

Research Article

Low Hb Density (LHD %) in the Detection of Latent Iron Deficiency in Non-Anemic Premenopausal Women

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Abstract

Hemoglobin (Hb) within the reference interval does not exclude iron deficiency, because there is a long period of reduced iron stores (latent iron deficiency) before anemia is present. Low Hb density (LHD %) reported by Beckman-Coulter analyzers, derived from the mean cell hemoglobin concentration, is related to iron availability and hemoglobin content. We study the potential utility of LHD% in the detection of the stages of latent iron deficiency, storage iron depletion (SID) and iron deficient erythropoiesis (IDE), when compared to well establish tests.

Methods: 1141 women on child-bearing age (mean 31.7 years) were recruited. Concordance of LHD % with ferritin and sTfR was studied with inter-rater agreement test and Pearson's correlation; the diagnostic performance with Receiver operating characteristic (ROC) curve analysis.

Results: the values of Hb, serum ferritin and sTfR, determined the groups: No Iron deficiency (n=450), SID (n=127), IDE (n=40). LHD% correlation with ferritin $R = -0.4896$; concordance $\kappa = 0.561$. Criteria for SID ferritin $< 20 \mu\text{g/L}$ ROC AUC 0.844 (95% CI 0.729-0.924), cut off 5.1 %, sensitivity 83.3 %, specificity 81.2 %, Correlation LHD% and sTfR $R = 0.7198$, concordance $\kappa = 0.665$. Criteria for IDE sTfR $> 2.2 \text{ mg/L}$ ROC AUC 0.899 (95 % CI 0.769 to 0.961), cut off 6.6 %, sensitivity 80.6 %, specificity 92.9 %

Conclusion: LHD % is a reliable parameter recognizing subsets of apparently healthy subjects, adding useful information for detecting subclinical iron deficiency in a group at special risk to develop iron deficiency anemia.

ABBREVIATIONS

AUC: Area Under Curve; Hb: Hemoglobin; LHD %: Low Hb Density; IDA: Iron Deficiency Anemia; IDE: Iron Deficient Erythropoiesis; ID: Iron Deficiency; MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Hemoglobin Concentration; MCV: Mean Cell Volume; RBC: Red Blood Cell Count; ROC: Receiver Operator Curve; Stfr: Soluble Transferrin Receptor; SID: Storage Iron Depletion

INTRODUCTION

Physiological iron deficiency is the most common type of iron deficiency both in the general population and in many clinical settings. The cause is a disparity between the physiological requirements for iron and the maximal absorption from the diet.

Due to the regular blood losses women of reproductive age a priori face a difficult situation concerning their iron balance. Natural mechanisms in women have not compensated for these

iron losses [1]. In addition, both in developed and especially in developing countries, women of reproductive age consume a diet containing an insufficient amount of iron to cover their needs [2].

On a worldwide scale, more than 468 million non-pregnant women suffer from anemia; of these women, ~ 80 millions live in Europe. In this continent the prevalence of anemia in women is 15%, among apparently healthy women of reproductive age, ~ 40% have low iron status (serum ferritin $< 30 \mu\text{g/L}$) [3].

Iron deficiency (ID) is a reduced content of total body iron. Iron deficiency anemia (IDA) occurs when the iron deficiency is sufficient to reduce erythropoiesis and therefore the hemoglobin (Hb) level falls.

Hb within the reference interval does not exclude ID, because individuals with normal body iron stores must lose a large amount of body iron during a long period before the Hb falls below the laboratory definition of anemia. According to the World Health

Organization's criteria, Laboratory definition of anemia is Hb< 120 g/L for female and < 130 g/L for male [4].

The transition from the normal iron-replete state to the development of IDA entails two sequential processes: depletion of the storage iron compartment (stage I), followed by its exhaustion and the consequent initiation of depletion of the functional iron compartment (stage II); the development of IDA is a sequel of progressive depletion of the functional compartment [5].

Non-anemic iron deficiency is sometimes termed 'latent iron deficiency' or subclinical iron deficiency. In the latent stage (depletion of iron reserves) Iron depots are slowly used up in the process, while red blood cells are continuously produced in the bone marrow; standard indices mean cell volume (MCV), mean cell hemoglobin (MCH) and red blood cell count (RBC) tend to decline, but in the initial phase the values can still remain in the lower limit of reference intervals and only minor changes can be detected.

Both serum ferritin and soluble transferrin receptor (sTfR) are known to undergo a characteristic sequence of changes, as body iron stores decrease from normal iron-replete levels to those found in IDA [5].

