_____

Health Sciences & Disease
The Journal of Medicine and Biomedical Sciences

Original Article

Added-Value of Reticulocyte Haemoglobin Equivalent in the Early Diagnosis of Iron Deficiency States among Blood Donors: A Pilot Study in Burkina Faso

Apport de l'équivalent en hémoglobine des réticulocytes dans le diagnostic précoce des carences en fer chez les donneurs de sang: Une étude transversale pilote au Burkina Faso

Sawadogo Salam[1,2*], Boureima Hassane[1], Koulidiati Jérôme[1], Nebie Koumpingnin[1,2], Coulibaly Abou[3], Sawadogo Abdoul-Guaniyi[2], Kima Donatien[4], Yooda Arzouma Paul[2], Deneys Veronique[5], Kafando Eléonore[1,4].

[1]Joseph Ki-Zerbo University, Ouagadougou, Burkina Faso
[2]National Blood Transfusion Center, Ouagadougou, Burkina Faso
[3]Research Institute of Health Sciences, Ouagadougou, Burkina Faso
[4]Charles de Gaulle Paediatric University Hospital Center, Burkina Faso
[5]CHU UCL Namur – Site de Sainte-Elisabeth, Namur, Belgium

Corresponding author:
Sawadogo Salam
01 BP 865 Ouagadougou 01, BF
Email:
salemserein@hotmail.com
Tel: +226 70032776

ABSTRACT

Background. Blood donor anemia is due to serum iron spoliation during blood donation. Haemoglobin (Hb) decrease occurs late compared to iron deficiency (ID). Also, Hb level seems to be bad parameter for early diagnosis of ID states in blood donor. Our study aimed to describe the usefulness of Reticulocyte Hemoglobin equivalent (Ret-He) in the early diagnosis of ID states. **Material and methods.** We conducted a cross-sectional study involving 125 blood donors. We evaluated performances of Ret-He to early diagnose ID states in comparison to Hb, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). **Results.** Anemia concerned 16.8% of study population. ID anemia and ID without anemia occurred in 5.6% and 14.4% respectively. Ret-He had moderate to strong correlation with Hb (r = 0.48), MCV (r = 0.81) and MCH (r = 0.82). At a cut-point of 32 pg/cell, Ret-He had better diagnostic performances for decreased iron stores (Se = 63.3%, Sp = 77.9% and AUC = 0.706; p = 0.001) and absent iron stores (Se = 76.5%, Sp = 75.0% and AUC = 0.757; p = 0.0006) compared to Hb, MCV, MHC and MCHC. **Conclusion.** Our study shows that Ret-He could be a usefulness tool in anemia and ID management among blood donors in low-income countries.

RÉSUMÉ

Introduction. L'anémie chez les donneurs de sang est due à la spoliation du fer lors du don. La diminution de l'hémoglobine (Hb) survient tardivement comparée à celle du fer. Aussi, le taux d'Hb apparaît être un mauvais paramètre de diagnostic précoce des carences en fer (CF). Notre étude visait à évaluer l'apport de l'équivalent en Hémoglobine des réticulocytes (Ret-He) dans le diagnostic précoce des CF. **Matériel et méthodes.** Nous avons mené une étude transversale prospective sur 125 donneurs. Nous avons évalué les performances du Ret-He dans le diagnostic précoce des CF par rapport à l'Hb, au Volume globulaire moyen (VGM), à la Teneur corpusculaire moyenne en Hb (TCMH) et à la Concentration corpusculaire moyenne en Hb (CCMH). **Résultats.** L'anémie concernait 16,8% de la population. Une anémie ferriprive et une CF sans anémie ont été notées respectivement chez 5,6% et 14,4%. Le Ret-He avait une corrélation modérée à forte avec le taux d'Hb (r = 0,48), le VGM (r = 0,81) et la TCMH (r = 0,82). Au seuil de 32 pg/cellule, le Ret-He avait de meilleures performances diagnostiques des déficits en fer (Se = 63,3%, Sp = 77,9% et ROC = 0,706; p = 0,001) et des cas de réserves en fer épuisées (Se = 76,5%, Sp = 75,0% et ROC = 0,757; p = 0,0006) par rapport au taux d'Hb, au VGM, la CCMH et la TCMH. **Conclusion.** Notre étude montre que le Ret-He pourrait être utile pour le diagnostic de l'anémie et de la CF chez les donneurs dans les pays à faible revenu.

INTRODUCTION

Blood donor anemia is a major concern, as it affects both the donor safety and the efficacy of blood component. It results not only from the decrease in donor haemoglobin (Hb) level with blood loss, but mainly, from the decrease in its iron stores. A donation of 450 mL of whole blood induces a spoliation of 230 to 250 mg of iron (equivalent to 100 days of dietary iron intake)[1] and a reduction of around 10 g/L of Hb concentration [2]. In the absence of iron supplementation, the times for donor Hb recovery (168 days for 80% recovery) and iron stores

reconstitution (180 days) exceed most inter-donation intervals observed in different countries. Around 2/3 of blood donors do not fully recover the iron loss before their next donation [2].

Anemia occurs late compared to iron deficiency (ID). Indeed, since the body regulates the absorption of iron for an effective erythropoiesis, Hb is kept normal as long as possible. Therefore, iron stores will be depleted before significant abnormal Hb concentration and cytological modifications occur [2, 3]. Which means that

Hb, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) are poor markers for early diagnosis of ID.

