

ARTICLE INFO

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Article history

Received	04-06-2026
Revised	06-08-2026
Accepted	29-09-2026
Available online	

Please cite this article in APA 7th edition style as:

Yanti, L. N., Rofinda, Z. D., Efrida, Rikarni, Husni, & Yusri, E. (2026). Predictive value of reticulocyte hemoglobin equivalent in screening iron depletion among regular blood donors. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, 15(2), 179–189.
<https://doi.org/10.30742/jikw.v15i2.5316>

Predictive Value of Reticulocyte Hemoglobin Equivalent in Screening Iron Depletion Among Regular Blood Donors

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Abstract

Background: Regular blood donors are at increased risk of iron depletion due to repeated blood loss and loss of hemoglobin-bound iron. Although hemoglobin testing can detect iron deficiency anemia, it may not identify early iron depletion. Assessment using bone marrow staining is invasive, while serum ferritin can be influenced by inflammation, highlighting the need for a practical and reliable alternative. **Objective:** This study aimed to evaluate the predictive value of Ret-He for detecting iron depletion in regular blood donors. **Methods:** An analytical cross-sectional study was conducted involving 168 regular blood donors at the Blood Centre of M. Djamil Hospital, Padang, from August to October 2025. Ret-He and serum ferritin levels were measured using flow cytometry and Enzyme-Linked Fluorescence Assay (ELFA), respectively. The diagnostic performance and optimal cut-off point of Ret-He were evaluated utilizing Receiver Operating Characteristic (ROC) curve analysis. **Result:** The study cohort was a median age of 30 years, with a predominantly male demographic (54.8%). Iron depletion was identified as 37.5% of the donors. The median Ret-He value in iron-depleted donors was significantly lower compared to non-depleted donors (30.8 pg vs. 32.1 pg; $p < 0.001$). ROC analysis established an optimal Ret-He cut-off point of < 31.75 pg, yielding an Area Under the Curve (AUC) of 0.702, alongside a sensitivity of 73.0%, specificity of 60.0%, positive predictive value (PPV) of 52.3%, and negative predictive value (NPV) of 78.8%. **Conclusion:** Reticulocyte Hemoglobin Equivalent with cut-off point of < 31.75 pg demonstrates moderate predictive ability in screening iron depletion among regular blood donors.

Keywords: Iron Depletion, Regular Blood Donors, Ret-He, Serum Ferritin

Original Research Article

INTRODUCTION

Blood donors are at high risk of iron deficiency anemia due to the loss of iron bound to hemoglobin (Hb), which is one of the criteria for donor rejection. One milliliter of blood contains 0.5 mg of iron, so a donor will lose 200-250 mg of iron for each blood donation of 450-500 mL (Gutierrez et al., 2021; Sweegers et al., 2020). Human blood and plasma products, as well as plasma-derived medicinal products, play an important role in healthcare and are fundamental to achieving universal health

coverage (World Health Organization, 2024). Blood Centres are responsible for the availability, quality, and safety of collected blood and blood components, as well as ensuring the safety of donors and recipients (Peraturan Menteri Kesehatan Nomor 91 Tahun 2015 Tentang Standar Pelayanan Transfusi Darah, 2015).

The prevalence of iron deficiency in regular blood donors from a study conducted in France, with a ferritin cut-off point of <26 ng/mL, was 29%, and with a ferritin cut-off point of <15 ng/mL, it was 13%. Studies in the United States (US) and Canada reported iron deficiency rates of 38% at a ferritin cut-off point of <26 ng/mL and 15% at <12 ng/mL. Study in low-resource countries such as South Africa showed a prevalence of iron deficiency of 16% in female donors with a ferritin cut-off point <12 ng/mL and in male donors of 19% with a ferritin cut-off point <20 ng/mL (Spencer & Mast, 2022). A study at the Indonesian Red Cross Blood Donation Unit in South Sumatra Province found a donor iron deficiency prevalence of 17.4% with a ferritin cut-off point of <15 ng/mL (Widiawati et al., 2021). Meanwhile, study at the Blood Centre of M. Djamil Hospital in West Sumatra Province reported a 39% prevalence using a ferritin cut-off point of <30 ng/mL (Yanti et al., 2025).

Iron deficiency is classified into 3 stages. Stage 1 iron deficiency is iron depletion, marked by progressively reduced iron stores. The production and development of erythrocytes are normal during this phase. Stage 2 iron deficiency is iron-deficient erythropoiesis, marked by iron in storage sites being depleted. Erythrocyte production continues relying on the iron available in the transport compartment and recycled from dead cells. Hemoglobin levels are normal at this stage. Stage 3 iron deficiency is iron deficiency anemia characterized by decreased hemoglobin levels. Iron stores level tests are not routinely performed during pre-donor screening, so donors with iron depletion (who are not anemic) may pass the screening and continue to donate blood. This condition can lead to iron deficiency anemia, which is one of the criteria for rejection in donor selection, thereby impacting the blood supply (Yanti et al., 2025; Mantadakis et al., 2022).

