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The clinical utility of new reticulocyte and erythrocyte parameters on the Sysmex XN 9000 for iron deficiency in pregnant patients

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Abstract

Introduction: Iron deficiency (ID) is a major cause of morbidity in pregnancy. Antenatal clinics use a hemoglobin to screen for ID, which delays the diagnosis of subclinical ID. The aim of this study was to investigate the clinical utility of the percentage of microcytic red cells (%Micro-R), percentage of hypochromic red cells (%Hypo-He), and reticulocyte hemoglobin content (Ret-He) on the Sysmex hematology analyzer in pregnant patients.

Methods: For this study, 102 nonanemic patients in the first trimester of pregnancy presenting for the first time to antenatal clinic were screened for ID. There were 50 (49.02%) patients with ID as defined according to iron studies. The independent *t* test and receiver operating characteristic (ROC) analysis were applied.

Results: There was a significant difference in the Ret-He, %Micro-R, and %Hypo-He between the ID and non-ID groups ($P < 0.001$). The area under curve (AUC) for the Ret-He (0.81, 95% CI 0.71-0.88) indicates that the Ret-He at a cutoff <31.2 pg is the best discriminator of ID ($P < 0.0001$). The AUC of %Hypo-He (0.78, 95% CI 0.69-0.86) was not superior to the mean cell hemoglobin (0.78, 95% CI 0.69-0.86). The %Micro-R (0.79, 95% CI 0.70-0.87) showed improved diagnostic accuracy compared to mean cell volume (0.75, 95% CI 0.65-0.83).

Conclusion: The new reticulocyte and erythrocyte parameters are reliable tests for the diagnosis of subclinical ID in pregnant patients. Further studies, however, are required to confirm the diagnostic utility of the erythrocyte parameters in pregnant patients. These tests will benefit the management of pregnant patients attending antenatal clinic.

KEYWORDS

iron deficiency, percentage of hypochromic red cells, percentage of microcytic red cells, pregnancy, reticulocyte hemoglobin content

1 | INTRODUCTION

Iron deficiency (ID) is the most common cause of anemia in pregnant women.[1] While rates of ID in the developed world have declined,[2] ID continues to be a public health concern in the developing world with estimated rates of 30%-40%.[3] Physiologically, iron requirements increase as pregnancy advances. Other causes include blood loss, nutritional deficiencies and several pregnancies close together. Laboratory diagnosis and treatment of ID are necessary to prevent maternal and neonatal complications. Maternal complications include anemia and postpartum infections, whereas neonates are at increased risk of premature delivery, low birthweight and poor growth in infancy.[4]

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Traditionally, iron studies, namely serum iron, transferrin, transferrin saturation, ferritin, and, more recently, a soluble transferrin receptor are used to diagnose ID.[5] However, these have significant limitations. A ferritin level of <30 μg/L in pregnancy is highly sensitive for the diagnosis of ID, but ferritin is also an acute-phase reactant showing an increase in the presence of infection or inflammation.[4,6] Further, serum iron and transferrin saturation show a significant diurnal variation and fluctuations with dietary intake making these impractical screening tests. The soluble transferrin receptor is a relatively expensive test and is not routinely available. Thus, in everyday clinical practice, antenatal clinics employ hematological tests such as the hemoglobin (Hb) to screen for ID. However, a decrease in the Hb level occurs late in ID. Thus, use of the Hb as a screen for ID will delay the diagnosis of ID in pregnant patients who are not yet anemic. In addition, red blood cell (RBC) indices, such as mean cell volume (MCV), mean cell hemoglobin (MCH) and red cell distribution width (RDW), are classically used to aid in the diagnosis of ID at a primary healthcare level. However, these too are indicators of long-standing ID and cannot reliably be used as a screening tool.[7] Further, RBC indices are nonspecific indicators of ID. There is a lack of a simple and reliable laboratory test to routinely detect ID in pregnant patients.

Automated hematology analyzers have evolved considerably over the last number of years. New reticulocyte and erythrocyte parameters are available on several automated hematology analyzers for the assessment of iron status. Parameters available on the Sysmex automated hematology analyzer (Sysmex Corporation, Kobe, Japan) include the percentage of microcytic red cells (%Micro-R), percentage of hypochromic red cells (%Hypo-He), and the reticulocyte hemoglobin content (Ret-He). These parameters provide information at a cellular level about iron availability for erythropoiesis in individual reticulocytes and RBC subsets. Several authors have demonstrated that these parameters offer a simple and cost-effective alternative to traditional biochemical tests.[8,9] Unlike biochemical tests, these parameters are not influenced by infection or inflammation and can be done on the same specimen used for full blood count (FBC) analysis. This may be of particular benefit in settings where resources are limited.

