



STUDY OF RETICULOCYTE PARAMETERS OF BLOOD DONORS OF AT THE NATIONAL BLOOD TRANSFUSION CENTER (NBTC) IN ABIDJAN

**Kamagaté Taïratou^{1,2*}, Yayo-Ayé Mireille^{1,3}, N'guessan-Blao Rebecca¹, Adjé Missa Louis³,
Meledje Marie-France³ and Sawadogo Duni^{1,3}**

¹Department of Hematology, Faculty of Pharmacy, Félix Houphouët Boigny University, BP V34 Abidjan, Ivory Coast.

²Center for Diagnosis and Research on AIDS and Other Infectious Diseases (CeDReS), Abidjan, Ivory Coast. BP V3
Abidjan, Ivory Coast.

³Hematology Unit, Central Laboratory, Yopougon University Hospital, Abidjan, Ivory Coast 21BP 632 Abidjan 21.



***Corresponding Author: Kamagaté Taïratou**

Department of Hematology, Faculty of Pharmacy, Félix Houphouët Boigny University, BP V34 Abidjan, Ivory Coast.

Article Received on 24/12/2023

Article Revised on 14/01/2024

Article Accepted on 04/02/2024

ABSTRACT

The occurrence of anemia in blood donors has always been a major concern, affecting both donor safety and the quality of blood components. Nowadays, automated blood analyzers offer new parameters, including reticulocyte hemoglobin content (RET-He), capable of diagnosing anemias. The objective of this study is to demonstrate the usefulness of RET-He in diagnosing anemias and iron deficiencies compared to the usual parameter (ferritin). This cross-sectional study included regular blood donors of both sex who consented to participate. Venous blood collected in EDTA tubes was used for hemogram analysis and RET-He measurement using the Sysmex XN 350 analyzer. Ferritin levels were measured from serum using the Maglumi 800 analyzer. The study population consisted of 196 donors, predominantly male (sex ratio=1.15). The prevalence of anemia was 29%, mostly of hypochromic microcytic type (59.92%). Using ferritin levels as the reference method, RET-He showed a sensitivity of 34% and specificity of 81% in diagnosing iron deficiencies in the study population. However, in cases of anemia, this parameter had a sensitivity of 59% and specificity of 61.8%. RET-He could be a less costly alternative in screening for iron deficiency and iron-deficiency anemia in association with the hemogram.

KEYWORDS: Iron Deficiency, Ferritin, Hemoglobin, RET-He.

1. INTRODUCTION

The prevention of anemia in blood donors is a crucial issue for donor safety and the quantitative and qualitative assurance of blood products. Indeed, a donation of 450 ml of whole blood results in a loss of 230 to 250 mg of iron, equivalent to 100 days of dietary iron intake.^[1] Approximately 2/3 of blood donors do not fully recover from iron loss before their next donation.^[2] Without iron supplementation, it would take the donor 186 days to recover 80% of the lost iron and 180 days to replenish iron reserves. This timeframe exceeds the typical 3-month inter-donation intervals observed in various countries.^{[2][3]} Therefore, it would be necessary to assess blood iron reserves before the next blood donation.

With the evolution of biological techniques, the exploration of the hemogram has enabled the diagnosis and typing of anemia, thanks to erythrocyte constants such as hemoglobin level (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).^[4] Serum ferritin concentration, serum iron

level, total iron binding capacity and transferrin saturation are biochemical tests that not only explore iron metabolism, but also reveal iron deficiency or alterations in its metabolism. Parameters of iron metabolism are not commonly used, as they are costly. They can also be influenced by certain physiological conditions, such as pregnancy, or pathological conditions, such as rheumatoid arthritis. Ferritin levels reflect the body's iron reserves, and very low values indicate iron deficiency.

Nowadays, with the development of biological techniques, automated complete blood count (CBC) analyzers offer a new reticulocyte parameter called reticulocyte hemoglobin content or RET-He. This parameter allows, alongside the blood count, to assess the contribution of iron to erythropoiesis, providing an insight into the quality of erythropoiesis. Indeed, reticulocytes are the precursors of mature erythrocytes. They migrate from the bone marrow to the peripheral blood, reaching maturity within 2 days and transforming into red blood cells. RET-He can be measured in less than 2 minutes on many hematology analyzers

simultaneously with a complete blood count and is less expensive than ferritin measurement. It thus provides timely information on bone marrow regeneration.^[5] Therefore, it could be used to reflect the body's iron status and identify iron deficiency with iron depletion on one hand, or inflammatory syndrome with iron overload on the other. The overall objective of this study was to demonstrate the usefulness of RET-He in identifying and monitoring anemias and iron-deficiencies compared to the usual parameter (ferritin).

