Reticulocyte Hemoglobin Content Helps Avoid Iron Overload in Hemodialysis Patients: A Retrospective Observational Study

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Abstract. *Background/Aim: Anemia in patients suffering from end-stage renal failure is currently treated with Erythropoiesis-Stimulating Agents (ESA). This treatment needs sufficient iron supplementation to avoid an inadequate dosage of ESA. Nowadays modern analytical instruments allow to accurately calculate the content of Hemoglobin (Hb) in reticulocytes (CHr), that can be used as a guide for prescribing patients with the appropriate amount of iron. Patients and Methods: Patients, undergoing hemodialysis, were retrospectively selected from the database and were divided in two groups: group A received intravenous (IV) iron and subcutaneously ESA, and their dosages were adjusted on the basis of the following parameters: Hb, Mean corpuscular haemoglobin (MCH), CHr with consequent MCH/CHr ratio and reticulocyte count determined by the ADVIA 120 Hematology System of Siemens; group B patients were administered IV iron and ESA monitoring iron storage, Hb and ferritin. The aforementioned parameters and the administered amount of iron and ESA were monitored at baseline, four and eight months from the begining of the study. Results: For ESA supplementation, no difference was observed between the groups at the various observed times. Despite similar Hb levels, the patients of group A needed significant lower doses of IV iron (–57.8%) avoiding risks of organ toxicity and obtaining consequent cost saving of nearly 1 €/patient/month. Conclusion: The use of CHr and its related parameters allows the avoidance of overdosage of IV iron, which can potentially damage organs, and the reduction of health care direct and indirect costs.*

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Patients suffering from End-Stage Renal Disease (ESRD) represent a major health care problem with a significant cost, should they undergo hemodialysis treatment. Patients lose up to 5-7 mg of iron during each dialysis treatment and this is a primary cause of their iron-deficiency anemia (1, 2). There is also an increased need for iron supplementation to maintain Hb levels within the optimal range and maximize the response to ESA (3). As oral iron supplementation is often ineffective due to both patient non-compliance and gastrointestinal adverse effects, most dialysis patients receive IV iron to fill sufficient iron stores (4, 5). However, iron excess is stored in the liver causing organ toxicity and inflammation as well as an increased risk of infections (6, 7).

ESRD patients generally need to be given ESA and IV iron together to achieve the optimal Hb concentrations (4). The majority of patients on hemodialysis receive an IV/subcutaneous ESA dose during each dialysis section (8). Values of transferrin saturation <20% and serum ferritin <100 ng/ml require iron supplementation. (9). There are not specific protocols for IV iron supplementation. Previous clinical studies have suggested the administration of iron saccharate at a total dose of 1g/14 days (two administrations of 500 mg or five of 200 mg) (10) or iron gluconate at a total

Table I. *Characteristics of enrolled patients at baseline.*

	Group A	Group B	*p*-Value
Number (M/F)	15 (8/7)	17 (8/9)	0.803
Age (years)	70.0 (62.0-73.7)	73.0 (64.2-77.7)	0.199
Duration of dialysis (years)	4.0 (3.0-6.5)	3.0 (2.0-6.0)	0.392
ESA dose (U/kg/patient/month)	425.3 (201.5-770.8)*	443.2 (217.6-612.9)**	0.682
Hb (mg/dL)	11.2±1.4	11.6±1.0	0.388
Ferritin (ng/mL)	286.0 (168.5-337.2)	230.0 (159.1-395.8)	0.737

*Eight on epoetin zeta, 4 on darbepoetin alfa and 3 on methoxy polyethylene glycol-epoetin beta; **Five on epoetin zeta, 7 on darbepoetin alfa, 4 on methoxy polyethylene glycol-epoetin beta and 1 on epoetin beta.

dose of 1g/month (four administrations of 250 mg) (11). This approach leads to a suboptimal correction of anemia with consequent strong variations of Hb levels. These Hb variations are responsible for the continuous adjustments of iron and ESA dosages.

Reticulocyte count can represent a simple and cheap indirect way for the diagnosis of anemia. The count of erythrocytes by automatic methodologies is important and some issues of this procedure, such as the imprecision and inaccuracy due to manual count, have been eliminated. The quantitative measurement of fluorescence intensity rate represents a current parameter that completes the diagnostic value of reticulocyte count.