The most widely established is the Ret-He, the hemoglobin content of the reticulocytes, which allows for early detection of ID.[10] Reticulocytes have a shorter life span (1-2 days) than mature RBC, and thus, the Ret-He provides an early indication of ID. In addition, the Sysmex analyzer reports the %Hypo-He and %Micro-R, which are derived parameters which correspond to the mature RBC with reduced hemoglobin content and volume, respectively. In contrast to the Ret-He, the %Hypo-He reflects the iron status of the RBC over the circulating life span (120 days). While the %Micro-R has not been well researched as a marker of ID, studies have demonstrated the clinical utility of the %Hypo-He in several patient groups with ID.[11,12] For example, the %Hypo-He is the most recognized parameter for identifying functional ID (iron stores are normal/increased with inadequate iron supply to erythrocytes) in patients with chronic renal failure treated with erythropoietin-stimulating agents.[13] However, this has not been evaluated locally in pregnant patients.

A prospective study was performed to determine the diagnostic utility of the Ret-He, %Hypo-He, and %Micro-R in pregnant patients attending the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) antenatal clinic, in which patients are routinely monitored for ID.

2 | METHODS

2.1 | Study design

Consecutive FBC and iron study samples from pregnant patients receiving antenatal care at the CMJAH, Johannesburg, South Africa (SA), were collected and prospectively analyzed at the Hematology Laboratory. The Ret-He, %Hypo-He, and % Micro-R were performed on the residual FBC sample. The study was conducted over a 6-month period.

2.2 | Study population

2.2.1 | Inclusion criteria

Consecutive healthy patients in the first trimester of pregnancy with no clinical symptoms of disease presenting for the first time to the antenatal clinic at CMJAH were eligible for this study. Information sheets were provided, and informed consent was obtained from those who agreed to participate in the study. Screening for ID was routinely performed at the first antenatal clinic visit. Samples with adequate volume (>2 mL) received within two hours were included.

2.2.2 | Exclusion criteria

Patients on iron supplementation, with a history of blood transfusion in the last 3 months or with an Hb <11 g/dL, were excluded. Participants were also excluded if they had a MCV >100 fL or a diagnosis of a hemoglobinopathy.[8] Thalassemia screening was performed by measurement of the RBC indices. Participants with a ferritin above the laboratory's reference range and clinical symptoms of disease were excluded.

2.2.3 | Patient classification

Bone marrow (BM) biopsy and iron staining are considered the gold standard tests for the diagnosis of ID. However, BM biopsy is an invasive procedure and is no longer considered the standard of care for assessment of iron stores.[14] In this study, ID was defined according to the traditional diagnostic criteria: serum iron of <9 μmol/L, transferrin saturation of <20%, and/or ferritin of <30 μg/L.

2.3 | Blood sampling

Patient screening results included a FBC, Ret-He, %Hypo-He, %Micro-R, and iron studies (serum iron, transferrin, transferrin saturation, and ferritin). Blood samples for Ret-He, %Hypo-He, and %Micro-R testing were collected in dipotassium (K_2) ethylenediaminetetraacetic acid (EDTA) tubes (1.5-2.2 mg of dipotassium EDTA per milliliter of blood) (Becton-Dickinson, Oxford, UK). Samples were stored at room temperature and analyzed by a dedicated technologist within four hours of collection. Demographics (age, ethnicity) were also collected from the laboratory information system.

2.4 | Analytical methods

The FBC, Ret-He, %Hypo-He, %Micro-R, and hemoglobin content of the RBC (RBC-He) were measured using the Sysmex XN 9000 hematology analyzers. Daily quality control was performed prior to patient analysis. The coefficient of variation (CV) for the Ret-He, %Hypo-He, %Micro-R, and RBC-He was 1.1%, 11.74%, 10.10%, and 0.6% respectively, for the normal quality control specimen (Sysmex Corporation, Kobe, Japan).These analyzers are compliant with local and international proficiency testing. In the reticulocyte channel, cells are stained with a nucleic acid dye and analyzed by fluorescent flow cytometry. Forward scattered light vs fluorescence is represented as a scattergram showing mature RBC and reticulocytes. The Ret-He is a log transformation of Ret-Y, which is derived from the forward scattered light of the reticulocyte. The %Hypo-He is determined from high angle forward scattered light, which is directly proportional to the hemoglobin content of the red cell (RBC-He). %Hypo-He refers to the percentage of RBC with a hemoglobin content of <17 pg. Iron studies were analyzed using the Cobas 1800 chemistry analyzer (Roche Diagnostics, Indianapolis, USA).