The gold standard for iron stores testing is bone marrow iron staining, but it is not routinely performed because it is invasive (Gelaw et al., 2019; Kulik-Rechberger & Dubel, 2024). Serum ferritin is a biomarker for measuring iron stores but is affected by inflammation (Salam et al., 2020). Reticulocyte hemoglobin equivalent (Ret-He) is a parameter available on almost all automated hematology instruments that describes the real-time iron availability 1-2 days prior to testing. A decrease in Ret-He values indicates iron-deficiency erythropoiesis. The Ret-He test offers several advantages, namely low cost, fast turnaround time, and is not affected by inflammation (Gelaw et al., 2019; Tiwari et al., 2018). This makes Ret-He a potential tool for identifying iron depletion in blood donors (Mantadakis et al., 2022).

Although the diagnostic performance of Ret-He for screening iron depletion in blood donors has been reported in various populations, such as India (De et al., 2025; Tiwari et al., 2018) and Burkina Faso (Salam et al., 2020), its optimal cut-off point, sensitivity, specificity, PPV, and NPV vary across populations due to differences in subject characteristics and ethnicity. In Indonesia, study on the diagnostic utility of Ret-He among regular blood donors has so far only been conducted in Yogyakarta; no similar study has been performed in the West Sumatra region. This study aims to determine the predictive value of Ret-He in screening iron depletion among regular blood donors, thereby providing additional validation evidence in a previously unstudied donor population and establishing a population-specific Ret-He cut-off relevant to this setting.

MATERIALS AND METHODS

Study Design, Location, and Time

This study is an analytical study with a cross-sectional design conducted at Blood Centre and the Central Laboratory Installation of M. Djamil Hospital Padang. This study was carried out from March 2025 to November 2025.

Study Population and Sample

The population of this study consisted of all regular blood donors who donated blood from August 2025 to October 2025 at the Blood Centre of M. Djamil Hospital, Padang. Regular blood donors were defined as those who donated blood at least twice a year (Anshori et al., 2021). The study sample comprised population members who met the specified inclusion and exclusion criteria. The inclusion criteria were regular blood donors who satisfied the donor selection requirements based on Indonesian Ministry of Health Regulation (Permenkes) No. 91 of 2015, were not currently taking iron supplements, and provided signed informed consent. The exclusion criteria were blood donors testing positive for C-reactive protein (CRP).

Sample Size Calculation and Sampling Method

The sample size is determined by the formula:

$$n = \frac{(Z\alpha)^2 \text{ Sen } (1 - \text{Sen})}{d^2P}$$

The $Z\alpha$ value corresponds to the confidence level based on the significance level (α). This study used an α of 5%, corresponding to a $Z\alpha$ of 1.96. The expected sensitivity was set at 95%, with an acceptable margin of error (precision) of 5% and an estimated prevalence (P) of 63.3% (from the literature of Salam et al., 2020). Based on the sample size formula, the minimum required sample size was 115 participants.

The study sample was selected using a consecutive sampling method. A total of 234 regular blood donors who presented for pre-donation screening were initially assessed. Among them, 52 donors were deferred for not meeting standard pre-donation criteria (21 due to blood pressure exceeding 160 mmHg and 31 due to hemoglobin levels below 12.5 g/dL). Furthermore, 14 donors were excluded because of a positive qualitative C-reactive protein (CRP) result, indicating systemic inflammation. As a result, a total of 168 eligible regular blood donors were included in the final analysis (Figure.1)

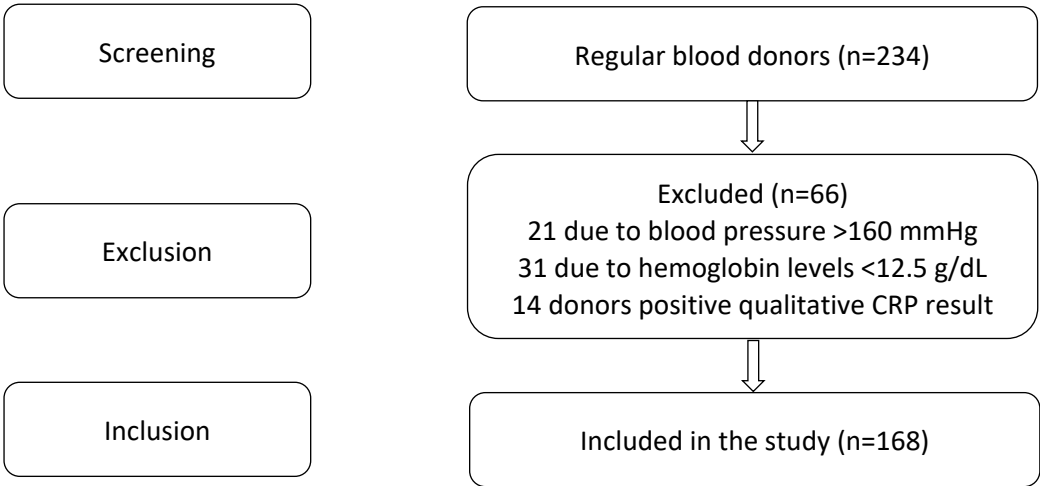


Figure 1. A flow chart of study samples

Operational Definition

Reticulocyte hemoglobin equivalent is a parameter that reflects the hemoglobin content in reticulocytes (Bo et al., 2023). In this study, Ret-He was measured using flow cytometry on a Sysmex XN-1000 analyzer. Currently, there are no established guidelines regulating the reference values for Ret-He.