Serum ferritin and transferrin among others, are considered as the most appropriate and accessible biomarkers for the diagnosis of ID [1, 4]. Several studies showed that the measurement of serum ferritin is theoretically feasible and effective in screening donors at risk for ID anemia [3, 5, 6]. But this biochemical assay is likely to be influenced by inflammatory states [1] and its high cost (around 16 USD) could be an obstacle for low-income countries [7]. In addition, there is no point-of-care that can be easily used in donors selection booth [1].

New erythrocyte parameters of some haematology analysers such as the Reticulocyte haemoglobin equivalent (Ret-He), have proven to be informative of iron status. But the diagnostic performances found in the different studies are quite disparate [8–12]. Moreover, there is no studies using Ret-He to explore ID in blood donors in sub-Saharan Africa. Our study aimed to assess the usefulness of Ret-He in the diagnosis of ID in blood donors at the Regional Blood Transfusion Center of Ouagadougou (CRTS-O).

METHODS

Study setting

We conducted a cross-sectional study from June 25 to July 2, 2018 at the CRTS-O which collects annually, around 40,000 blood units from voluntary unpaid blood donors. Ouagadougou, the capital of Burkina Faso, is located between 12°21′56″ North latitude and 1°32′01″ West longitude, at 300 m above sea level. It is part of the Soudano-Sahelian area characterized by an endemic transmission of malaria and frequent cereal deficits.

Study population and inclusion criteria

The study included voluntary unpaid blood donors of both gender, aged 18 to 60 years, accepted for blood donation after pre-donation selection. This medical selection consisted of an interview and a physical examination focused on donor behaviours and medical conditions, measurement of blood pressure and body weight. It was carried out by a trained medical staff, using standardized selection criteria. Donors with either any infection or major hemoglobinopathy, history of surgery or bleeding less than three months, general chronic illness, pregnancy or breastfeeding less than six months and donors who received iron therapy in the last six months before the study were not included. No pre-donation biological test has been carried out to select donors. Indeed, in Burkina Faso, due to limited resources, the pre-donation haemoglobin is not systematically checked, both in first-time donors and regular donors. Also, donors with asymptomatic anemia could be accepted for blood donation.

For donors who donated blood in the past, a minimum time of 80 days for male and 110 days for female were required for inclusion in the study.

The variables studied were among other, age, gender, number of donations, erythrocyte indices on complete blood count, reticulocytes haemoglobin equivalent, serum iron and ferritin.

Blood samples and biological analyses

We used a standardized protocol for samples collection and storage, in order to minimize inter-individual and nycthemeral variations. The samples were taken between 8 and 12 am and analyses performed within 4 hours of collection. For each donor, two five-mL samples of blood, one without anticoagulant and the other with EDTA/K3 anticoagulant, were took from the diversion pouch of first 30 mL of blood for biochemical and haematological analyses. Complete blood count was performed on SYSMEX XN1000 automated haematology analyser (Sysmex Corporation, Kobe, Japan) which uses a combination of several technical principles (impedance and hydrodynamic focusing, flow fluoro-cytometry, chemical lysis and spectrophotometry) for multi-parametric analyses.

Absolute reticulocyte count is determined in "reticulocyte channel" of the equipment by fluorescence flow cytometry. The haemoglobinisation level (haemoglobin content of the reticulocytes) of newly formed red blood cells is given by the parameter Ret-He. It derives from forward scattered light signals measured in the reticulocyte channel. The intensity of these signals correlate strongly with the haemoglobin content of the reticulocytes. Also, they are used to determine the average 'reticulocyte haemoglobin equivalent' (RET-He) reported in pictogram (pg).

Ferritin measurement was performed using immunoturbidimetric methods on the Thermo Scientific Indiko biochemistry analyser (Thermo Fisher Scientific Oy, Clinical Diagnostics Systems Finland).

The measurement of the serum iron concentrations is carried out by colorimetric assay. The absorbance of the colored complex was measured at the wave of 600 nm (580-620 nm).

To guarantee the quality and reliability of the results, the automated systems used for analyses were calibrated and quality controls periodically carried out according to the manufacturers' instructions. The analytical and biological validations were respectively carried out by trained biomedical technologists and biologists.

Operational definitions

The different stages of ID were defined as follows [13, 14] :

- ID anemia (IDA): Hb < 130 g/L in male or Hb < 120 g/L in female and ferritin <12 µg/L in both genders.
- ID without anemia (IDWA): Hb ≥ 130 g/L + ferritin < 30 µg/L in male or Hb < 120 g/L + ferritin < 20 µg/L in female.
- Non IDA (NIDA): Hb < 130 g/L in male or Hb < 120 g/L in female and ferritin ≥ 12 µg/L in both genders.
- Normal: Hb ≥ 130 g/L + ferritin ≥30 µg/L in male or Hb ≥ 120 g/L + ferritin ≥20 µg/L in female.

For the evaluation of the diagnostic performances of the Ret-He and the old erythrocytes parameters, we define two stages of ID regardless of donor Hb:

- Decreased iron stores (DIS): ferritin <30 µg/L in male and <20 µg/L in female
- Absent iron stores (AIS): ferritin <12 µg/L in both genders.