2.5 | Statistical analysis

Data from data collection sheets were analyzed using Statistica Statistical Software version 13.2 (Statistica, Tulsa, USA) and MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium). Normality was assessed using the Shapiro-Wilk test. Statistical comparisons were performed using the parametric independent *t* test. Correlations between continuous variables were estimated by Pearson's correlation coefficient (*r*). The diagnostic performance of the tests for ID was assessed using receiver operating characteristic (ROC) curves. Traditional diagnostic criteria for ID for ROC curve generation included serum iron of <9 μmol/L, transferrin saturation of <20%, and/or ferritin of <30 μg/L. The cutoffs for the Ret-He, %Hypo-He, %Micro-R, and RBC indices were established based on the optimal combination of sensitivity and specificity. Sensitivity was defined as the probability that a test result would be positive when ID was present, and specificity as the probability that a test result would be negative when ID was absent. The area under the curve (AUC) and 95% confidence intervals were determined by the Wilcoxon rank-sum test. Statistical significance was set at a *P* value of 0.05 or less.

2.6 | Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M-1704104).

3 | RESULTS

3.1 | Study population

During the study period, 106 pregnant patients in the first trimester were screened, of which 102 were included in the final analysis. There were four exclusions: three patients with MCV >100 fL and one patient with the diagnosis of a hemoglobinopathy on RBC indices. The mean age was 32 ± 6 years. All patients were of black ethnicity by self-report. There were 50 (49.02%) patients with ID and 52 (50.98%) with non-ID. The diagnosis of ID was based on the combination of decreased serum iron, transferrin saturation, and ferritin in 36 (72%) of these patients. In 14 (28%) patients, ID was based on serum iron and transferrin saturation. Iron status was assessed only in healthy pregnant women without clinical symptoms of disease and a ferritin below the upper limit of the reference range to minimize false-negative results.

3.2 | Comparison of parameters

The biochemical and hematological parameters in the ID and non-ID groups are presented in Table 1. Ret-He levels were normally distributed with mean ± SD values of 30.4 ± 3.3 and 33.4 ± 2.2 pg in the ID and non-ID groups, respectively ($P < 0.001$) (Figure 1). Similarly, %Micro-R levels showed mean ± SD values of 4.4 ± 4.3% in the ID group and 1.5 ± 1.2% in the non-ID group ($P < 0.001$) (Figure 2). A strong correlation between %Micro-R and the MCV was observed ($r = -0.81$, 95% confidence interval (CI) −0.87 to −0.73, $P < 0.001$). An increase in hypochromic red cells, however, only correlated moderately with a decline in MCH ($r = -0.64$, 95% CI −0.74 to −0.51 $P < 0.001$). A possible reason is the %Hypo-He values were within the laboratories' reference range. The %Hypo-He, however, was significantly higher in the ID group (1.0 ± 1.7) as compared to the non-ID group with no overlap (0.2 ± 0.2) ($P < 0.001$) (Figure 2). In addition, the mean values for Hb and MCV were significantly lower in the ID group as compared to the non-ID group ($P < 0.012$ and $P < 0.001$). The mean RDW was significantly higher in the ID group as compared to the non-ID group ($P < 0.001$). Although the differences between the two groups were statistically significant, the ranges for Hb, MCV, and RDW showed considerable overlap. There was no difference for MCH.

3.3 | Receiver operating characteristic (ROC) curve

The optimal cutoff values for the diagnosis of ID are presented along with sensitivity, specificity, and AUC in Table 2.