Iron depletion represents the early stage of iron deficiency characterized by depleted iron stores, evaluated through serum ferritin measurement. In this study, serum ferritin was measured

using the enzyme-linked fluorescent immunoassay (ELFA) method on a VIDAS automated analyzer. Although normal serum ferritin thresholds vary across laboratories, selecting an optimal cut-off requires balancing sensitivity and specificity; for screening purposes, a cut-off with higher sensitivity was preferred. Accordingly, iron depletion was defined as a ferritin level of <30 ng/mL for both men and women (World Health Organization, 2020; Nemeth et al., 2024). Inflammatory status was evaluated using a qualitative latex agglutination CRP assay with a detection threshold of 5 mg/L, in accordance with WHO guidelines on ferritin interpretation in populations with potential inflammation (World Health Organization, 2020).

Data Collection

The study subjects were regular blood donors who had undergone pre-donor screenings. They were informed about the study and signed an informed consent form. The subjects filled out the questionnaire form guided by the researcher and recorded it on a separate sheet.

Venous blood was collected during the donation process. Three milliliters of venous blood was placed into a clot activator tube with a gel separator, and 3 mL was drawn into a K2EDTA tube. Blood samples in the K2EDTA tube were tested immediately for Ret-He. The specimen in the clot activator tube was centrifuged at room temperature at 3,500 rpm for 15 minutes to obtain serum. The serum was then aliquoted into two portions for qualitative CRP and serum ferritin testing. Quality control was performed for qualitative CRP, serum ferritin, and Ret-He assays prior to specimen analysis.

Data Analysis

Analysis of numerical variables began with a data normality test using the Kolmogorov-Smirnov test (sample size >50). Numerical variables were presented as medians (minimum-maximum) because the data were not normally distributed. Categorical variables were presented as frequencies and percentages (%). The difference in Ret-He values between iron-depleted and non-iron-depleted regular blood donors was analyzed using the Mann-Whitney U test because the data were not normally distributed. A p-value < 0.05 considered statistically significant. To evaluate the diagnostic performance of Ret-He, receiver operating characteristic (ROC) curve analysis was performed. The ROC curve plotted sensitivity against 1-specificity. The area under the curve (AUC), sensitivity, specificity, PPV, and NPV were evaluated. AUC values were interpreted as follows: ≥0.9 (excellent), 0.8 to <0.9 (good), 0.7 to <0.8 (moderate), 0.6 to <0.7 (poor), and 0.5 to <0.6 (very poor) (Çorbacioğlu & Aksel, 2023). Finally, the optimal cut-off point was determined using the Youden Index. Internal validation (bootstrap) was performed to evaluate the reliability and stability of diagnostic performance and to correct sample optimism bias.

Ethical Statement

This study has obtained ethical approval from the Research Ethics Committee of M Djamil Hospital, Padang, with the number: DP.04.03/D.XVI.10.1/308/2025.

RESULTS

Subjects ages ranged from 19 to 61 years, with a higher proportion of male than female subjects. The most common donation interval was ≤3 months.

Table 1. Characteristics of study subjects (n=168)

Variables	f (%)	Median (Min-Max)
Age (years)	-	30 (19 – 61)
Gender		
Male	92 (54.8)	-
Female	76 (45.2)	-
Last donation interval		-
≤ 3 months	101 (60.1)	
> 3 months	67 (39.9)	
Donation frequency		
2-5 times	50 (29.8)	-
6-10 times	48 (28.6)	-

Variables	f (%)	Median (Min-Max)
11-20 times	37 (22.0)	-
>20 times	33 (19.6)	-
Iron status		
Iron depletion	63 (37.5)	-
No iron depletion	105 (62.5)	-
Iron status based on gender		
Iron depletion		
Male	29 (46.0)	
Famale	34 (54.0)	
No iron depletion		
Male	63 (60.0)	
Famale	42 (40.0)	
Hb level (g/dL)		
Male		15.1 (12.5-16.0)
Famale		12.7 (12.5-15.0)
Ret-He (pg)		
Male		32.1 (22.2-35.0)
Famale		31.1 (23.2-33.8)

The frequency of blood donations was evenly distributed across the study population. Overall, iron depletion was identified in 63 of 168 subjects (37.5%), showing a higher prevalence among females compared to males. Additionally, male subjects exhibited higher Hb levels and Ret-He values than females (Table 1).

Table 2. Correlation between Hb levels and Ret-He values

Variable	p-value		r
	Kolmogorov smirnov	Spearman	
Hb Level	<0.001	<0.001	0.421
Ret-He Value	<0.001		

Both Hb levels and Ret-He values across all donors were not normally distributed ($p < 0.001$). The correlation between Hb levels and Ret-He values were analyzed using the Spearman correlation test, yielding $p < 0.001$ with $r = 0.421$ (Table 2).