Statistical analysis

Data were double-entered, verified for completeness and aberrant data. Analyses were done using Stata 13. Mean ± 2SD (standard deviation) or median with the interquartile range (IQR) were used to describe quantitative variables and proportions were used to describe qualitative variables. Correlation between Ret-He and certain variables was assessed using the Pearson correlation coefficient. Sensitivity, specificity, positive and negative predictive values and the area under the ROC curve (AUC) were used to compare the performances of Ret-He and those of the classic erythrocyte indices (MCV, MCH and MCHC) to diagnose DIS and AIS. The empirical cut-point of 32 pg/cell, estimated by Liu X. method [15] was used. Chi-square and non-parametric tests of Kruskall Wallis and Wilcoxon rank-sum were used when appropriated. Any difference was considered significant for p ≤ 0.05.

Ethical considerations

The study was authorized by the Internal Scientific Review Committee of the National Blood Transfusion Center of Burkina Faso. Each blood donor, before inclusion in the study gave informed consent. The confidentiality and anonymity of the data collected were respected. Each donor was informed of his biological results. Donors with clinical or biological abnormalities were referred to a medical center for further investigations and treatment.

RESULTS

Baseline characteristics

A total of 125 blood donors, comprising of 97 males (77.6%) and 28 females (22.4%) were included. The mean age was 30 ± 8.3 years. The median number of donations per donor at the time of blood sampling was 3 (IQR: 2 - 7). One third made more than 5 donations and almost half made at least one donation in the last 12 months. Anemia noted in 16.8% (21/125) of all donors, was more common in female (39.3% versus 10.3%, p <0.001). IDA, IDWA and NIDA concerned respectively 5.6%, 14.4% and 11.2% of donors. Means Ret-He of 33.6 ± 2.6 pg, 26.8 ± 4.3 pg, 32.1 ± 2.8 pg and 31.1 ± 2.9 pg was noted respectively in normal, IDA, IDWA and NIDA cases. The mean ferritin decrease from 98.3 µg/L for zero donation to 54.4 µg/L for at least 3 donations. In the same way, mean Ret-He decreased from 32.93 pg/cell to 30.79 pg/cell. The coefficient of linear regression of serum ferritin and Ret-He with the number of donations was respectively – 1.61 (CI 95% [-3.05; - 0.19]; $F_{(1, 123)} = 5.01$; p = 0.027) and – 0.1 (CI 95% [- 0.17; - 0.03], $F_{(1,123)} = 8.62$; p = 0.004).
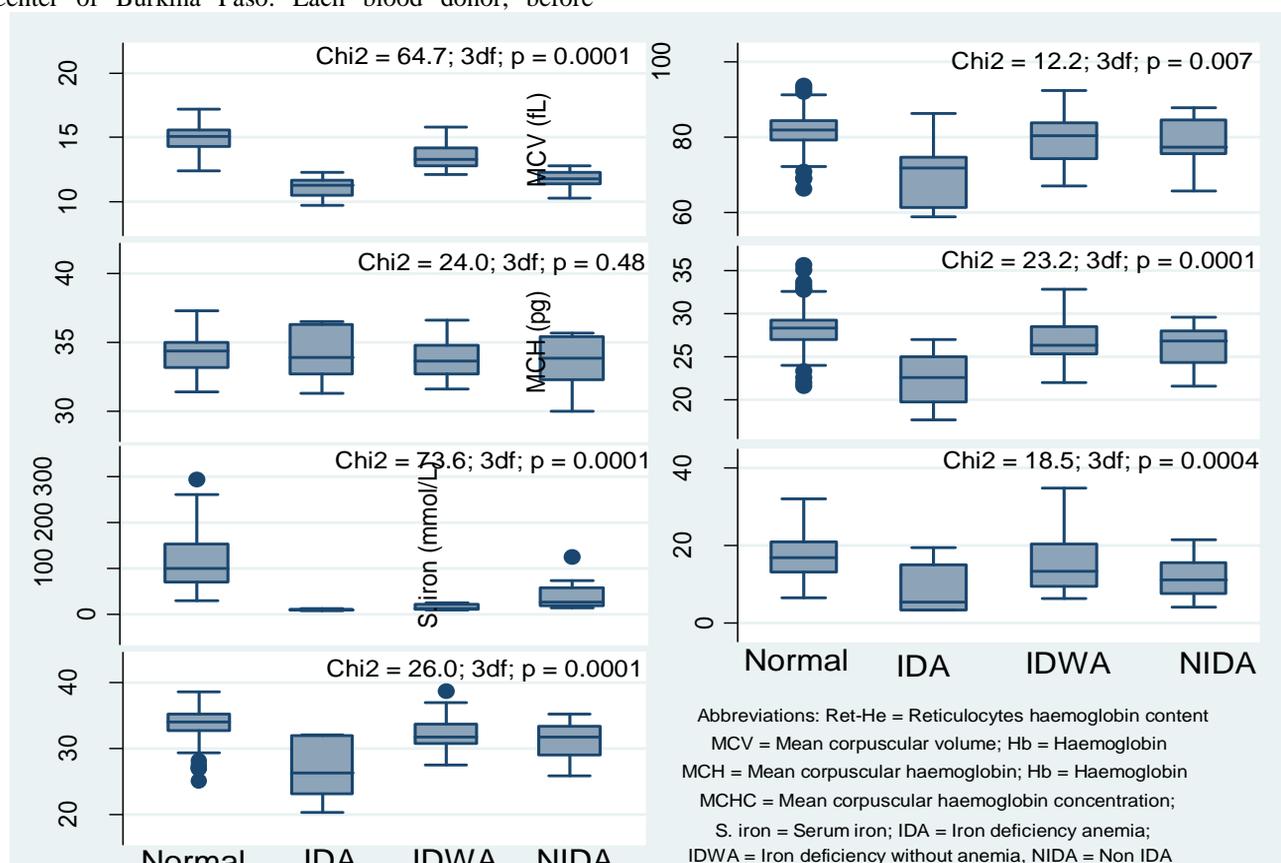