Thus, these modern analytical instruments allow to carefully calculate the CHr: CHr is related to MCH and the corresponding MCH/CHr ratio is influenced both by the entity of erythropoiesis stimulation of the marrow and the iron availability for the Hb synthesis. In this study we have evaluated the usefulness of CHr (by using MCH/CHr ratio) as a guide for the prescription of iron in order to reduce the fluctuations of Hb concentrations in patients undergoing hemodialysis. Testing ferritin levels is a routinely approach used to adjust iron supplementation in ESRD patients.

The aims of this work were: a) to compare the usefulness and appropriateness of CHr, evaluated by MCH/CHr ratio, *versus* ferritin in order to optimally set the iron supplementation in ESRD patients on dialysis; b) to consequently assess the cost saving analysis.

Materials and Methods

Population. Fifty Caucasian patients on stable hemodialysis treatment without high C reactive protein levels were retrospectively studied based on the data of their records. Eighteen patients were excluded not having filled the entire database, from which the laboratory parameters were drawn. Thirty two patients formed the final population. The selected patients were broken into two groups. The well matched characteristics of the population were reported in the Table I.

Table II. *Amount of administered ESA (U/kg/patient/month) in both groups at observed times.*

	T0	T4	T8
Group A	425.3 (201.5-770.8)	387.4 (196.5-677.1)	463.8 (248.4-571.3)
Group B	443.2 (217.6-612.9)	442.5 (350.5-529.2)	385.9 (342.1-447.9)

ANOVA 2X2 Design: groups: F=0.99, *p*=0.323; time: F=0.51, *p*=0.60; groups*time: F=0.32, *p*=0.72.

Laboratory parameters. Group A received iron and ESA, adjusting their dosages on the basis of the following parameters: Hb, MCH/CHr ratio, reticulocyte count (expressed as both absolute and relative values) by an automated hematology system count (ADVIA 120 Hematology System of Siemens Healthcare GmbH, Erlangen, Germany). It is a flow-cytometric analyser using low-angle and high-angle scatter for the determination of individual cell volume and Hb concentration (12). CHr is determined by measuring the volume and Hb concentration of each reticulocyte and it represents the mean value of Hb mass of each cell (13). This allows calculation of the following indexes: MCH, CHr with consequent MCH/CHr ratio and the reticulocyte production index (IPR). The MCH/CHr ratio guides iron supplementation. A value <1.0 is indicative of physiologic availability of iron by the erythrone. A ratio >1.0 reveals iron deficiency and a consequent need for iron supplementation. Reticulocyte Production Index (RPI) addresses the administration of ESA; in particular RPI<2.0 indicates a reduced production of reticulocyte by the marrow with consequent need of ESA treatment; RPI>2.0 indicates a hyperplastic erythropoiesis and reduction until the withdrawal of ESA is performed. *Vice versa*, patients of group B were administered iron and ESA on the basis of the recommended guidelines for the treatment of anemia in chronic renal failure of the Italian Society of Nephrology (14) monitoring iron storage, Hb and ferritin. The afore mentioned parameters as well as the administered amount of iron and ESA were monitored at baseline, at four and eight months of the study.

Statistics. First of all, data of every studied variable were analyzed in order to assess the distribution by the Shapiro-Wilk (S-W) test.

Table III. *Hematologic parameters investigated in both groups at observed times.*

Group A	T0	T4	T8	Group B	T0	T4	T8
Hb (mg/dL)	11.2±1.4	11.1±1.4	10.8±0.9	Hb (mg/dL)	11.6±1.0	10.7±0.6	11.6±0.6
MCH/CHr	0.95 (0.94-0.96)	0.91 (0.88-0.93)	0.88 (0.87-0.92)	MCH/CHr	0.89 (0.87-0.91)	0.88 (0.87-0.89)	0.88 (0.85-0.91)
RPI	5.78 (3.87-7.38)	5.00 (4.15-5.83)	6.00 (4.79-6.87)	RPI	6.14 (3.24-7.69)	4.00 (2.55-5.83)	5.87 (4.59-7.70)
Ferritin (ng/mL)	286.0 (168.5-337.2)	116.0 (48.5-165.0)	153.0 (48.5-198.0)	Ferritin (ng/mL)	230.0 (159.1-395.8)	213.0 (171.7-345.3)	285.5 (241.2-368.5)

Hb ANOVA 2X2 Design: groups: F=1.56, $p=0.214$; time: F=2.17, $p=0.120$; groups*time: F=2.93; $p=0.06$; MCH/CHr ANOVA 2X2 Design: groups: F=18.26, $p \leq 0.001$; time: F=5.28, $p=0.007$; groups*time: F=2.25, $p=0.11$; RPI ANOVA 2X2 Design: groups: F=0.015, $p=0.903$; time: F=2.19, $p=0.118$; groups*time: F=0.132, $p=0.876$; Ferritin ANOVA 2X2 Design: groups: F=9.66, $p=0.0003$; time: F=3.739, $p=0.028$; groups*time: F=2.563, $p=0.083$.

