

# Extended erythrocyte and reticulocyte parameters in the diagnosis of iron deficiency in hemodialysis patients

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## Abstract

**Objective:** Iron deficiency (ID) is a frequent complication in end stage renal insufficiency. These patients have to be diagnosed and treated to reduce the prevalence of anemia. Functional iron deficiency (FID) is a situation that can disrupt biochemical iron tests and mask an eventual association with ID. In this study, we tried to prove the ability of extended parameters of red cells and reticulocytes to diagnose ID without being influenced by FID. **Design and methods:** 164 chronic hemodialysis patients (CHP) in end stage renal disease were enrolled. Research parameters of red cells and reticulocytes determined on ADVIA 2120i were studied in the diagnosis of ID associated or not with chronic inflammation. **Results:** Parameters such as corpuscular hemoglobin of mature red cells (CHm), corpuscular hemoglobin of reticulocytes (CHr), cellular concentration of hemoglobin in mature red cells (CHCm), cellular concentration of hemoglobin in reticulocytes (CHCmr) and percentage of microcytic and hypochromic red cells (HYMI) showed a high sensitivity to diagnose ID. However, the distinction of combined iron deficiency (CID) from other entities was not possible with all parameters. In chronic inflammatory states, the decrease of CHm, CHCm and CHCmr with the rise of percentage hypochromic mature red cells (HYPOm) and reticulocytes (HYPOr) is in favor of CID. So, determination of inflammatory state is needed to complete research parameters of blood count in CHP. **Conclusion:** Extended erythrocyte and reticulocyte parameters can be useful to check iron status in CHP.

**Keywords:** Hematology analyzer, hemodialysis, iron deficiency anemia.

## INTRODUCTION

Anemia is the most serious complication in CHP. It is found in 70% of these patients and is responsible of important degradation of quality of life [1-3]. In end stage renal insufficiency, anemia is a consequence of accumulation of toxic metabolites, ID, FID and reduced erythropoietin production by renal failure, this situation is also observed in patients with a glomerular filtration rate less than 40 mL/min/1.73 m<sup>2</sup> [4, 5].

Iron is the most important element for the development and the growth of the majority of living organisms. It plays a role in the transport of oxygen, synthesis of ATP and ADP and regulation of the cell cycle [1].

ID is accentuated by chronic blood loss in hemodialysis patients and is found in about 47% of them [1, 2]. In this case, iron stores and iron absorbed by enterocytes are insufficient to maintain a

normal erythropoiesis. Patients with ID have to be treated by oral or parenteral iron administration for more efficiency.

FID is a particular situation where iron is not available for erythropoiesis despite normal or important stores secondary to reduced iron carriers [6]. FID is present in about 50% of CHP, it is considered as a complication of inflammatory states and the use of erythropoiesis stimulating agents [3, 7]. In this situation, biochemical parameters (serum iron, total iron binding capacity and serum ferritin) are unable to be used in the determination of iron status [2, 7]. A great interest was made to develop biological parameters permitting determination of iron status without being influenced by inflammatory state.

Many studies have shown the interest of CHr and HYPOm in the exploration of iron metabolism and erythropoiesis. Actually, many other parameters are provided by recent hematology analyzers described as research parameters of red cells and reticulocytes. So in this work, we tried to demonstrate the

interest of these research parameters in determination of iron status in CHP.

## MATERIAL AND METHODS

This prospective study was performed in the department of hemobiology of the 1st November hospital of Oran. Overall 164 chronic hemodialysis patients were enrolled from two hemodialysis center. All patients were in end stage renal disease and are treated by hemodialysis for more than one year. Has been excluded all patients recently transfused or receiving iron therapy. Patients were orally informed about the purpose of this study and approved their consent.

Complete blood count was realized on ADVIA 2120i (Siemens Healthcare). Serum ferritin and C reactive protein (CRP) were performed by immunoturbidimetric latex essay (BioSystems).

Patients with anemia received intravenous iron therapy and hemoglobin rate was controlled after one month. Patients with a rise of hemoglobin greater than 10 gr/l were considered as having an ID as recommended by Hershko [9]. According to the inflammatory status, patients were classified in 4 groups: ID, FID, CID and normal.

Volumetric parameters studied were the mean corpuscular volume of mature erythrocytes and reticulocytes (MCVm and MCVr respectively), the report MCVr/MCVm, the red cell factor

(RCf) calculated by the equation  $RCf = \sqrt{(MCVm \times MCVr)}$  [10] and percentage of microcytic red cells and microcytic reticulocytes (MICROm and MICROr respectively).

Parameters of hemoglobin studied were CHm, CHCMm, CHr, CHCMr, the report CHr/CHm, HYPOm, HYPOr and HYMI.

The immature reticulocyte fraction (FRI) was also included in the study.

## Statistical analysis

Collected data was analyzed with SPSS 20 (IBM statistics). ROC curves were established for all parameters in the screening of ID and completed with the determination of sensitivity and specificity. Student t test for mean comparison of all parameters was used to search statistical difference between ID, FID, CID and normal.