The AUC for the Ret-He (0.81, 95% CI 0.71 −0.88) indicates that the Ret-He is the best discriminator of ID in this population ($P < 0.001$). Figure 2 illustrates the ROC curve for the Ret-He test as compared to the %Hypo-He and %Micro-R for the diagnosis of

TABLE 1 Laboratory parameters of patients with iron deficiency and noniron deficiency

Parameter	Reference interval (Lawrie et al, 2009)	Iron deficiency (n = 50)	Noniron deficiency (n = 52)	*P*
RBC ($\times 10^{12}$/L)	3.9-5.4	4.5 ± 0.4	4.3 ± 0.42	0.011
Hb (g/dL)	11.6-16.4	12.25 ± 0. 89	12.73 ± 1.02	0.012
HCT (L/L)	0.34-0.48	0.34 ± 0.03	0.34 ± 0.03	0.396
MCV (fL)	78.9-98.5	87.4 ± 6.5	92.0 ± 5.1	0.001
MCH (pg)	26.1-33.5	27.7 ± 2.6	33.4 ± 26.1	0.112
MCHC (g/L)	32.7-34.9	31.7 ± 1.3	32.4 ± 0.9	0.001
RDW (%)	12.4-17.3	14.5 ± 1.6	13.7 ± 0.9	0.001
Ret-He (pg)	32.1-38.8	30.4 ± 3.31]	33.4 ± 2.2	0.001
%Hypo-He (%)	0.1-1.1	1.0 ± 1.7	0.2 ± 0.2	0.001
% Micro-R (%)	0.3-2.8	4.4 ± 4.3	1.5 ± 1.2	0.001
RBC-He (pg)	27.0-32.6	27.4 ± 2.8	29.8 ± 2.0	0.001
Fe (μmol/L)	9.0 -30.4	10.2 ± 6.35	18.8 ± 8.6	0.001
Transferrin saturation (%)	15-50	12.0 ± 7.2	26.3 ± 12.0	0.001
Ferritin (μg/L)	15-150	31.6 ± 25.7	74.4 ± 28.1	0.001

Hb, hemoglobin; HCT, hematocrit; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; MCH, mean cell hemoglobin; RDW, red cell distribution width; Ret-He, reticulocyte hemoglobin equivalent; %Micro-R, percentage of microcytic red cells; %Hypo-He, percentage of hypochromic red cells; RBC-He, hemoglobin content of the red cell; Fe, iron.
Data are expressed as mean ± SD for parametric tests.

ID. The Ret-He cutoff with the most optimal sensitivity and specificity to distinguish ID from non-ID was <31.2 pg. However, despite the excellent specificity of 86.44%, the corresponding sensitivity of 62.50% was low. This can be explained on the basis of the selected Ret-He threshold (Table 3). ID was missed in 20 of the 50 (19.61%) patients using a Ret-He cutoff of <31.2 pg. These false-negative cases had a significantly higher mean MCV of 92.3 ± 5.31 fL as compared to the true positives ($P < 0.004$). The distribution of Ret-He values for this pregnant patient population with ID showed a shift to the right. Further, eight (7.84%) of the false negatives, defined as ID on the basis of a low serum iron (6.18 ± 1.93 μmol/L) and transferrin saturation (8.83 ± 2.51%), had an elevated ferritin level of 71.69 ± 27.4 μg/L. No other markers of inflammation or chronic disease were available to confirm the absence of an acute-phase response. We further investigated a combination of ≥2 diagnostic criteria using the Ret-He, %Hypo-He, or %Micro-R. This was associated with an improved sensitivity of 76.0%, however, at the expense of a lower specificity of 71.7%.

4 | DISCUSSION

The prevalence of subclinical ID in this study, defined according to biochemical iron studies, was ~50%. A ferritin level of <30 μg/L was used to diagnose ID. Several studies have demonstrated that this is the most clinically relevant threshold at which treatment should be initiated.[10,15,16] However, most studies estimating the prevalence of ID have applied the World Health Organization criteria of a Hb level of <11 g/dL and ferritin levels <12 μg/L. The sensitivity and specificity at this ferritin cutoff, however, are 25.0% and 100.0%, respectively. A prevalence of ID and anemia of 6% to 15% has been reported applying these diagnostic criteria.[2,17] As such, the reported rates of ID and anemia vary according to the applied criteria.