Table 3. Ret-He values regular blood donors with iron depletion and without iron depletion

Variable	Iron Depletion		p-value
	Yes Median (min - max)	No Median (min - max)	
Ret-He, (pg)	30.8 (22.2 – 33.9)	32.1 (28.2 – 35.0)	<0.001*

***Mann-Whitney test**

The median Ret-He in regular blood donors with iron depletion was lower than that in regular blood donors without iron depletion, and the difference was statistically significant ($p < 0.001$) (Table 3).

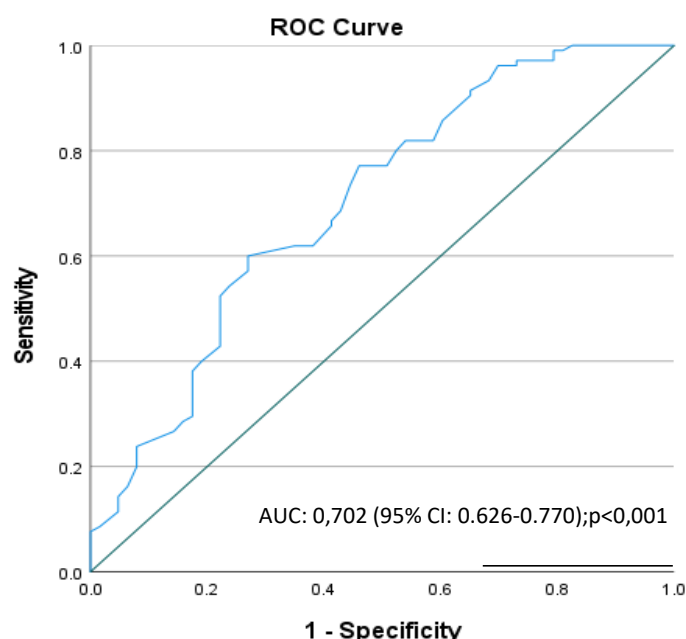


Figure 2. ROC curve of Ret-He values in detecting iron depletion in regular blood donors

The AUC from the ROC curve analysis of Ret-He for detecting iron depletion in regular blood donors was 0.702 (95% CI: 0.626-0.770), with $p < 0.001$. Through internal validation based on the bootstrap resampling method (1,000 replications), a bootstrap of 95% CI of 0.611 to 0.780 was obtained.

Based on the highest Youden's Index J, the optimal cut-off value for Ret-He levels were set at ≤ 31.75 (with a bootstrap 95% CI: ≤ 30.10 to ≤ 32.0) pg. At this optimal cut-off point, the diagnostic performance of Ret-He levels in predicting iron depletion is shown as follows:

Table 4. Ret-He cut off point in routine blood donors with iron depletion

Ret-He Cut-Off Point (pg)	Sensitivity (%)	Specificity (%)
<31.75	73.0	60.0
(95% CI: <30.10 - <32.0)	(95% CI: 60.3-83.4)	(95% CI: 50.0-69.4)

Table 5. Predictive values of Ret-He in screening iron depletion among regular blood donors

Variable		Ferritin		Total
		Iron Depletion f (%)	No Iron Depletion f (%)	
Ret-He	Iron Depletion	46 (52.3)	42 (47.7)	88 (100)
	No Iron depletion	17 (21.2)	63 (78.8)	80 (100)
Total		63 (37.5)	105 (62.5)	168 (100)

PPV: 52.3 (95% CI: 45.3 – 59.1) %; NPV: 78.8 (95% CI: 70.6 – 85.1) %

The sensitivity obtained was 73.0 (95% CI: 60.3 – 83.4) %, specificity was 60.0 (95% CI: 50.0 - 69.4)%, positive predictive value (PPV) was 52.3 (95% CI: 45.3 – 59.1) %, and negative predictive value (NPV) was 78.8 (95% CI: 70.6 – 85.1)% (Table 4 and 5).

DISCUSSION

Characteristics of Study Subjects

The median age of the subjects in this study was 30 (19-61) years. These age characteristics are consistent with previous studies by Anshori et al. (Anshori et al., 2021), Widiwawati et al. (Widiwawati

et al., 2021), and Triyono & Pratiwi (Triyono & Pratiwi, 2024), which reported median age of 31 (18-60) years, 35 (21-52) years, and 30 (17-77) years, respectively. Age is a fundamental criterion in blood donor screening (Anshori et al., 2021). The age range of 31 to 40 years is considered a productive period because the body is physiologically fully developed. This enables optimal function, ensuring that blood donation does not interfere with systemic health (Widiawati et al., 2021).

Male subjects (54.8%) outnumbered female subjects (45.2%). Studies by Widiawati et al. (Widiawati et al., 2021), Nurulita et al. (2022), and Zeng et al. (2023) also found a higher proportion of male donors at 66.3%, 72.4%, and 77.6%, respectively. This relates to the maximum allowed donation frequency, which is higher for men (6 times per year) than for women (4 times per year). Furthermore, physiological factors such as menstruation, pregnancy, and breastfeeding often lead to donation deferral among women (Nurulita et al., 2022).