MATERIAL AND METHODS

Study Type, Setting, and Period

This is a cross-sectional study initiated by the Department of Hematology, Immunology, and Cellular Biology of the Faculty of Pharmaceutical and Biological Sciences at Abidjan, Ivory Coast. The study was conducted in collaboration with the National Blood Transfusion Center (NBTC) of Abidjan, which served as the setting for patient recruitment and sample collection. The Ivoir-German Bio-Diagnostic Laboratory (DAS) provided the setting for hemogram analysis, while biochemical examinations were conducted at the Emergency Medical Assistance Service (EMAS). This study spanned from January 2021 to January 2022.

Study Population

The study population consisted of regular blood donors, aged 18 to 60 years, weighing over 50 kg, who had given their informed, signed consent and were in good general condition after the medical examination.

Study Procedure

The survey form used included donor identification, sociodemographic data, medical history, and clinical data. The confidentiality of study data was preserved through strict adherence to the required measures to ensure respect for privacy.

After enrolment, samples were taken from the clamped bag tubing during blood donation. Biological tests were carried out using 4 tubes, including 2 violet-capped tubes and 2 red-capped dry tubes.

The violet-capped tubes, containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, were used for hemogram analysis and RET-He measurement on the Sysmex XN 350.

The red-capped tubes, without any anticoagulant, were used to obtain serum and were employed for biochemical analyses, specifically ferritin measurement on the Maglumi 800.

EDTA tube samples for the hemogram were transported to the Ivoir-German DAS laboratory in less than 6 hours.

Anemia was defined by a hemoglobin value below 12 g/dL for women and 13 g/dL for men. It was considered

normocytic if the MCV was between 80 and 98 fL and microcytic if it was below 80 fL. It was termed normochromic if the MCHC values were between 32 and 36%, and hypochromic if the MCHC was less than 31%.

The normal value of serum iron is between 0.65 and 1.75 mg/l in men and between 0.5 and 1.7 mg/l in women. Serum ferritin levels range from 14 to 250 ng/ml in men and between 5 and 200 ng/ml in women. Iron deficiency was defined as a ferritin level < 14 ng/ml in men and < 5 ng/ml in women. The simultaneous presence of anemia and iron deficiency was considered iron-deficiency anemia. RET-He had a normal value of between 28 and 35 pg. Sensitivity (Se) is defined as the ability of the test evaluated to correctly detect samples containing iron deficiency. Specificity (Sp) is defined as the ability of the test evaluated to correctly detect samples with no iron deficiency.

Data processing and analysis

All data were collected on individual survey forms, entered and processed by Excel 2013 software. Descriptive statistics were used to calculate the mean and standard deviation of biological variables. The expected results were presented in the form of tables and graphs created using Microsoft Word software. Two tests were employed; the Pearson Chi-square test to verify the association between RET-He and ferritin, and the diagnostic tests: sensitivity (Se) and specificity (Sp) to assess the reliability of the new test.

RESULTS

Socio-demographic Data

The mean age of the study population was 39 years (± 10 years), with extremes ranging from 20 to 60 years. The study population was predominantly male, with a sex ratio of 1.15 (Table 1).

Table 1: Socio-demographic parameters.

Parameters	Frequency (n)	Percentage (%)
Age		
[18-25]	27	13.8
[26-35]	44	22.4
[36-45]	74	37.8
[46-60]	51	26.0
Total	196	100
Sex		
Male	105	53.6
Female	91	46.4
Total	196	100

Biological Data

Overall, 29% of the population was anemic (Fig. 1). Hypochromia and microcytosis were present in 20% of donors with no anemia (Table 2). Microcytic hypochromic anemia predominated in anemic subjects (58.92%) (Table 3).