Statistical significance was defined as  $P < 0.05$

## RESULTS

Anemia was present in about 83% of CHP and ID was confirmed in 14%. Concerning inflammatory status, CRP was elevated in 23% of patients so we concluded that 5.7% of patients had CID. In table 1 our data are compared with other studies, the frequency of ID is less important in our series.

**Table 1:** Prevalence of anemia, ID and FID in hemodialysis patients. A comparison between studies (ND: not determined).

Study	Anemia	ID	FID
Our results	82.9%	14.3%	23.3%
Benmansour [3]	89.7%	39.7%	51%
Barde [2]	77.3%	25.33%	ND
KDOQI [11]	ND	25 – 38%	ND
Biniiaz [12]	ND	ND	41.3%
Valderrabans [13]	ND	39%	21%

ROC curve and analysis of area under curve are summarized in table 2. Cutoff, sensitivity and specificity were calculated only for

parameters presenting an area under curve upper than 0.7.

**Table 2:** Results of ROC curve analysis of all parameters in the diagnosis of ID.

Parameter	Area under curve	Cutoff	Sensitivity	Specificity
CHm	0.909	26.4 pg	100%	70%
CHr	0.888	27.5 pg	100%	75%
CHCMm	0.823	31.7 g/dl	85.70%	85%
CHCMr	0.909	26.9 g/dl	71.40%	95%
ferritin	0.754	478 µg/l	100%	42.50%
RCf	0.707	90.3 fl	85.70%	55%
MICROm	0.722	0.95%	93.30%	38.60%
HYPOm	0.776	6.45%	93%	34.10%
HYPOr	0.755	59.70%	86.70%	58%
HYMI	0.821	0.15%	80%	72.70%
MICROr	0.597	ND	ND	ND
MCVm	0.695	ND	ND	ND

MCVr	0.663	ND	ND	ND
CH ratio	0.554	ND	ND	ND
MCV ratio	0.661	ND	ND	ND
IRF	0.555	ND	ND	ND

Cutoff, sensitivity and specificity were calculated just for parameters with an area under curve upper than 0.7.

Comparisons between groups are summarized in table 3. We identified parameters with statistical difference between all situations; ID was clearly identified by many parameters but CID was more difficult to be distinguished.

**Table 3:** A comparison of different cutoffs of CHr and HYPO determined by some authors and confronted to our results.

Study	CHr	HYPO
Our cutoffs	27.5 pg	6.45%
Locatelli <sup>[14]</sup>	29 pg	10%
Urrechaga (2016) <sup>[15]</sup>	30.8 pg	2.4%
Urrechaga (2012) <sup>[16]</sup>	ND	3.6%
KDOQI <sup>[11]</sup>	29 pg	ND
Karlsson <sup>[17]</sup>	30.5 pg	ND
Vidyadhar <sup>[18]</sup>	ND	2.45%
Buttarelo <sup>[19]</sup>	31.2 pg	5.8%
Luo <sup>[20]</sup>	ND	1.55%
Agarwal <sup>[21]</sup>	25 pg	ND
Thomas <sup>[8]</sup>	28 pg	ND
Mitsuiki <sup>[22]</sup>	32 pg	ND
Igartua <sup>[23]</sup>	30 pg	ND
Lorenz <sup>[24]</sup>	29 pg	ND
Chinudomwong <sup>[25]</sup>	30pg	ND