The current practice in SA is to screen pregnant patients presenting for the first time to antenatal clinic with Hb testing. The Hb, however, is an unsuitable screening test for subclinical ID. Anemia is a late stage of ID when the absent iron stores restrict Hb synthesis. In the first stage of ID, iron stores are depleted and the ferritin is low. UK guidelines on the management of ID in pregnancy therefore recommend routine screening for ID with ferritin in high-risk populations.[18] However, biochemical iron studies are expensive tests at a primary healthcare level. Further, in antenatal clinics in SA, there is a high burden of chronic infections such as human immunodeficiency virus (HIV) and tuberculosis. A ferritin level within the reference range therefore does not exclude accompanying ID. This was illustrated in this study whereby ID was diagnosed based on the combination of low serum iron and transferrin saturation in 14 (28%) patients. As such, pregnant patients in SA are not routinely screened for subclinical ID with ferritin. The consequence is that therapeutic iron supplementation is only initiated in pregnant patients in whom anemia is already present. Other developing countries are also faced with similar diagnostic challenges for ID. A study performed in Bangladesh investigated the diagnostic utility of inexpensive and widely available RBC indices.[19] In the second stage of ID, RBC becomes hypochromic and microcytic as a result of iron-restricted erythropoiesis. In this study, however, the RBC indices were of

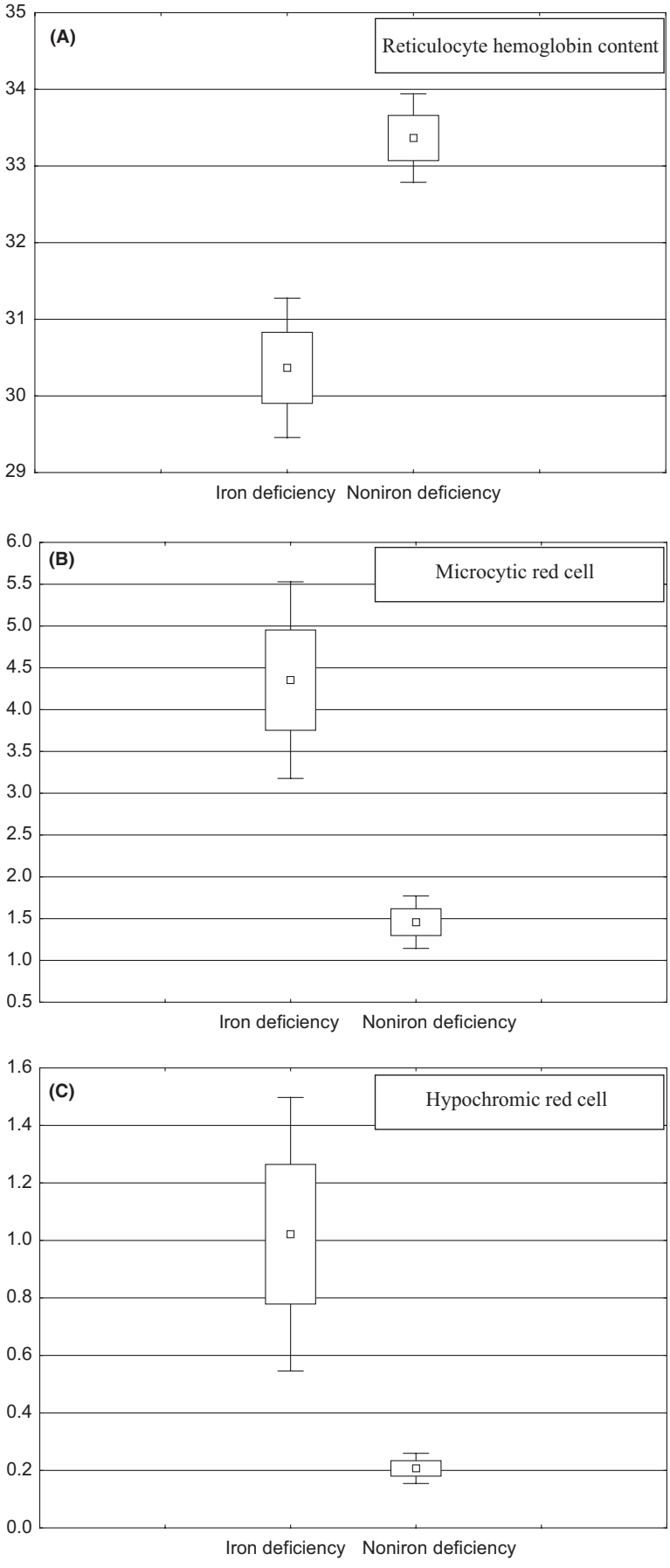

FIGURE 1 Boxplot of values in the iron deficiency and noniron deficiency groups. Horizontal box represents the mean values, larger boxes represent the standard deviations, and whiskers represent the 95% confidence interval

