Sysmex XE-5000)	
Ret% (%)	49	0.665 – 2.099	0.769-1.511				0.48 – 2.25
Ret# (x10 ⁶ /μL)	49	0.031-0.098	0.035-0.073	0.033-0.098	0.023-0.093	0.023-0.101	0.02 – 0.09
Ret-He (pg)	49	26.524-32.574		29.3-35.4	30.90-35.70	29.40-35.98	29.81 – 36.54

Ret-He represents the hemoglobin content in reticulocytes. This value reflects the functional availability of iron used for heme synthesis in erythroblasts. Ret-He value is known to be very sensitive to iron status change. Therefore, this parameter is a valuable marker to evaluate iron status and the haematopoiesis process itself (Aedh *et al.*, 2023). Our study showed the range of Ret-He between 26.524 and 32.574 pg. This range is lower than the previously published studies shown in **Table 4**. This discrepancy might be due to the different characteristics of the population used among the studies. A recent study showed that the Ret-He value varied among age groups, especially in children under 5 years. Children of a younger age displayed a relatively lower range of Ret-He compared to the older age groups (Tung *et al.*, 2024). This study also employed a younger subject population (ages 14-19 years), resulting in a lower reference interval compared to other studies that employed older and a wider range of ages in their populations (Buttarello *et al.*, 2017; L. Van Pelt *et al.*, 2022; Scherer *et al.*, 2015).

The values of Ret-He from 28 to 36 pg are generally considered normal. A study in 2022 demonstrated that Ret-He value of ≤ 27.0 pg represents a diagnostically relevant cut-off, as subjects with Ret-He levels below or equal to 27 pg suggested IDA in adolescent females in Thailand (Thimthong *et al.*, 2022).

Among all the reticulocyte parameters explored in this study, the absolute reticulocyte count (Ret#) might be the most effective parameter for monitoring anaemia. The absolute reticulocyte count represents the length of time reticulocytes remain in circulation prior to maturation. In general, reticulocytes mature within one day after being released from the bone marrow. However, under conditions of impaired erythropoiesis, such as in the presence of elevated erythropoietin levels (e.g., in patients with severe anaemia), reticulocytes are prematurely released from the bone marrow into the circulation. This prolongs their time in circulation (several days until maturation) and results in a markedly elevated reticulocyte count (Prchal, 2021). Similar to the role of hematocrit in correcting reticulocyte percentages, the erythrocyte count is inherently factored into the calculation of the absolute reticulocyte count. As a result, no further correction is required for Ret# (Paltrinieri *et al.*, 2017). Furthermore, with the use of automated hematology analyzers, the absolute reticulocyte count is automatically provided.

Table 5. Conditions associated with increased and decreased reticulocyte counts

Increased reticulocyte count	Decreased reticulocyte count
Blood loss (e.g., trauma, gastrointestinal bleeding, menorrhagia)	Nutritional Deficiency: Vitamin B12, folate, and iron deficiency (megaloblastic anemia, pernicious anemia, iron deficiency anemia)
Haemolytic anaemia (e.g., autoimmune hemolytic anaemia, hemolytic disease of the new-born)	Reduced erythropoietin levels (chronic kidney disease)
Response to therapy (e.g., iron, vitamin B12, or folate supplementation; erythropoietin therapy; bone marrow recovery after chemotherapy or bone marrow transplantation)	Aplastic anemia or bone marrow failure syndromes
	Post-radiation therapy
	Bone marrow replacement due to benign processes (e.g., metabolic storage disorders, infections, sarcoidosis) or malignant infiltration (e.g., leukemia, lymphoma, metastatic tumors)

In terms of appropriate evaluation of bone marrow response in anaemia, both the absolute reticulocyte count and the corrected reticulocyte percentage are valuable indicators of red blood cell (RBC) production and provide an initial assessment to determine whether

anemia is due to increased RBC loss or insufficient RBC production (Marks & Glader, 2009; Prchal, 2021), as summarized in **Table 5** (Rai *et al.*, 2023). Concurrently, Ret-he showed a more useful parameter in assessing the amount of functional iron available for hemoglobin synthesis in erythrocytes over the preceding 3-4 days (Rana *et al.*, 2026). Automated hematology analyzers can also provide measurements of reticulocyte hemoglobin content, reported as the mean cellular hemoglobin of reticulocytes (CHr) or as reticulocyte hemoglobin equivalent (Ret-He), depending on the instrument used. CHr and Ret-He are comparable but not identical parameters. Moreover, these parameters have been reported as the strongest predictors of iron deficiency anaemia in children (Ringoringo *et al.*, 2023; Russo *et al.*, 2020; Tung *et al.*, 2024).

In complex clinical settings, reticulocyte haemoglobin content (CHr or Ret-He) is particularly valuable for detecting functional iron deficiency, for example, in chronic inflammation and chronic kidney disease, where serum ferritin may be falsely elevated as an acute-phase reactant despite depleted body iron stores, and where physiologic fluctuations in serum iron and total iron-binding capacity limit the usefulness of conventional iron panels (Cai *et al.*, 2017; Kılıç *et al.*, 2022). Additionally, CHr or Ret-He may serve as a more reliable predictor of iron levels in the bone marrow than traditional serum iron parameters in non-macrocytic individuals (Cai *et al.*, 2017).

4. Conclusion

This study successfully established specific reference intervals for reticulocyte parameters, including absolute reticulocyte counts, reticulocyte percentage, and Ret-He, specifically in adolescent females in Indonesia, using the Sysmex XN-1000 hematology analyzer. These reference intervals will serve as a useful reference for clinical decision-making when dealing with female patients in their adolescent ages in Indonesia.

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