Figure 1: Box plots (median with 3rd and 4th quartiles) of erythrocyte indices (Hb, MCV, MCHC and MCH), reticulocytes haemoglobin content, serum ferritin and serum iron according to iron deficiency stages among blood donors at the CRTS-O, Burkina Faso in 2018 (Kruskall wallis test)

Value of the Ret-He in the diagnosis of ID states

The Figure 1 showed that Hb ($\chi 2$ = 64.7; p = 0.0001), MCV ($\chi 2$ = 12.2; p = 0.007), MCH ($\chi 2$ = 23.2; p = 0.0001), Ret-He ($\chi 2$ = 26; p = 0.0001), ferritin ($\chi 2$ = 73.6; p = 0.0001) and serum iron ($\chi 2$ = 18.5; p = 0.0004) were statistically lower in IDA than other situations.

Ret-He had moderate to strong correlation with Hb (r = 0.48; p < 0.001), MCV (r = 0.81; p < 0.001) and MCH (r = 0.82; p < 0.001) as showed on figure 2. Ret-He at a cut-point of 32 pg/cell had a sensitivity and specificity of 63.3% and 77.9% in the diagnosis of DIS and 76.5 and 75.0% for AIS. The AUC were 0.706 and 0.757 respectively for DIS and AIS (Table I).

DISCUSSION

Our study that aimed to evaluate the value of Ret-He in the diagnosis of ID states in blood donors shows that at a cut-point of 32 pg/cell, this parameter had an acceptable discrimination level for the diagnosis of DIS (Se = 63.3%, Sp = 77.9% and AUC = 0.706) and AIS (Se = 76.5%, Sp = 75.0% and AUC = 0.757).

It is the first study conducted on this topic in our country and in West Africa. It provides therefore one more tool for the management of the IDs. Our findings could guide implementation of measures for the protection and promotion of donor health.
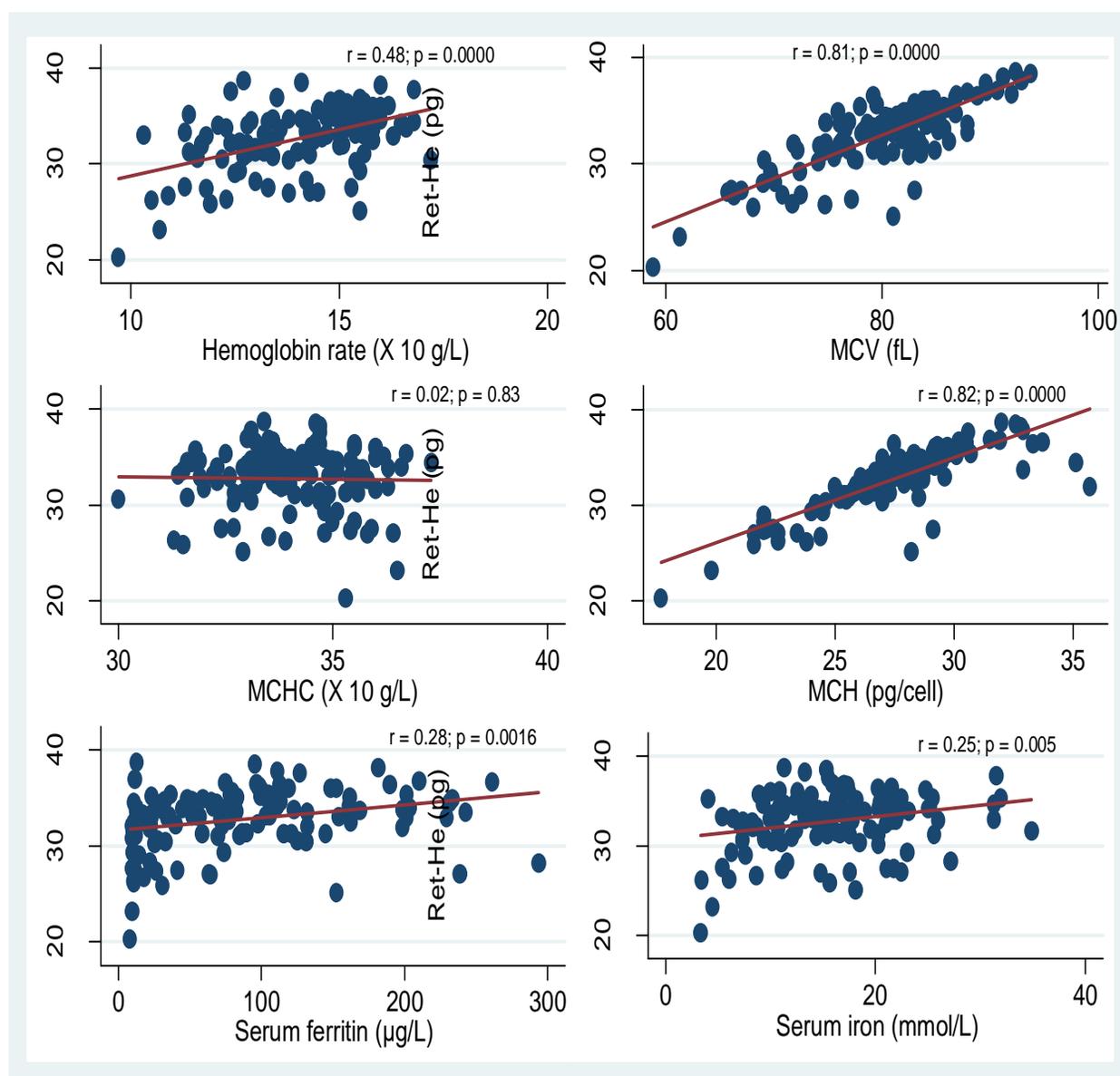