Most study subjects (60.1%) had a last donation interval of ≤ 3 months, and the remaining (39.9%) had a last donation interval of > 3 months. A study by Anshori et al. similarly found that 69% of donors had a last donation interval of ≤ 3 months and 30% had a donation interval of > 3 months (Anshori et al., 2021). Minister of Health Regulation No. 91 of 2015 states that whole blood donors in Indonesia may donate again after a minimum of 2 months after their previous donation (Peraturan Menteri Kesehatan Nomor 91 Tahun 2015 Tentang Standar Pelayanan Transfusi Darah, 2015). International Guidelines and European Legislation also regulate this interval, permitting donation every 56 days across many countries worldwide (Spencer & Mast, 2022; World Health Organization, 2024).

The frequency of donation was relatively evenly distributed: 2-5 times (29.8%), 6-10 times (28.6%), 11-20 times (22.0%), and >20 times (19.6%). These results align closely with previous studies by Anshori et al. (Anshori et al., 2021) and Anju et al. (2022). Overall, most regular blood donors donated fewer than 10 times. Whole blood donation is well-known to cause iron deficiency, which can delay subsequent donations and ultimately lead to a decrease in donors who give blood more than 10 times (Reddy K et al., 2020; World Health Organization, 2024).

The iron status of donors based on ferritin levels showed that 37.5% of regular donors had iron depletion, with a higher prevalence observed in females compared to males. A study by Reddy et al. (Reddy K et al., 2020) involving 374 donors found that the prevalence of regular blood donors with serum ferritin levels ≤ 30 ng/mL was 25.9%. Similarly, a study by Suria et al. (2022) on 205 regular blood donors revealed that 29.9% had serum ferritin levels ≤ 26 ng/mL. Furthermore, De et al. (2025) reported that among 292 male regular blood donors in India, 17.5% had serum ferritin levels ≤ 15 ng/mL.

The variation in the percentage of regular blood donors with iron depletion in the results of these studies may be due to differences in sample characteristics and ferritin cut-off points for determining iron depletion. Specifically, studies by Suria et al. (Suria et al., 2022) and De et al. (2025) included a higher proportion of male subjects and utilized lower ferritin cut-off point to define iron depletion.

Median of Hb levels and Ret-He values were higher in males compared to females. The Spearman correlation test between Hb levels and Ret-He values yielded $p < 0.001$ with $r = 0.421$. This indicates a significant positive correlation between Hb levels and Ret-He values, with a moderate strength of correlation (r -value in the range of 0.400–0.599).

Ret-He Values in Regular Blood Donors with Iron Depletion and Without Iron Depletion

The median Ret-He value in regular blood donors with iron depletion was significantly lower than in those without iron depletion ($p < 0.001$). The median Ret-He in regular blood donors with iron depletion was 30.8 (22.2-33.9) pg, while in regular blood donors without iron depletion was 32.1 (28.2-35.0) pg. Similarly, previous study by Salam et al. (2020) involving 125 blood donors reported a lower mean value in donors with iron depletion compared to those without iron depletion (32.1 ± 2.8 pg vs 33.6 ± 2.6 pg).

Blood donation (especially regular blood donation) increases the body's iron requirements and can deplete iron stores, leading to varying degrees of iron deficiency (Tiwari et al., 2018). An imbalance between iron demand and supply reduces cellular hemoglobin content in newly produced reticulocytes. This parameter, known as reticulocyte hemoglobin equivalent (Ret-He), reflects the iron availability for hemoglobin synthesis (Bó et al., 2023). Ret-He decreases progressively alongside the severity of iron depletion, a trend clearly demonstrated across children with normal iron status, non-anemic iron deficiency, and iron deficiency anemia (Neef et al., 2021).

The reticulocyte hemoglobin equivalent is clinically very helpful in detecting the early changes in red blood cell indices that reflect iron-deficiency erythropoiesis (Auerbach et al., 2021). Ret-He is a *real-time* parameter that reflects iron-restricted erythropoiesis over the preceding 48 hours and correlates directly with the functional iron available for heme synthesis in erythrocyte precursors (Aedh et al., 2023).

Predictive Value of Ret-He in Screening Iron Depletion among Regular Blood Donors

In this study, the optimal Ret-He cut-off point for screening iron depletion in regular blood donors was <31.75 (95% CI: <30.10 - <32.0) pg, yielding an AUC of 0.702 (95% CI: 0.626-0.770), a sensitivity of 73 (95% CI: 60.3-83.4) %, a specificity of 60 (95% CI: 50.0-69.4) %, a PPV of 52.3 (95% CI: 45.3 – 59.1) %, and an NPV of 78.8 (95% CI: 70.6 – 85.1) %. In comparison, Salam et al. (2020) studied 125 donors using ferritin cut-off point of <30 ng/mL for men and <20 ng/mL for women, with a Ret-He cut-off point of 32 pg, they reported an AUC of 0.706, sensitivity of 63.3%, and specificity of 77.9% (Salam et al., 2020). Similarly, a study by De et al. (2025) involving 292 blood donors applied a ferritin cut-off point of <15 ng/mL and a Ret-He cut-off point of 32 pg, achieving an AUC of 0.822, sensitivity of 72.5%, and specificity of 77.8% (De et al., 2025).