During the depletion phase (stage I), in which sTfR concentration remains stable, there is a progressive decrease in serum ferritin. A normal Hb level with MCH in the lower limit of reference range, point to mild ID without anemia, but the main laboratory finding is low ferritin [6].

When the storage deficit (SID) is sufficient to restrict the synthesis of Hb and other functional iron compounds, iron-deficient erythropoiesis (IDE) ensues (stage II). The indicator of early IDE is the compensatory elevated sTfR concentration [5].

In iron deficient erythropoiesis, synthesis Hb is severely impaired, direct consequence of the imbalance between the erythroid marrow iron requirements and the actual supply, leading to the production of hypochromic red cells.

Beckman-Coulter (Beckman Coulter Inc., Miami FL, USA) has proposed a parameter, Low HemoglobinDensity(LHD %), derived from the traditional mean cell hemoglobin concentration (MCHC), using the mathematical sigmoid transformation:

$$LHD\% = 100 * \sqrt{1 - \left(1 / (1 + e^{1.8(30 - MCHC)})\right)}$$

MCHC is an estimation of the availability of iron over the preceding 90–120 days. In the same way LHD%, is related to iron availability and the Hb present of the mature red cells.

The reference and the clinical reliability as marker of iron availability for erythropoiesis have been already established [7-9]. Defined to highlight minimal changes in the erythrocyte indices, our hypothesis was that LHD% could reflect the progressive depletion of the iron stores and the lack of iron for the synthesis of Hb (hypochromic cells), in the initial stages of ID, before Hb level drops and IDA is established.

In this study we investigate the utility of LHD% in the detection of the sequential stages of iron deficiency in women of

reproductive age and normal Hb levels, in terms of concordance with serum ferritin (storage iron deficiency, SID) and sTfR (iron-deficient erythropoiesis, IDE).

MATERIALS AND METHODS

The study was conducted according to the Hospital ethic's guidelines and after being approved by the Committee of Ethics and Good Practice of the Hospital.

Criteria of inclusion: the study focuses on non-anemic women of child-bearing age. A group of 1141 women, age range 18-40 years (mean 31.7 years), who's analysis being requested by general practitioners for routine health control, were studied in our Laboratory. Pregnants, thalassemia carriers, patients with inflammation or kidney disease, and subjects registered of Hospital admission in the previous 3 months were excluded.

The samples were obtained in the course of routine analysis, collected in K₂EDTA anti coagulant tubes (Vacutainer TM Becton-Dickinson, Rutherford, NJ, USA) and were analyzed on LH 780 analyzers (Beckman Coulter Inc., Miami FL, USA). Serum ferritin and sTfR were measured with a chemical analyzer Cobas c 711 (Roche Diagnostics, Manhein, Germany) using FERR3 and sTfR reactive (Roche Diagnostics, Manhein, Germany), respectively.

Statistical evaluation of analytical results

Kolmogorov-Smirnoff was applied to verify the distribution of the values of the different tests under study.

Independent samples t test was applied in order to detect statistical deviations between the groups of patients; p values less than 0.05 were considered to be statistically significant. Correlation between LHD %and the standard tests were calculated by Pearson method.

Cohen's Kappa Index of Inter-rater Reliability (κ index) was calculated to determine the concordance between LHD% and serum ferritin (storage iron deficiency, SID) and sTfR (iron-deficient erythropoiesis, IDE). κ has a range from 0-1.0, the larger values indicate better reliability; $\kappa > 0.7$ is considered satisfactory.

Receiver operating characteristic (ROC) curve analysis was utilized to illustrate the diagnostic performance of LHD% in the assessment of SID and IDE.

RESULTS

All parameters had a Gaussian distribution. Table (1) shows the analytical data in the groups generated in base to the values of Hb, serum ferritin and sTfR: Different decision limit for ferritin has been proposed; we have selected 20 μ g/L, in the range 12-30 μ g/L, which is considered a state of "latent anemia "in healthy subjects [10]. No Iron deficiency: Hb>120g/L and serum ferritin within reference interval. Patients with Hb>120g/L and serum ferritin < 20 μ g/L were considered to have latent or subclinical iron deficiency; in this group sTfR was measured. Patients with sTfR< 2.2 mg/L were included in the depletion of storage iron group (stage I, SID). A value sTfR>2.2 mg/L defined iron deficient erythropoiesis (stage II, IDE).

In the group of 1141 patients Hb values ranged 84-149 g/L, 617 of them had Hb>120 g/L; in this group 450 women were considered No Iron deficient (normal serum ferritin); the other

Table 1: Analytical data of the groups of patients in the stages No Iron deficiency (No ID, n=450), latent iron deficiency 167 patients, divided in storage iron depletion (SID, n=127) and iron deficient erythropoiesis (IDE, n=40). Values are informed as mean (standard deviation).