Figure 2: Correlation of Ret-He with erythrocyte indices (Hb, MCV, MCHC, and MCH), serum ferritin and serum iron among blood donors at the CRTS-O, Burkina Faso in 2018 (Pearson correlation test)

MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Ret-He = Reticulocyte haemoglobin equivalent

Table I: Performances of Ret-He compared to Hb, MCV, MCHC and MCH for the diagnosis of (a) DIS (ferritin <30 µg/L for male and < 20 µg/L for female) and (b) AIS (ferritin <12 µg/L for both male and female) in blood donors at the CRTS-O, Burkina Faso in 2018

	Parameter	Cut-off	Sensitivity (%) [IC 95]	Specificity (%) [IC 95]	PPV (%) [IC 95]	NPV (%) [IC 95]	AUC [IC 95]	χ^2 ; p-value*
(a)	Ret-He (pg)	< 32	63.3 [43.9 – 80.1]	77.9 [68.2 – 85.8]	47.5 [31.5 – 63.9]	87.1 [78.0 – 93.4]	0.706 [0.61 – 0.80]	16.46 ; 0.0025
	Hb (g/L)**	130/120	40.0 [22.7 – 59.4]	90.5 [82.8 – 95.6]	57.1 [34.0 – 78.2]	82.7 [74.0 – 89.4]	0.653 [0.56 – 0.75]	
	MCV (fL)	< 80	56.7 [37.4 – 74.5]	64.2 [53.7 – 73.8]	33.3 [20.8 – 47.9]	82.4 [71.8 – 90.3]	0.604 [0.50 – 0.71]	
	MCHC (g/L)	< 320	10.0 [2.11 – 26.5]	91.6 [84.1 – 96.3]	27.3 [6.0 – 61.0]	76.3 [67.4 – 83.8]	0.508 [0.45 – 0.57]	
	MCH (pg)	< 27	63.3 [43.3 – 80.1]	73.7 [63.6 – 82.2]	43.2 [28.3 – 59.0]	86.4 [77.0 – 93.0]	0.685 [0.58 – 0.78]	
(b)	Ret-He (pg)	< 32	76.5 [50.1 – 93.2]	75.0 [65.7 – 82.8]	32.5 [18.6 – 49.1]	95.3 [88.4 – 98.7]	0.757 [0.65 – 0.87]	17.96 ; 0.0013
	Hb (g/L)**	130/120	41.2 [18.4 – 67.1]	87.0 [79.2 – 92.7]	33.3 [14.6 – 57.0]	90.4 [83.0 – 95.3]	0.641 [0.52 – 0.77]	
	MCV (fL)	< 80	52.9 [27.8 – 77.0]	61.1 [51.3 – 70.3]	17.6 [8.4 – 30.9]	89.2 [79.8 – 95.2]	0.570 [0.44 – 0.70]	
	MCHC (g/L)	< 320	17.6 [3.8 – 43.4]	92.6 [85.9 – 96.7]	27.3 [6.0 – 61.0]	87.7 [80.3 – 93.1]	0.551 [0.52 – 0.76]	
	MCH (pg)	< 27	70.6 [44.0 – 89.7]	70.4 [60.8 – 78.8]	27.3 [15.0 – 42.8]	93.8 [86.2 – 98.0]	0.705 [0.58 – 0.82]	

*: Equality test of Ret-He AUC with MCV, MCHC, MCH and Hb;   **: < 130 g/L for male and < 120 g/L for female
DIS = Decreased iron stores; AIS = Absent iron store; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Ret-He = Reticulocyte haemoglobin equivalent; Hb = Haemoglobin rate; PPV= Positive predictive value; NPV= Negative predictive value; AUC = Area under ROC curve; CI 95 = 95% confidence interval.

The prevalence of anemia was 16.8%. Females were more affected than males (39.3% versus 10.3%). In 2009, with less restrictive haemoglobin thresholds (120 g/L in male and 110 g/L in female), Tagny *et al*. [16] found in the same population, prevalence of 3% in male and 10% in female. In Nigeria, two studies, using a threshold of 110 g/L for both genders, observed a prevalence of 25.7% and 16% [17, 18]. Bakrim *et al*., using the same thresholds as in current study, found a prevalence of 8.5% in Morocco [19]. In South Africa, the prevalence of anemia was 7.7% for a threshold of 125 g/L for both genders [20].

Taking into account these different haemoglobin thresholds, anemia seems considerably less frequent among donors in Morocco and South Africa than in our population. The disparities in socio-economic level and epidemiological context could explain these differences. Burkina Faso is a low-income country with high prevalence of genetic RBCs pathologies (sickle cell anemia, G6PD deficit, thalassemia) [21], nutritional deficits [22], parasitic diseases (malaria, helminthiasis)[23]. Indeed, in African inter-tropical countries, sickle cell anemia and sickle cell trait affect around 10-40% of the general population, while it is only 1-2% in North Africa and less to 1% in South Africa [24]. A study in Ghana, a neighbouring country with the same facets of hemoglobinopathy as in Burkina Faso, found a prevalence of the sickle cell trait at 11.3% [25]. The same trend is observed with malaria and nutritional deficits. These genetic abnormalities of RBCs and parasitic diseases could explain the high frequency of NIDA (11.2%) in our study.