The results of this study yielded an AUC 0.702 (95% CI: 0.626-0.770), indicating a moderate diagnostic ability for screening iron depletion among regular blood donors (Çorbacioğlu & Aksel, 2023). Through internal validation based on the bootstrap resampling method (1,000 replications), a bootstrap of 95% CI of 0.611 to 0.780 was obtained, indicating that the diagnostic performance of this parameter is stable, consistent, and free from sample optimism bias.

A sensitivity of 73 (95% CI: 60.3-83.4) % indicates that a Ret-He value <31.75 pg can screen 60.3% until 83.4% of regular blood donors who have iron depletion. A specificity of 60 (95% CI: 50.0-69.4) % indicates that a Ret-He value ≥31.75 pg can screen 50% until 69.4 % of regular blood donors who do not actually have iron depletion. A positive predictive value of 52.3 (95% CI: 45.3 – 59.1) % means that of all regular blood donors who are considered to have iron depletion based on the Ret-He cutoff point <31.75 pg, 45.3% until 59.1% have iron depletion. A negative predictive value of 78.8 (95% CI: 70.6 – 85.1) % indicates that of all regular blood donors who are considered not to have iron depletion based on the Ret-He value ≥31.75 pg, 70.6% until 85.1% do not have iron depletion. Variations in AUC, sensitivity, and specificity across studies may be due to differences in subject characteristics, ethnicity/ population, and ferritin cut-off points used to define iron depletion.

Iron deficiency progresses through three distinct stages. The first stage is iron depletion, marked by progressively reduced iron stores. Red blood cell production and development remain normal during this phase. Continuous red blood cell production can lower iron stores, which can be detected through iron staining in the bone marrow. This test isn't done routinely because people without anemia appear healthy. Instead, reduced serum ferritin serves as the primary indicator. The second stage is iron-deficiency erythropoiesis, occurs when iron stores are completely exhausted. Red blood cell production continues by relying on iron available in the transport compartment and recycled from dead red blood cells. During this phase, Ret-He value decreased rapidly, reflecting restricted iron supply, but since most circulating red blood cells were produced during periods of adequate iron availability, hemoglobin levels are still within the normal range. Consequently, regular blood donors often pass routine pre-donation hemoglobin screenings, placing them at high risk for developing stage 3 iron deficiency, which is overt iron-deficiency anemia (Rechberger & Dubel, 2024).

Serum ferritin and Ret-He detect iron deficiency at different stages. Serum ferritin can detect iron deficiency at the earliest stage, namely during iron depletion, whereas Ret-He detects iron deficiency at the stage of iron-deficient erythropoiesis. Consequently, Ret-He cannot entirely replace serum ferritin for detecting iron depletion in regular blood donors. However, there are several considerations that make Ret-He a potential alternative for screening iron depletion when serum ferritin testing is not feasible during pre-donor blood screening.

Serum ferritin is an acute phase reactant. Its levels increase during inflammation, infection, malignancy, and certain diseases. Measuring inflammatory markers such as CRP helps identify coincidence inflammatory conditions. Inflammation is indicated if CRP levels are >5 mg/L (World Health Organization, 2020; Fletcher et al., 2022). Consequently, serum ferritin testing must be accompanied by CRP assessment to accurately detect iron depletion, which increases the total screening cost per donor unit. This requirement poses a significant financial challenge for countries with limited healthcare budgets.

Ret-He examination has the potential to be used as an alternative when serum ferritin testing is not feasible, however, the Ret-He cut-off point for predicting iron deficiency has not been established globally. Reticulocyte hemoglobin equivalent is the latest additional parameter in the continuously expanding list of biomarkers. Compared to other biochemical markers such as serum iron and ferritin, Ret-He is a more stable parameter (not affected by inflammation). Serum iron is influenced by circadian variation, whereas ferritin is affected by inflammation, making it inaccurate in reflecting changes in iron kinetics. Compared to hemoglobin levels, Ret-He quickly reflects the actual iron supply to erythrocytes in real time, allowing Ret-He to detect iron deficiency earlier. Therefore, this parameter serves as a valuable supplementary tool for screening iron depletion among regular blood donors (Gelaw et al., 2019; Mouleeswaran et al., 2022).

This study has several limitations that should be acknowledged. First, as a single-center study, the generalizability of the findings may be limited. Second, no sex-specific analysis. Although this study applied a single cut-off based on the WHO framework and instrument specifications, future large-scale prospective studies are recommended to evaluate the potential of sex-specific Ret-He cut-offs using sex-adjusted ferritin thresholds. Third, the ferritin reference standard is not the gold standard and is affected by inflammation and no bone marrow iron staining was performed. Fourth, CRP was measured only qualitatively, although a CRP cut-off of > 5 mg/L directly aligns with the WHO recommendation for identifying clinically relevant inflammation that influences ferritin levels, very low-grade subclinical inflammation below 5 mg/L could not be ruled out.

CONCLUSION

Iron stores testing is not routinely performed during pre-donor screening. Consequently, donors with iron depletion remain unidentified and continue to donate blood. Although bone marrow iron staining is the gold standard for assessing iron stores, its invasive nature limits routine application. Meanwhile, serum ferritin testing is quite expensive because it must be paired with inflammatory markers, such as CRP, to accurately assess iron depletion. Reticulocyte hemoglobin equivalent shows potential for the identification of iron depletion in blood donors, offering lower testing costs, faster turnaround times, and results unaffected by inflammation.