**Table 4:** Means and references intervals of all parameters in different situations.

Parameters	ID mean	FID mean	CID mean	Normal mean	Signification
MCVm (fl)	83.98 (71.7 – 96.2)	85.96 (72.9 – 101.2)	82.50 (69.2 – 94.8)	86.67 (77.3 – 97.2)	-
MCVr (fl)	103.93 (90.8 – 114.6)	103.83 (88.1 – 121.9)	100.55 (93.6 – 113.2)	103.6 (93.3 – 116.4)	-
CHm (pg)	25.82 (22.4 – 29.9)	27.51 (23.6 – 31)	25.17 (20.9 – 29.7)	28.25 (25.1 – 31.4)	1, 4
CHr(pg)	27.48 (24.3 – 30.4)	28.98 (23.1 – 33.6)	26.38 (24.2 – 30.9)	29.54 (26.1 – 33)	3, 6
CHCMm (g/dl)	31.01 (29.1 – 33.4)	32.24 (29.2 – 34.8)	30.65 (29.5 – 31.7)	32.35 (29.6 – 35.4)	1, 3, 4, 6
CHCMr (g/dl)	26.60 (25 – 28.5)	28.02 (26.3 – 31)	26.28 (25.1 – 27.7)	28.70 (25.4 – 31.9)	1, 3, 4, 6
MICROm (%)	3.93 (0.2 – 10.4)	2.42 (0.2 – 12)	6.08 (1.1 – 24.9)	1.61 (0.2 – 4.4)	3
MICROr (%)	0.29 (0 – 1.1)	0.33 (0 – 2.4)	0.38 (0 – 0.7)	0.37 (0 – 1)	
HYPOm (%)	16.13 (6.7 – 33.7)	6.96 (0.8 – 27.9)	18.15 (9.4 – 25.8)	6.56 (0.2 – 26.1)	1, 3, 4, 6
HYPOr (%)	72.47 (47.5 – 92)	52.91 (11.9 – 79.7)	71.70 (59.8 – 87.9)	42.15 (7.6 – 83.5)	1, 3, 4, 6
Ferritin (µg/l)	224.37 (3.8 – 120)	437.71 (83.6 – 795)	233.85 (14.1 – 464.2)	335.49 (72 – 718.8)	1, 3
HYMI (%)	0.47 (0.1 – 0.9)	0.20 (0 – 1.2)	0.92 (0.1 – 4.1)	0.08 (0 – 0.4)	1, 3
RCf (fl)	91.8 (79.7 – 102.1)	92.71 (82.02 – 105.5)	88.29 (80.4 – 97.2)	93.77 (84.69 – 104.4)	6
MCV ratio	1.24 (1.18 – 1.28)	1.21 (1.07 – 1.31)	1.22 (1.13 – 1.38)	1.20 (1.15 – 1.25)	3
CH ratio	1.07 (1.02 – 1.11)	1.05 (0.9 – 1.19)	1.05 (0.96 – 1.16)	1.06 (1.02 – 1.1)	-
IRF (%)	24.85 (1.42 – 55.02)	25.15 (2.01 – 52.6)	34.57 (9.38 – 54.61)	23.28 (2.97 – 49.6)	-

Statistical differences between groups are noticed: 1: ID vs FID, 2: ID vs CID, 3: ID vs controls, 4: FID vs CID, 5: FID vs controls, 6: CID vs controls.

## DISCUSSION

Many studies evaluated the interest of red cell and reticulocyte parameters in erythropoiesis disorders especially ID even before the development of anemia [27-31]. In this work, we tried to demonstrate the interest of some cellular parameters determined by novel hematology analyzer in the diagnosis of ID in hemodialysis patients with or without FID. End stage renal insufficiency patients present a high frequency of anemia, ID and inflammation so the discrimination of these situations is essential for the follow-up. Frequencies of anemia and FID were close to results of other studies. However, ID was less frequent. Probably it was necessary to stop iron therapy for more than three months.

Research parameters of red cells and reticulocytes are considered as reflecting erythropoiesis activity in relation with iron supply to erythroid precursors. Red cells become smaller and contain less hemoglobin as they age, a reduction in the rate of red cells turnover allows smaller and hypochromic cells to continue circulating to extend their lifespan [32].

Many studies have demonstrated the interest of CHr and HYPO in the diagnosis of ID while other cellular parameters were little studied.

Serum ferritin is widely used in routine determination of iron status [33, 34] and we also found a significant efficiency to diagnose ID. However, being an acute phase protein, serum ferritin rate tends to be increased in inflammatory states so in our study, serum ferritin was unable to distinguish CID from other groups.

The rise of hemoglobin rate after intravenous iron therapy was used as gold standard to diagnose ID as recommended by Hershko [9]. Thus, the most significant parameters in the diagnosis of ID were CHm, CHr, CHCMm, CHCMr and HYMI. In contrast with many authors, HYPO was less significant as well as for RCf, MICRO. Parameters reflecting hemoglobin content seem to be more interesting in determination of iron status than parameters in relation with size of cells.

Different cutoffs of ID were assigned for CHr and HYPO and there is a great variability between authors. These variations can be explained by the interlaboratory heterogeneity and the need of more standardization of analyzers. The technical differences and calculation algorithms used in different analyzers can explain these different cutoffs values reported [35]. Actually, the usefulness of cellular parameters is widely proved but each laboratory has to set its own normal values and cutoffs.

In end stage renal insufficiency patients, cellular parameters were able to diagnose patients with ID and distinguish them from FID especially CHm, CHCMm, CHCMr, HYPOm, HYPOr, HYMI. However, any test could separate patients with CID from FID and ID, so it is important to complete cellular analysis with inflammatory tests as recommended by Vidiyadhar [19].

According to these results, an algorithm can be developed. Negative CRP excludes FID and CID and patients have to be classed as normal or ID. For this, parameters such as CHr, CHCMm, CHCMr, MICROm, HYPOm, HYPOr and HYMI can be used or only serum ferritin. When CRP is increased it's important to differentiate between FID and CID. The decrease of CHm, CHCMm and CHCMr with the rise of HYPOm and HYPOr is in favor of CID.

## CONCLUSION

Simple and quickly available, extended erythrocyte and reticulocyte parameters seem to be useful for checking iron status in CHP. However, determination of inflammatory state is needed.

### Conflict of interest

None to disclose.

### Authors' contributions

All authors have approved the final article. MN BENNAOUM wrote the manuscript with support from the remaining authors.

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