ID states were found in 20% of donors (IDWA in 14.4% and IDA in 5.6%). ID is one of main adverse effects of blood donation. The serum iron loss is important in repeated donors and in case of short inter-donation interval [26, 27]. In our study, the serum ferritin decreased by 1.61 µg/L if the number of donations increased by one. Consequences resulting from ID are considerable. Firstly, ID even without anemia, induces often a decline in cognitive functions, physical and intellectual performances [28] that could lead to temporary exclusion or self-exclusion of blood donor; this will impact blood supply. Secondly, blood taken from anaemic blood donors will result in ineffective blood transfusions. A previous study showed that 16.8% of blood units in Burkina Faso had an haemoglobin content below to required level [29].

At to date, the pre-donation haemoglobin screening is the main strategy used to mitigate donor anemia. It is an essential measure to avoid anaemic blood donation and to preserve donor's safety. Unfortunately, it appears to be a non-reliable biomarker of ID and early stage of anemia (in our study, Hb had a sensitivity around 40%). Indeed, by the time haemoglobin will drop below normal values, the donor will have practically exhausted its iron stores [3, 17]. The most reliable biochemical markers of ID (bone marrow iron staining, serum ferritin and transferrin, transferrin saturation, transferrin iron binding capacity, soluble transferrin receptor) are unsuitable in blood donation context. They are expensive and not easy to implement.

Several studies evaluated the diagnostic performances of Ret-He as a parameter for the early diagnosis of ID states in different clinical situations (Table II). Their results accredited its usefulness, despite high divergences of diagnostic performances. These divergences are probably due to the differences in the cut-points used or to the socio-demographic, clinical and biological factors which can affect haematological parameters [30].

Table II: Diagnostic performances of Ret-He in several studies in different clinical situations

Authors (Year)	Population	Ret-He (pg)	Iron deficiency definition criteria	Se / Sp (%)
Our study (2018)	Blood donors	32	- Hb (F/M) <120/130 g/L - Ferritin (F/M) <20/30 µg/L (DIS) or Ferritin <12 µg/L (AIS)	63.3/77.9 (DIS) 76.5/75 (AIS)
Brugnana et al. (2006) [8]	Patients of internal medicine	27.2	- Hb <110g/L ; - TSAT < 20% ; - Ferritin <100ng/mL	93/83
Nadarajan et al. (2008) [32]	Blood donors	28	- Hb (F/M) <120/130g/L - TSAT < 20% - Ferritin <100 µg/L	69/93
Semmelrock et al. (2012) [33]	Blood donors	28	- sTfR-F >1.5 - Hb (F/M) <12.5/13.5 g/L	27/98 (ND) 41/98 (DR)
Urrechaga et al. (2013) [34]	CKD	29.8	- sTfR-F >21 NM	91/83
Peerschke et al. (2014) [35]	Oncology	32	- TSAT < 20% - Serum iron <100 µg/L	78/70
Rungngu et al. (2016) [36]	Children	27.8	- Ferritin <12 µg/L	44/85
Buttarello et al. (2016) [9]	Patients of internal medicine	30.6	- TSAT < 16% - Ferritin (F/M) <12/15µg/L	93/95
Dalimunth et al. (2016) [11]	CKD	31.65	- Hb <100g/L - TSAT < 20% - Ferritin <100 µg/L	81/62
Sanyoto et al. (2017) [37]	Patients of internal medicine	25	- Hb <100g/L - Ferritin <20 µg/L	97/67
Poffenroth et al. (2017) [38]	Patients of internal medicine	26	- Hb (F/M) <120/130 g/L - Ferritin <20 µg/L	100/94
Cai et al. (2017) [10]	Patients of internal medicine	27.2	- Negative bone marrow iron staining - Hb (F/M) <120/130 g/L	87/93
Jarc et al. (2017) [39]	Blood donors	28.2	- TSAT < 20% - Ferritin <200 µg/L	76/100
Toki et al. (2017) [13]	Patients of internal medicine	30.9	- Hb <120g/L - Ferritin <12 µg/L	92/81
Urrechaga et al. (2017) [12]	Patients of internal medicine	30	- Hb <120g/L - Ferritin <30 µg/L	84/71
Tiwari et al. (2018) [7]	Blood donors	28	- sTfR >3 ng/mL; - Hb <12.5g/L	91/97

Abbreviation: CKD: Chronic kidney disease; Hb: Hemoglobin rate; DIS: Decrease iron store; AIS: Absent iron store; TSAT: Transferrin saturation coefficient; sTfR: Soluble receptor of transferrin; M: male; F: female; g: gram; µg: microgram; L: Liter, DR: repeated donor; ND: new donor

In our study, Ret-He had a positive correlation with MCV and MCH (strong correlation), ferritin and serum iron (weak correlation). It also had an acceptable discrimination capacity for the diagnosis of iron deficiency states, supporting the idea that Ret-He could be a useful indicator of ID. As serum ferritin, we found that Ret-He had a negative linear correlation with the number of donations (the coefficient of correlation was -0.1). Ret-He represents the Hb of the young RBCs that were just released from the bone marrow. Therefore, it offers real-time information on the bioavailability of iron for effective erythropoiesis. Also, its measurement can detect changes in iron status earlier than Hb of mature RBCs. A low value of Ret-He indicates that iron is not bioavailable for erythropoiesis. In our study, the diagnostic performances of Ret-He were better in AIS as noted by Buttarello *et al.* [9]. This observation is normally expected, since haemoglobin synthesis may not be compromised yet in DIS.