This study evaluated the predictive value of Ret-He for screening iron depletion in regular blood donors. The median Ret-He value in regular blood donors with iron depletion was significantly lower than in regular blood donors without iron depletion ($p<0.001$). At an optimal cut-off point of <31.75 pg, Ret-He demonstrated moderate predictive ability in screening iron depletion among regular blood donors, yielding an AUC of 0.702 (95% CI: 0.618–0.785), a sensitivity of 73%, a specificity of 60%, a PPV of 52.3%, and an NPV of 78.8%.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in this research

ACKNOWLEDGEMENTS

The authors would like to thank all participants and regular blood donors who contributed to this study. We also acknowledge the support of the laboratory staff and research team for their valuable assistance. Finally, we thank the institution for providing facilities and support throughout the research process.

REFERENCES

- Aedh, A. I., Khalil, M. S. M., Abd-Elkader, A. S., El-Khawanky, M. M., Alshehri, H. M., Hussein, A., Alghamdi, A. A. L., & Hasan, A. (2023). Reticulocyte Hemoglobin as a Screening Test for Iron Deficiency Anemia: A New Cut-Off. *Hematology Reports*, 15(1), 201-211. <https://doi.org/10.3390/hematolrep15010021>
- Anju, J., Abhishekh, B., Debdatta, B., Bobby, Z., & Sharan, M. (2022). Assessment of iron status in regular blood donors in a tertiary care hospital in Southern India. *Asian Journal of Transfusion Science*, 16(2), 186. <https://doi.org/10.4103/ajts.ajts.119.21>
- Anshori, F., Ratnaningsih, T., & Triyono, T. (2021). Hematology and Iron Status Evaluation Based on Donation Characteristics in Dr. Sardjito Hospital, Yogyakarta. *INDONESIAN JOURNAL OF CLINICAL PATHOLOGY AND MEDICAL LABORATORY*, 27(3), 249–253. <https://doi.org/10.24293/ijcpml.v27i3.1876>
- Auerbach, M., Staffa, S. J., & Brugnara, C. (2021). Using Reticulocyte Hemoglobin Equivalent as a Marker for Iron Deficiency and Responsiveness to Iron Therapy. *Mayo Clinic Proceedings*, 96(6), 1510–1519. <https://doi.org/10.1016/j.mayocp.2020.10.042>
- Bó, S. D., Fragoso, A. L. R., Farias, M. G., Hubner, D. P. G., & de Castro, S. M. (2023). Evaluation of RET-He values as an early indicator of iron deficiency anemia in pregnant women. *Hematology, Transfusion and Cell Therapy*, 45(1), 52–57. <https://doi.org/10.1016/j.htct.2021.05.006>
- Çorbacioğlu, Ş. K., & Aksel, G. (2023). Receiver operating characteristic curve analysis in diagnostic accuracy studies. *Turkish Journal of Emergency Medicine*, 23(4), 195–198. <https://doi.org/10.4103/tjem.tjem.182.23>
- De, R., Deepak, M. B., Stephen, L., Lakshmi, K., Mammen, J., & Edison, E. S. (2025). Exploring the Relationship between Extended Red Blood Cell Indices and Platelet Indices in Voluntary Blood Donors. *The Journal of Applied Laboratory Medicine*, 10(5), 1119–1132. <https://doi.org/10.1093/jalm/jfaf078>
- Fletcher, A., Forbes, A., Svenson, N., & Wayne Thomas, D. (2022). Guideline for the Laboratory Diagnosis of Iron Deficiency in Adults (Excluding Pregnancy) and Children. *British Journal of Haematology*, 196(3), 523–529. <https://doi.org/10.1111/bjh.17900>
- Gelaw, Y., Woldu, B., & Melku, M. (2019). The Role of Reticulocyte Hemoglobin Content for Diagnosis of Iron Deficiency and Iron Deficiency Anemia, and Monitoring of Iron Therapy: a Literature Review. *Clinical Laboratory*, 65(12). <https://doi.org/10.7754/Clin.Lab.2019.190315>
- Gutierrez, C.Y.M., Pérez, R.D.S., Arias, J.A.C. (2021). Prospective Study on The Effect of Blood Donation In The Iron Status. *Investigaciones Andina*, 42 (23), 37-50. <https://doi.org/10.33132/01248146.2057>
- Kulik-Rechberger, B., & Dubel, M. (2024). Iron deficiency, iron deficiency anaemia and anaemia of inflammation – an overview. *Annals of Agricultural and Environmental Medicine*, 31(1), 151–157. <https://doi.org/10.26444/aaem/171121>
- Mantadakis, E., Panagopoulou, P., Kontekaki, E., Bezirgiannidou, Z., & Martinis, G. (2022). Iron Deficiency and Blood Donation: Links, Risks and Management. *Journal of Blood Medicine*, 13, 775–786. <https://doi.org/10.2147/JBM.S375945>
- Mouleeswaran, K.S., Einstien, D., Aprathiba. (2022). Usefulness of Reticulocyte Haemoglobin Equivalent in the Evaluation of Iron Deficiency Status: A Cross-sectional Study. *Journal of Clinical and Diagnostic Research*, 16(6), EC10-EC12. <https://doi.org/10.7860/JCDR/2022/56493.16491>