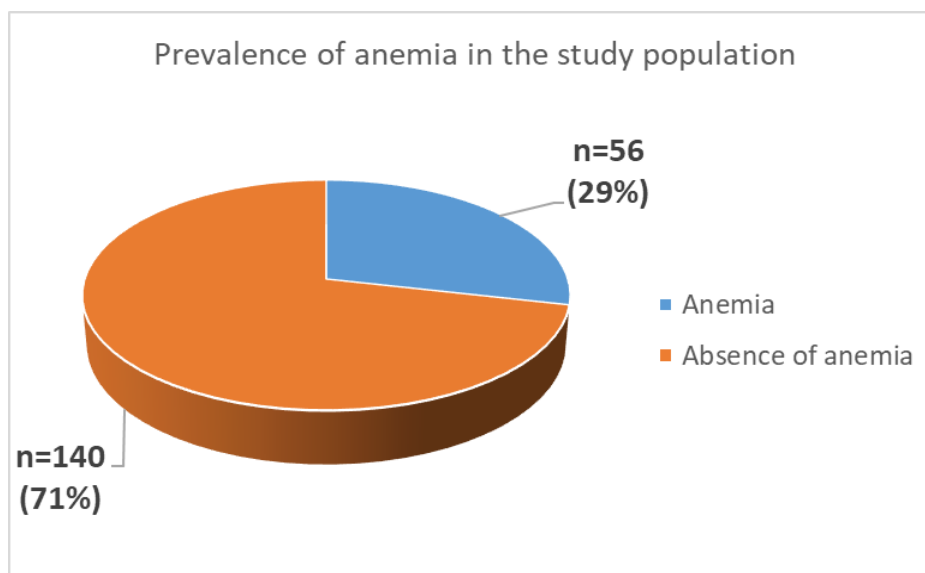


Figure 1: Prevalence of anemia in the study population.

Table 2: Variation in erythrocyte parameters in donors without anemia.

	Parameters	Frequency (n)	Percentage (%)
No variations	Normal MCHC	90	64,29
	Normal MCV		
Hypochromia and isolated microcytosis	Low MCHC	28	20,00
	Low MCV		
Isolated hypochromia	Low MCHC	15	10,71
Isolated microcytosis	Low MCV	07	5,00
Total		140	100

MCV :Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration

Table 3: Distribution of the study population by anemia type.

	Parameters	Frequency (n)	Percentage (%)
Microcytic hypochromic anemia	Low Hb	33	58,92
	VGM lowered		
	CCMH lowered		
Normocytic normochromic anemia	Low Hb	22	39,28
	Increased MCV		
	Normal MCHC		
Macrocytic anemia	Low Hb	1	1,79
	Normal MCV		
	Normal MCHC		
Total		56	100

Hb: Hemoglobin; MCV :Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration

The mean values of ferritin and RET-He were within the normal range. The minimum ferritin value was low, while the maximum value was very high (Table 4).

Table 4: Description of ferritinemia and RET-He variables.

Parameters	m ± s.d	Min	Max
Ferritinemia (ng/ml)	52,1 ± 43,0	0,635	210
RET-He (pg)	29,6 ± 2,96	20,2	36,8

ng/ml : Nanogram/milliliter, pg : Picogram, m : Mean, s.d : Standard deviation, Min : Minimum, Max : Maximum

There was a significant difference between the average ferritin levels of anemic and non-anemic donors ($p < 0.05$).

There was a significant difference between the mean RET-He of donors with anemia and the mean RET-He of donors without anemia ($p < 0.05$) (Table 5).

Table 5: Ferritin and RET-He Description Based on the presence or absence of Anemia.

Variables	Absence of anemia	Presence of anemia	p-value
Ferritinemia (ng/ml)	56,80	40,49	0,024
RET-He (pg)	30,37	27,67	5.138 10 ⁻⁰⁷

All values in this table are mean values.

RET-He: reticulocyte hemoglobin content, ng/ml: Nanogram/milliliter, pg.: Picogram

Significant difference in mean compared with control at $p < 0.05$.

Taking serum ferritin as the reference method, RET-He has a sensitivity of 34% and a specificity of 81% in the diagnosis of iron deficiency in the study population

(Table 6). However, in the case of anemia, this parameter has a sensitivity of 59% and a specificity of 61.8% (Table 7).

Table 6: Sensitivity (Se) and Specificity (Sp) in the diagnosis of iron deficiency by ferritin levels and RET-He in the General Population.