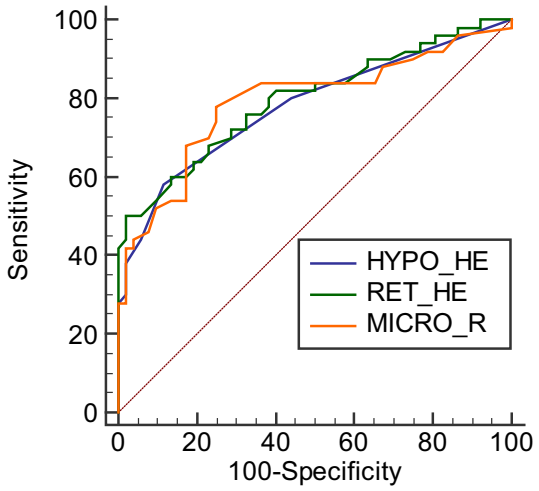

FIGURE 2 Receiver operating characteristic curve for reticulocyte hemoglobin content, microcytic red cell, and hypochromic red cell as indicators of iron deficiency [Colour figure can be viewed at wileyonlinelibrary.com]

limited diagnostic utility for the detection of subclinical ID. Analysis of the MCH was not found to be significantly different between the ID and non-ID pregnant groups ($P = 0.112$). A significant difference was observed for the RDW and MCV ($P < 0.001$). However, values in both groups were within the laboratory's reference range.

The new erythrocyte and reticulocyte parameters represent a cost-effective alternative to traditional red cell indices and biochemical iron studies. This study confirms that patients in the ID group had a significantly lower Ret-He and a significantly higher %Micro-R and %Hypo-He than those in the non-ID group ($P < 0.001$). In conjunction with previous studies, it is evident that these parameters also accurately reflect the severity of ID and anemia.[9] There are few reports of the new reticulocyte and erythrocyte parameters as markers of iron status in pregnancy in the English literature.[20–22] Studies by Schoorl et al have evaluated pregnant patients with iron deficiency anemia (IDA) in the third trimester owing to the physiological increase in iron requirements as pregnancy advances.[20,21,23] These studies recommend the Ret-He as a useful diagnostic and monitoring tool of iron status. In the study of 114 pregnant patients, the Ret-He in conjunction with measurement of zinc protoporphyrin reliably distinguished IDA from anemia due to hemodilution.[21] Another study of 25 pregnant patients with suspected IDA measured the Ret-He and Ret-He/RBC-He ratio after 1 month of iron supplementation. The Ret-He and Ret-He/RBC-He ratio showed a significant increase indicating these parameters are also reliable monitoring tools in pregnancy.[23]

In this study, Ret-He at a cutoff of <31.2 pg, corresponding to a sensitivity of 62.50% and a specificity of 86.44%, was superior to standard hematological tests for the diagnosis of ID ($P < 0.001$). The sensitivity, however, was poorer when compared to reported diagnostic iron studies (sensitivity of 90.0%), soluble transferrin receptor (sensitivity of 86%), and recent studies of Ret-He.[10,16,17] Ret-He has been widely studied, within numerous population groups including pregnancy.[8,9,12,23] The reported diagnostic Ret-He cutoff values vary from 27 to 32 pg according to the study population and diagnostic inclusion criteria.[8,9,22,24] This highlights the importance of determining cutoffs specific to each patient population. Urrechaga et al reported a lower Ret-He cutoff of 29.9 pg, corresponding to an improved sensitivity of 86.80% and a specificity of 85.70%, in a group of premenopausal women with subclinical ID. Although the pregnant patients enrolled in this study were clinically well, markers of inflammation and HIV status were not measured and clinical records were not reviewed, which is a limitation of this study. Chronic disease resulting in a reticuloendothelial iron blockade may have contributed to the high false-negative rate.