These findings suggest that Ret-He could be used as early predictor of ID in low-income countries blood collection setting where ferritin assay is not accessible. In this case, Ret-He could replaces ferritin in the previous algorithms applied in some developed countries [2]. So, Ret-He should be measured in donors with 1°) a

finger stick pre-donation Hb level lower than the lower limit authorized for donation, 2°) a decrease of 20 g/L in baseline finger stick pre-donation Hb, 3°) in any female first-time donors and 4°) in any first-time donors with a finger stick pre-donation Hb level equal or just greater than the higher limit authorized for donation.

However, our study had obvious limitations that should be noted. Firstly, our gold standard was serum ferritin only, a biomarker known to be influenced by inflammation [31]. The absence of C reactive protein does not allow us to eliminate inflammatory situations. The reliable test for ID states was bone marrow iron staining [10]. But it's a very invasive test and difficult to perform in the donors selection booth. Also, some authors propose algorithms combining serum ferritin, serum transferrin and soluble transferrin receptors [31]. Due to financial constraints, we have not been able to implement these algorithms. Secondly, the absence of pre-donation Hb, gender unbalance, possible congenital or acquired red blood cells' disorders or malaria in blood donors, etc. could have altered reliability of our findings.

CONCLUSION

Our study shows that Ret-He could be a reliable tool for early diagnose of ID in blood donors. It can be measured

in less than 2 minutes on many haematology analysers at the same time as the complete blood count. It appears to be less expensive than the ferritin assay. It also has the advantage of not being affected by inflammatory reactions, pregnancy or other severe pathologies, unlike ferritin or transferrin. However, further studies with more laboratory tests and a large study sample remain necessary to validate any algorithm based on Ret-He.

ACKNOWLEDGMENTS

SYSMEX PARTEC – Burkina Faso for its contribution to the acquisition of reagents.

AUTHORSHIP CONTRIBUTION

S. S., B. H., K. D., K. E. designed the study and collected the data, B. H., S. S., K. D., N. K., analysed the data, B. H., S. S., K. E., C. A., S. A-G., Y. A. P., D. V., interpreted the results,
S. S., B. H., K. D., K. E., drafted the manuscript, N. K., S. A-G., C. A., Y. A. P., D. V., critically revised the manuscript.
All the authors approved the manuscript final version and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Goldman M. Iron depletion and routine ferritin measurement in blood donors. ISBT Sci Ser. 2015;10(S1):124-128.
2. Fillet A-M, Gross S. Prévention de l'anémie chez les donneurs de sang. Transfus Clin Biol. 2017;24(3):143-147.
3. Mittal R, Marwaha N, Basu S, Mohan H, Kumar AR. Evaluation of iron stores in blood donors by serum ferritin. Indian J Med Res. 2006;124:641-646.
4. Ziegler AK, Grand J, Stangerup I, et al. Time course for the recovery of physical performance, blood hemoglobin, and ferritin content after blood donation: Time Course of Blood Donation Effects. Transfusion (Paris). 2015;55(4):898-905.
5. Stone M, Brambilla D, Murcia K, et al. Feasibility of routine ferritin testing for donor management: validation of delayed processing and demonstration of within donor reproducibility over time: FERRITIN TESTING VALIDATION FOR DONOR MANAGEMENT. Transfusion (Paris). 2016;56(10):2422-2425.
6. O'Meara A, Infanti L, Stebler C, et al. The value of routine ferritin measurement in blood donors: FERRITIN IN BLOOD DONORS. Transfusion (Paris). 2011;51(10):2183-2188.
7. Tiwari AK, Bhardwaj G, Arora D, et al. 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 Sang. 2018;113(7):639-646.
8. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. Clin Lab Haematol. 2006;28(5):303-308.
9. Buttarello M, Pajola R, Novello E, Mezzapelle G, Plebani M. Evaluation of the hypochromic erythrocyte and reticulocyte hemoglobin content provided by the Sysmex XE-5000 analyzer in diagnosis of iron deficiency erythropoiesis. Clin Chem Lab Med CCLM. 2016;54(12):1939–1945.
10. Cai J, Wu M, Ren J, et al. Evaluation of the Efficiency of the Reticulocyte Hemoglobin Content on Diagnosis for Iron Deficiency Anemia in Chinese Adults. Nutrients. 2017;9(540):6. doi:10.3390/nu9050450