	No ID	SID	IDE	P
RBC, 10 ¹² /L	4.8 (0.8)	3.99 (0.33)	4.02 (0.29)	1,2
Hb, g/L	148 (6)	122 (1.4)	121 (0.8)	1,2
MCV, fL	91.8 (2.1)	90.3 (5.5)	85.2 (4.5)	1,2,3
MCH, pg	30.4 (0.7)	30.6 (2.2)	27.1 (3.3)	1,3
MCHC, g/L	337 (3.5)	339 (3.8)	319 (6.1)	1,2,3
RDW, %	13.1 (0.6)	14.0 (1.1)	15.7 (1.2)	1,3
Ferritin µg/L	86 (3)	16 (4.6)	5 (1.9)	1,2,3
sTfR, mg/L	----	1.3 (0.38)	2.6 (0.32)	3
LHD %	2.2 (1.2)	4.5 (3.2)	12.2 (4.0)	1,2,3

Abbreviations: RBC: Red Blood Cells; Hb: Hemoglobin; MCV: Mean Cell Volume; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; RDW: Red Cell Distribution Width; Stfr: Soluble Transferrin Receptor; % LHD: Low Hb Density.

P statistical significance between groups

1 statistical significance between No ID and IDE P<0.05

2 statistical significance between No ID and SID P<0.05

3 statistical significance between SID and IDE P<0.05

167 women (27 % of this non-anemic group) had subclinical iron deficiency; the mean s-ferritin in this group was 16 µg/L. Thirty eight percent of patients in this group of 167 women with latent iron deficiency had LHD% > 4.4 %, above the reference interval. Gold standard for detecting SID was ferritin < 20 µg/L.

ROC analysis for LHD% AUC 0.844(95 % CI 0.729-0.924), cut off 5.1 % sensitivity 83.3 % and specificity 81.2 %. The percentage of patients with LHD% >5.1 % was 33.3 %. With this cut off concordance LHD% / Serum ferritin κ =0.561. Pearson's correlation R=- 0.4896 (P=0.0036, 95 % Confidence Interval CI-0.5953 to -0.0361). In this group with latent iron deficiency sTfR was analyzed to find out patients with depletion of iron stores (SID, stage I) and those in the stage II, iron deficient erythropoiesis (IDE). Based on the values of sTfR, 127 were classified in stage I and 40 patients (24 %) were diagnosed of I

DE. 84.5 % of the patients in the IDE group presented pathologic values for LHD %. Gold standard for detecting IDE was sTfR>2.2 mg/L. ROC analysis for LHD% AUC 0.899 (95 % CI 0.769 to 0.961); in the limit of the reference interval (4.4 %) sensitivity 68.5 % and specificity 90.0 %; the best Youden index was obtained at cut off 6.6 % sensitivity 80.6 % and specificity 92.9 %, positive predictive value 92.0 %. The agreement LHD %/ sTfR (cut off 6.6 %), κ =0.695, Pearson's correlation R=- 0.7198 (P<0.0001, 95 % CI 0.5398 to 0.8242).

DISCUSSION

Iron deficiency remains the most prevalent micronutrient deficiency worldwide, with substantial consequences for affected individuals and the economic productivity of their societies [11,12]. It appears from many surveys that the major risk groups for IDA are young children and, after childhood, individuals of female gender, pregnant with an increased need for iron, and lactating women [2]. Most of the literature on this topic deals with iron deficiency anemia, and there is less information on iron deficiency with normal hemoglobin levels. A recent article in which more than 12,000 patients in a develop country were studied, suggests iron deficient erythropoiesis in more subjects than those anemic [13].

The diagnosis of mild forms of ID and initial depletion of iron stores presents a great challenge because the deleterious effects of ID seem to begin before anemia develops [2,11]. In non-anemic patients, the most important clinical clue of ID is the symptom of chronic fatigue (iron is required for the enzymes involved in oxidative metabolism). However, it is of little screening value because clinicians rarely consider the presence of ID in patients who are not anemic, and therefore ID is invariably diagnosed in the Laboratory.

The identification of iron deficiency as a potential cause of fatigue and decrease vitality may prevent inappropriate diagnosis, reducing the unnecessary use of health care resources, including inappropriate pharmacologic treatments [14].