Reticulocyte counts, however, are not readily available at a primary healthcare level. As such, we investigated the role of the %Hypo-He and %Micro-R, which are available with a FBC request on the Sysmex analyzer. In this study, the mean %Hypo-He and %Micro-R values were considerably lower compared to previous reports.[9,10] This can possibly be attributed to the low incidence of microcytic (n = 7, 6.86%) and hypochromic RBC indices (n = 14, 13.73%) in patients who were not yet anemic. The best combination of sensitivity and specificity for % Hypo-He and %Micro-R was at a cutoff of >0.2% and >1.4%, respectively. Similarly, the reported diagnostic cutoff values vary according to the study population and diagnostic inclusion criteria. A cutoff of >1.6% has been proposed for %Hypo-He in premenopausal patients with subclinical ID,[10] whereas a cutoff of >3.5% has been suggested in patients on

TABLE 2 Test characteristics based on optimal cutoff values for the diagnosis of iron deficiency determined by receiver operating characteristic curve analysis

Parameter	Sensitivity (%)	Specificity (%)	AUC	(95% CI)	Cutoff	*P*
Ret-He (pg)	62.50	86.44	0.81	0.71-0.88	<31.2	<0.001
%Hypo-He (%)	58.00	88.46	0.78	0.69-0.86	>0.2	<0.001
% Micro-R (%)	78.00	75.00	0.79	0.70-0.87	>1.4	<0.001
Hb (g/dL)	74.00	57.69	0.66	0.56-0.75	<126	0.004
MCV (fL)	50.00	92.30	0.75	0.65-0.83	<86.5	<0.001
MCH (pg)	68.00	86.54	0.78	0.69-0.86	<28.4	<0.001
RDW (%)	54.00	94.23	0.73	0.63-0.81	>14.3	<0.001

AUC, area under curve; Ret-He, reticulocyte hemoglobin equivalent; %Micro-R, percentage of microcytic red cells; %Hypo-He, percentage of hypochromic red cells; Hb, hemoglobin; MCH, mean cell hemoglobin; MCV, mean cell volume; RDW, red cell distribution width.

TABLE 3 Reticulocyte hemoglobin content cutoff values for the diagnosis of iron deficiency determined by receiver operating characteristic curve analysis

Cutoff	Sensitivity (%)	(95% CI)	Specificity (%)	(95% CI)
≤30.0	40.0	24.9-56.7	100.0	93.9-100.0
≤31.0	55.0	38.5-70.7	89.8	79.2-96.2
≤31.2	62.5	45.8-77.3	86.44	75.0-94.0
≤31.6	67.5	50.9-81.4	79.6	67.2-89.0
≤32	72.5	56.1-85.4	67.8	54.4-79.4
≤33.2	82.5	67.2-92.7	59.3	45.7-71.9
≤33.9	90.0	76.3-97.2	33.9	22.1-47.4

hemodialysis.[9] The %Hypo-He and %Micro-R have been less frequently studied in a pregnant population. Further, the %Micro-R has predominantly been investigated as a %microcytic/%hypochromic ratio index for discriminating ID and thalassemia in population groups where thalassemia is prevalent.[25] There is a need for additional studies to confirm the clinical usefulness of the %Micro-R as a diagnostic tool for subclinical ID.

The Ret-He, %Hypo-He, and %Micro-R as single parameters or in combination are of particular benefit in an outpatient setting in a patient population with limited resources. These tests do have some limitations for routine use. The main limitation is their reduced availability. However more recently, similar clinical applications on other hematology analyzers have shown good agreement.[26,27] Further, the Ret-He is calculated from forward scattered light, which is a measure of cell size. It is thus important to interpret the Ret-He in the context of the patient's RBC indices, vitamin B12, red cell folate levels, and Hb electrophoresis findings. Another factor which also needs to be considered for outpatient testing is that the %Hypo-He and %Micro-R are stable for less than 12 hours after collection.[28]

5 | CONCLUSION

In conclusion, this study provides further insight into the clinical efficacy of the Ret-He, %Hypo-He, and %Micro-R for detecting ID in nonanemic pregnant patients.

Early diagnosis of ID is important in parts of the world with a high prevalence of ID. The performance of the Ret-He was superior to standard hematological parameters. Further studies are required to confirm the diagnostic utility of the %Micro-R and %Hypo-He in pregnant patients. The findings of this study suggest that these erythrocyte and reticulocyte parameters are a cost-effective alternative, which will benefit the management of pregnant patients attending antenatal clinic.

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CONFLICT OF INTERESTS

The author(s) declare no conflict of interests with respect to the authorship and/or publication of this article.

AUTHOR'S CONTRIBUTION

S Levy (Dept of Molecular Medicine and Haematology, Charlotte Maxeke Johannesburg Academic Hospital) designed the study, wrote the manuscript, performed data entry, and analyzed the data. E Schapkaitz (Dept of Molecular Medicine and Haematology, Charlotte Maxeke Johannesburg Academic Hospital) critically reviewed the manuscript.

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