RET-He		Ferritinemia		
		Iron deficiency	No iron deficiency	Total
	Iron deficiency	16	28	44
	No iron deficiency	31	121	152
Total		47	149	196

RET-He : Teneur en hémoglobine des réticulocytes

$Se = [True\ positive / (True\ positive + False\ negative)] \times 100$

$Sp = [True\ negative / (False\ positive + True\ negative)] \times 100$

The sensitivity and the specificity were 34 % and 81.2% respectively.

Table 7: Sensitivity and specificity in the diagnosis of iron deficiency by ferritinemia x RET-He in anemia.

RET-He		Ferritinemia		
		Iron deficiency	No Iron deficiency	Total
	Iron deficiency	13	13	26
	No iron deficiency	9	21	30
Total		21	34	56

The sensitivity and the specificity were 59 % and 61.8% respectively.

DISCUSSION

Sociodemographic Data

In the present study, the studied population was predominantly male, with a sex ratio of 1.15. This male predominance can be explained by the fact that women were mostly ineligible for blood donation due to physiological reasons (pregnancy, breastfeeding, recent menstruation).

The mean age of the study population was 39 years (± 10 years), with a minimum of 20 years and a maximum of 60 years. This average age was higher than those in the studies by Sekongo et al. and Traore in Mali, which had mean ages of 26 years and 37 years, respectively.^[6, 7]

Our results are not in agreement with those of Sekongo et al., who found that the age group of 18-45 age group was the most represented among blood donors (82%); this was confirmed by the study of Tagny et al., who obtained a prevalence of 77.2% among blood donors aged 18 to 39 years.^[6, 8]

Biological data

This study revealed that anemia was frequent in 29% of blood donors. Our results remain very high compared with those reported by Sekongo and Diarra, who respectively reported a prevalence of 4.17% and 10% among blood donors. However, Nzengu-Lukusa et al. in the Democratic Republic of Congo reported in their study a higher prevalence of anemia at 36.5%.^[6, 9, 10] These differences could be explained by a difference in the hemoglobin threshold used in the different studies.

This anemia was predominantly microcytic hypochromic, with a value of 58.92%. These findings align with those of Sekongo and Igala, who obtained a majority of hypochromic microcytic anemia (62% and 61.8%, respectively). This suggests a potential iron deficiency as the origin of the anemia.^[6, 11]

To date, pre-donation hemoglobin screening is the main strategy used to mitigate donor anemia. It is an essential measure to prevent anemic blood donation and preserve donor safety. Unfortunately, it appears to be an unreliable biomarker of iron deficiency and the early stage of anemia.^[12]

Indeed, By the time hemoglobin levels fall below normal values, the donor has likely depleted their iron reserves.^[3,8] The most reliable biochemical markers for iron deficiency (staining of iron in the bone marrow, serum ferritin and transferrin, transferrin saturation, transferrin iron-binding capacity, soluble transferrin receptor) are not suitable in the context of blood donation. They are costly and difficult to implement.^[12] We aimed to use RET-He as an indicator to detect iron deficiency. Thanks to new analyzers, this parameter was more readily accessible than ferritin and less costly.

There was a statistically significant difference between mean ferritinemia in the presence of anemia and mean ferritinemia in the absence of anemia ($p < 0.05$); the same applied to mean RET-He. In fact, these parameters were elevated in the absence of anemia and lowered in the presence of anemia. These results are in agreement with those of Sawadogo and colleagues, who showed that RET-He ($p = 0.0001$) and ferritin ($p = 0.0001$) were statistically lower in anemia than in other situations.^[12]

However, RET-He had a sensitivity of 34% and a specificity of 81% in the study population. But in the case of anemia, sensitivity was higher (59%), with a specificity of 61.8%. This observed sensitivity could be due to the size of the study sample or the numbers of subjects with iron deficiency, or to the RET-He threshold used, which was 28 pg/cell. Indeed, the study by Sawadogo et al. with a RET-He threshold of 32 pg/cell, gave better diagnostic performance for iron deficiency (Sensitivity = 63.3%, Specificity = 77.9%) and cases of depleted iron reserves (Sensitivity = 76.5%, Specificity = 75.0%).^[12] Furthermore, according to Nebie et al. several studies have evaluated the diagnostic performance of RET-He as an early diagnostic parameter for iron deficiency states in different clinical situations. Their results have confirmed its usefulness, despite wide divergences in diagnostic performance.