11. Dalimunthe NN, Lubis AR. Usefulness of Reticulocyte Hemoglobin Equivalent in Management of Regular Hemodialysis Patients with Iron Deficiency Anemia. Rom J Intern Med. 2016;54(1):31-36.
12. Urrechaga Igartua E, Hoffmann JJML, Izquierdo-Álvarez S, Escanero JF. Reticulocyte hemoglobin content (MCHr) in the detection of iron deficiency. J Trace Elem Med Biol. 2017;43:29-32.
13. Toki Y, Ikuta K, Kawahara Y, et al. Reticulocyte hemoglobin equivalent as a potential marker for diagnosis of iron deficiency. Int J Hematol. 2017;106(1):116-125.
14. AABB. Updated strategies to limit or prevent iron deficiency in blood donors. AABB Association Bulletin. http://www.aabb.org/programs/publications/bulletins/Docs/ab17-02.pdf. Published 2017. Accessed January 10, 2020.
15. Liu X. Classification accuracy and cut point selection. Stat Med. 2012;31(23):2676-2686.
16. Dignass A, Farrag K, Stein J. Limitations of Serum Ferritin in Diagnosing Iron Deficiency in Inflammatory Conditions. Int J Chronic Dis. 2018;2018(ID 9394060):1-11.
17. Tagny CT, Diarra A, Yahaya R, et al. Characteristics of blood donors and donated blood in sub-Saharan Francophone Africa. Transfusion (Paris). 2009;49(8):1592-1599.
18. Jeremiah ZA, Koate BB. Anaemia, iron deficiency and iron deficiency anaemia among blood donors in Port Harcourt, Nigeria. Blood Transfus. 2010;8:113-117.
19. Erhabor O, Imrana S, Buhari H, et al. Prevalence of anaemia among blood donors in Sokoto, North Western, Nigeria. Int J Clin Med Res. 2014;1(3):85-89.
20. Bakrim S, Ouarour A, Jaidann K, Benajiba M, Masrar A. Profil de l'hémogramme et intérêt de la mesure de l'hémoglobine pré-don chez des donneurs de sang de la région Nord-Ouest du Maroc. Transfus Clin Biol. 2018;25(1):35-43.
21. van den Berg K, Swanevelder R, Ingram C, et al. The iron status of South African blood donors: balancing donor safety and blood demand. Transfusion (Paris). 2019;59(1):232-241.
22. Kafando E, Sawadogo M, Cotton F, Vertongen F, Gulbis B. Neonatal screening for sickle cell disorders in Ouagadougou, Burkina Faso: a pilot study. J Med Screen. 2005;12(3):112-114.
23. De Benoist B, World Health Organization, Centers for Disease Control and Prevention (U.S.). Worldwide Prevalence of Anaemia 1993-2005 of: WHO Global Database of Anaemia. World Health Organization; 2008. Accessed January 12, 2020. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf
24. Tagny CT, Owusu-Ofori S, Mbanya D, Deneys V. The blood donor in sub-Saharan Africa: a review. Transfus Med. 2010;20(1):1-10.
25. OMS. Drépanocytose. In: Rapport du secrétariat. Cinquante-neuvième Assemblée Mondiale de la santé. Organisation Mondiale de la Santé; 2006:310. Accessed January 12, 2020. http://www.apps.who.int/gb/archive/pdf files/WHA59/A59 9-fr.pdf
26. Antwi-Baffour S, Asare RO, Adjei JK, Kyeremeh R, Adjei DN. Prevalence of hemoglobin S trait among blood donors: a cross-sectional study. BMC Res Notes. 2015;8(1):583.
27. Goldman M, Yi Q, Steed T, O'Brien SF. Changes in minimum hemoglobin and interdonation interval: impact on donor hemoglobin and donation frequency. Transfusion (Paris). 2019;59(5):1734-1741.
28. Di Angelantonio E, Thompson SG, Kaptoge S, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. The Lancet. 2017;390(10110):2360-2371.
29. Bialkowski W, Bryant BJ, Schlumpf KS, et al. The strategies to reduce iron deficiency in blood donors

_____

randomized trial: design, enrolment and early retention. Vox Sang. 2015;108(2):178-185.

30. Nebie K, Sawadogo S, Kafando E, 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.

31. Archer NM, Brugnara C. Diagnosis of iron-deficient states. Crit Rev Clin Lab Sci. 2015;52(5):256-272.

32. Nadarajan V, Sthaneshwar P, Eow GI. Use of red blood cell indices for the identification of iron deficiency among blood donors. Transfus Med. 2008;18(3):184-189.

33. Semmelrock MJ, Raggam RB, Amrein K, et al. Reticulocyte hemoglobin content allows early and reliable detection of functional iron deficiency in blood donors. Clin Chim Acta. 2012;413(7-8):678-682.

34. Urrechaga E, Borque L, Escanero JF. Erythrocyte and reticulocyte indices in the assessment of erythropoiesis activity and iron availability. Int J Lab Hematol. 2013;35(2):144-149.

35. Peerschke EIB, Pessin MS, Maslak P. Using the Hemoglobin Content of Reticulocytes (RET-He) to Evaluate Anemia in Patients With Cancer. Am J Clin Pathol. 2014;142(4):506-512.

36. Rungngu SLP, Wahani A, Mantik MFJ. Reticulocyte hemoglobin equivalent for diagnosing iron deficiency anemia in children. Paediatr Indones. 2016;56(2):90-94.

37. Sanyoto A, Suega K, Adnyana L, Bakta IM. Diagnostic Test Equivalent Hemoglobin Reticulocyte in Iron Deficiency Anemia. Indones Biomed J. 2017;9(3):143-146.

38. Poffenroth C, Mabbett C. The reticulocyte haemoglobin equivalent (RET_He) and laboratory screening for iron deficiency. N Z J Med Lab Sci. 2017;71:120-123.

39. Jarc E, Preložnik Zupan I, Buturović Ponikvar J, Snoj N, Podgornik H. Comparison of erythrocyte and reticulocyte indices for the diagnosis of iron deficiency. Zdr Vestn. 2017;86(1-2):19–27.