- Nemeth, E, Hollenhorst, M., Goodnough, T. L. (2024). Iron Deficiency and Related Disorders. *Wintrobe's Clinical Hematology, 15th Edition, Chapter 25*. Philadelphia: Wolters Kluwer, 1272–1301
- Neef, V., Schmitt, E., Bader, P., Zierfuß, F., Hintereder, G., ...& Piekarski, F. (2021). The Reticulocyte Hemoglobin Equivalent as a Screening Marker for Iron Deficiency and Iron Deficiency Anemia in Children. *Journal of Clinical Medicine, 10*(16), 3506. <https://doi.org/10.3390/jcm10163506>
- Nurulita, Purnamaningsih, N., & Hardjo, K. (2022). Gambaran Hasil Seleksi Pendonor Darah Sukarela di UDD PMI Kota Pangkalpinang Tahun 2020. *Jurnal Kesehatan, 15*(1), 23–29. <https://doi.org/10.23917/jk.v15i1.16039>
- Peraturan Menteri Kesehatan Nomor 91 Tahun 2015 Tentang Standar Pelayanan Transfusi Darah, Pub. L. No. 91 (2015)
- Reddy K, V., Shastry, S., Raturi, M., & Baliga B, P. (2020). Impact of Regular Whole-Blood Donation on Body Iron Stores. *Transfusion Medicine and Hemotherapy, 47*(1), 75–79. <https://doi.org/10.1159/000499768>
- Rechberger, B.K & Dubel, M. (2024). Iron Deficiency, Iron Deficiency Anaemia and Anaemia of Inflammation – an Overview. *Annals of Agricultural and Environmental Medicine, 31* (1), 151–157. <https://doi.org/10.26444/aaem/171121>
- Salam, S., Hassane, B., Koulidiati, J., Nebie, K., Abou, C., ...& Kafando, E. (2020). Added-Value of Reticulocyte Haemoglobin Equivalent in the Early Diagnosis of Iron Deficiency States among Blood Donors: A Pilot Study in Burkina Faso. *Health Sciences and Disease, 21*(10), 1–8. <https://doi.org/10.5281/HSD.V21I10.2323>
- Spencer, B. R., & Mast, A. E. (2022). Iron status of blood donors. *Current Opinion in Hematology, 29*(6), 310–316. <https://doi.org/10.1097/MOH.0000000000000733>
- Suria, N., Kaur, R., Mittal, K., Palta, A., Sood, T., ...& Kaur, G. (2022). Utility of reticulocyte haemoglobin content and immature reticulocyte fraction in early diagnosis of latent iron deficiency in whole blood donors. *Vox Sanguinis, 117*(4), 495–503. <https://doi.org/10.1111/vox.13216>
- Sweegers, M. G., van Kraaij, M. G. J., & van den Hurk, K. (2020). First do no harm: iron loss in whole blood donors. *ISBT Science Series, 15*(1), 110–117. <https://doi.org/10.1111/voxs.12527>
- Tiwari, A. K., Bhardwaj, G., Arora, D., Aggarwal, G., Pabbi, S., ...& Sethi, M. (2018). Applying newer parameter Ret-He (reticulocyte haemoglobin equivalent) to assess latent iron deficiency (LID) in blood donors–study at a tertiary care hospital in India. *Vox Sanguinis, 113*(7), 639–646. <https://doi.org/10.1111/vox.12700>
- Triyono, T., & Pratiwi, D. (2024). Analysis of donor characteristics in a Tertiary Hospital Blood Center in Indonesia. *Journal of Medicinal and Pharmaceutical Chemistry Research, 7*, 507–515. <https://doi.org/10.48309/jmpcr.2025.466324.1319>
- Widiawati, R., Hafy, Z., & Liana, P. (2021). Hubungan Kadar Komponen Besi Darah Pendonor terhadap Kualitas Packed Red Cells (PRC) di UDD PMI Provinsi Sumatera Selatan. *Jurnal Surya Medika, 7*(1), 66–71. <https://doi.org/10.33084/jsm.v7i1.2303>
- World Health Organization. (2024). Guideline on haemoglobin cutoffs to define anaemia in individuals and populations.
- World Health Organization. (2020). WHO Guideline on Use of Ferritin Concentrations to Assess Iron Status in Individuals and Populations. Geneva: World Health Organization. 1-82
- Yanti, L.N., Rofinda, Z.D., Syofiaty, Rikarni, Husni, Yusri, E. (2025). Impact of Donation Frequency on Iron Stores and Hemoglobin Levels in Regular Blood Donors. *Bioscientia Medicina: Journal of Biomedicine and Translational Research, 9*(5). 1576-1587. <https://doi.org/10.37275/bsm.v9i5.1282>
- Zeng, X., Meng, X., Wang, Y., Li, S., Liao, Q., ...& Rong, X. (2023). A reference interval study of ferritin and transferrin levels in donors from two blood centers. *Experimental and Therapeutic Medicine, 25*(5), 218. <https://doi.org/10.3892/etm.2023.11916>