CONCLUSION

RET-He emerges as a frontline tool in diagnosing iron deficiencies and iron-deficiency anemias due to its cost-effectiveness compared to ferritin measurement and its ability to be determined more rapidly, simultaneously with the complete blood count.

It would therefore be advisable to deepen this study with a larger sample of anemic subjects presenting iron deficiency to better establish its algorithm for the benefit of the healthcare system.

REFERENCES

- Goldman M. Iron depletion and routine ferritin measurement in blood donors. *ISBT Sci Ser*, 2015; 10(S1): 124-128.
- Fillet AM, Gross S. Prevention of anemia in blood donors. *Transfus Clin Biol*, 2017; 24(3): 143-147
- Mittal R, Marwaha N, Basu S, Dur M, Kumar UR. Evaluation of iron stores in blood donors by serum ferritin. *Indian J Med Res*, 2006; 124: 641-646.
- Concentration en hémoglobine des réticulocytes - RET-He <https://www.sysmex.fr/academie/centre-dexpertise/les-parametres-du-xn/teneur-en-hemoglobine-des-reticulocytes-ret-he.html>
- Hönemann C, Hagemann O, Doll D, Luedi M, Ruebsam ML, Meybohm P. Reticulocyte haemoglobin as a routine parameter in preoperative iron deficiency assessment. *Endocrinol Metab Open Access*, 2021; 5(1): 154. <https://www.imedpub.com/articles/reticulocyte-haemoglobin-as-a-routine-parameter-in-preoperative-iron-deficiency-assessment>.
- Sekongo YM, Kabore S, Yao K D, Dembele B, Konate S, Dasse SR. (2020) Anemia to Blood Donors at the National Blood Transfusion Center of Abidjan Cote d'Ivoire. *Eur. j. clin. bioimed. Sci*, 2020; 6(1): 1-4.
- Traoré M. Intérêt de l'index de production réticulocytaire en zone urbaine et rurale du Mali. Thèse, Faculté de Médecine, de Pharmacie et d'Odontostomatologie, Université Bamako, 2008. <https://bibliosante.ml/bitstream/handle/123456789/6765/03P37.pdf?sequence=1&isAllowed=y>
- Tagny CT, Diarra A, Yahaya R, Hakizimana, M, Nguessan A, Mbensa, G, et al. (2009) Le centre de transfusion, le donneur de sang, et le sang donné dans les pays d'Afrique francophone. *Transfus Clin Biol*, 2009; 16: 431-438.
- Diarra A. Anémie chez les donneurs de sang réguliers au CNTS de Bamako. Thèse, Faculté de Médecine, de Pharmacie et d'Odontostomatologie, Université Bamako, 2006; <https://bibliosante.ml/bitstream/handle/123456789/6933/06P72.pdf?sequence=1&isAllowed=y>.
- Nzengu-Lukusa F, Yuma-Ramazani S, Sokolua-Mvika E, Dilu-Keti A, Malenga-Nkanga B, Shuli JB, et al. Carence en fer, anémie et anémie ferriprive chez les donneurs de sang à Kinshasa, République Démocratique du Congo. *Pan Afr. med. J*, 2016; 23: 174.
- Igala M, Ledaga LE, Kouegnigan RL, Diop LS, Guarisco M, Rebienot PO, et al. Anemia in voluntary blood donors after verification of pre-donation hemoglobin at the Libreville Blood Transfusion Center. *Bull Med Owendo*, 2022; 20(51): 18-23.
- Sawadogo S, Boureima H, Koulidiati J, Nebie K, Coulibaly A, Sawadogo A et al. Added-Value of Reticulocyte Haemoglobin Equivalent in the Early Diagnosis of Iron Deficiency States among Blood Donors: A Pilot Study in Burkina Faso. *Health Sci. Dis*, 2020; 21(10): 1-8.
- Nebie K, Sawadogo S, Kafando E, Bationo EM, Dahourou H, Ouattara S et al. Apports du contrôle qualité produits finis dans l'amélioration de la qualité des produits sanguins labiles au Centre régional de transfusion sanguine de Ouagadougou (Burkina Faso), 2014. *Transfus Clin Biol*, 2017; 24(4): 431-439.