Thus, Laboratory tests for investigating ID fall into two categories: measurements providing evidence of iron depletion in the body, and measurements reflecting iron-deficient erythropoiesis [15]. The appropriate combination of these laboratory tests will help to establish a correct diagnosis of anemia and ID status [16].

The Laboratory role in nutritional management of the patient has increased while there have been remarkable changes in technology in the last years [17]. Technological progress has meant that the modern hematology counters report new parameters which provide further information from the traditional count. Among the new test the "markers of hypochromia", related to the availability of iron for erythropoiesis, seem promising [18]. In the initial phases of iron deficiency and restricted erythropoiesis, limited iron supply to the bone marrow yield decreased Hb production resulting in reticulocytes, and after mature red cells, with low Hb concentration [19]. LHD % can be considered a marker of hypochromia, reflecting a period of time with reduced supply of iron for erythropoiesis, as occurs during the progressive depletion of iron stores and iron of the functional compartment before IDA is established.

New algorithms have been proposed to improve the diagnosis of anemia in population of risk like children and adolescents; studies reveal the advantageous cost- effectiveness that supposes

to introduce reticulocyte derived parameters (Hb content) in the modern algorithms [20,21]. Nevertheless, in a context of primary health care, with no clinical suspicion of iron deficiency, the clinicians don't request reticulocyte counts; from this point of view a marker related to RBC, rather than reticulocyte, should fit better to the purpose of detecting the latent deficiency. As is showed in the table 1, there is a progressive decline in the RBC indices along the stages of iron deficiency progression, but the extensive overlap in those values makes it difficult to recognize SID and IDE.

The goal is the detection of a negative iron balance before functional iron compartment is impaired; our study focuses on the reliability of the added information of LHD % for detecting subclinical iron deficiency, in correlation with the biochemical tests.

Our results in a group of premenopausal women are in concordance with recent reports on children and young adults, where MCV, MCH, and MCHC were only moderately accurate in diagnosing empty iron stores and values within reference ranges of these indices do not exclude empty iron stores [22].

On the other hand LHD% was considered useful in the screening of iron-deficiency anemia in children, with the cut off LHD% <6.0 % [23], and in a large cohort of both pediatric and adult patients [24].

According to our results, SID group presented Hb and RBC indices within reference ranges, even MCH had no statistically difference when compared to the group with no iron depletion (Table 1); 38 % of those patients had LHD % values higher than the reference range.

The correlation with ferritin was poor, may be because LHD % is related to the functional iron compartment and iron availability rather than to iron stores. Nevertheless, ROC results are in good agreement with previous reports evaluating laboratory tests in the assessment of iron deficiency in blood donors [25].

On the contrary, 84.5 % of the patients in the IDE group presented pathologic values for LHD % and thus presence of hypochromia, due to the reduced iron supply in the subclinical stage previous to anemia, but only minor changes can be detected in the Wintrobe's indices.

sTfR is recommended as sensitive and accurate test of ID when only a single indicator can be used [26], and has been applied by the National Health and Nutrition Examination Survey (USA) to the evaluation of children and young women [27].

But this is a expensive test, not widely available nor requested by general practitioners but the unrestricted access to requests sTfR analysis in the screening for iron deficiency may be costly [28]. From this point of view the new parameters of the hemogram seem promising. This could be an interesting strategy not only in the developing countries where iron deficiency is a Public Health problem [29], mainly among children and women on child bearing age, but also in the western world due to general cut of budgets.

CONCLUSION

The present study shows that LHD% can add a value for

detecting iron deficiency in a subclinical phase; a value higher than 6.6% presents a high positive predictive value to identify latent iron deficiency in a group of apparently healthy young women. LHD% is a reliable test for detecting subclinical iron deficiency in this group: it is useful recognizing subsets of apparently healthy subjects, therefore improving the diagnosis and management of iron deficiency. Iron depleted women can quickly develop IDA if not detected quickly, the inclusion of LHD % could be considered an strategy to prevent anemia a group at special risk of iron deficiency.

The value of LHD % can be calculated automatically and simultaneously in the course of routine blood counts, with no incremental costs and no additional needs of more blood sampling, adding information on iron status easily incorporated into the report released from the analyzer. This information could be extremely useful to the primary care physician in the early detection of a negative iron balance and start treatment before IDA is established.

More studies are needed to verify our findings in other groups of population at risk of iron deficiency. We propose to use LHD% in the initial investigation of iron status in the groups of risk, together with the standard hemogram; LHD % could aid to selected samples for further investigation, including at least reticulocyte counts with derived parameters and serum ferritin assay. In conjunction with standard blood cell counts and iron parameters could enable the diagnosis to be made rapid and accurately.

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