The normally distributed variables (S-W, $p>0.05$) were expressed as mean (M)±standard deviation (SD). *Vice versa* the abnormally distributed variables (S-W, $p<0.05$) were expressed as median values (InterQuartile Range, IQR). To study frequencies, Chi squares were used. For the variable Hb, which was normally distributed, the unpaired *t*-test was performed to assess the difference between two groups. When dealing with not normally distributed variables, the Mann-Whitney test was adopted in order to evaluate the difference between two groups.

An ANOVA 2×2 analysis was performed to evaluate differences between the two groups at the investigated times.

A p-value <0.05 was considered significant.

Results

In Table I the characteristics of the studied population at baseline were reported.

First of all, concerning the ESA supplementation, no difference was observed between groups and at the observed times (Table II). Stable values of Hb were present in both groups at the observed times and no significant difference was found between the groups A and B (Table III). Despite these similar Hb levels, the patients of group A needed significant lower doses of IV trivalent iron (−57.8%). In fact, the total amount of administered iron in patients of groups A and B were 11,500 and 19,875 mg, respectively. For ferritin, although the basal values in both groups were similar, in the course of treatment its values tended to significantly decrease in the group A, confirming the minor need for iron supplementation. Accordingly, MCH/CHr baseline values were superior to those of group B, with a trend to decrease during the follow up.

When analyzing the RPI values, no difference was observed between groups and regards to times, in agreement with the used doses of ESA.

Furthermore, a pharmacoeconomic evaluation showed a reduction in the cost of iron therapy of nearly 1 €/patient/month.

Discussion

The loss of blood due to hemodialysis procedures but also laboratory testing is responsible for the anemia that usually is normocytic, normochromic and slightly regenerative. This condition needs ESA therapy, which should be associated with the administration of iron, as its efficacy is strongly linked to the adequate supplementation of this important trace metal. Optimal iron dosage is mandatory in order to avoid inappropriate ectopic storage (particularly liver, spleen, hearth, endocrine system) with related specific organ injury (15). In fact, overdose leads to an epidemic overload of iron in the ESRD population; IV iron bypasses the biological safeguards for the transport and handling of iron and helps to intensify chronic kidney disease-associated oxidative stress and inflammation. As a consequence, indiscriminate use of IV iron can accelerate cardiovascular disease, promote microbial infections, aggravate eventual viral hepatitis, and worsen diabetes and diabetic complications in such patients. For these reasons IV iron should be judiciously used in this vulnerable population (6).

Thus, adequate supplementation of both ESA and iron is central to reaching the Hb target concentrations. With regard to iron supplementation, IV iron is required as iron absorption is reduced along the gastro-intestinal tract due to elevation of hepcidin causing iron deficiency in ESRD patients. Even slight excess iron as a result of prolonged exposure can lead to toxicity. This approach could seem to be exaggerated, but it is mandatory to prevent long-term complications of iron overload and maintain serum iron and total body iron levels within a normal range. In this regards, the CHr could represent a valid diagnostic alternative although conflicting results exist in the literature. In fact, a previous study reported that the analysis of CHr is not superior to MCH when screening for iron deficiency in elderly anemic hospitalized patients (16). Conversely, other investigations carried out in animals and humans demonstrated an important predictive

role of CHr in the diagnosis of iron deficiency (17). In our population, patients of group A required minor iron supplementation based on this recent analytical approach, and this finding is in agreement with the latter reported study.

Administering the ideal iron dosage avoids important fluctuations in ESA dosage, identifies no-responders and improves health care costs. The cost reduction, due to tailored iron therapy, could be considered an apparently inconsistent issue but, taking into account the large number of patients on haemodialysis in Italy and the prolonged mean time of treatment (various years), this saving is noteworthy. Direct medical costs (laboratory tests including also false positive or true positive results, instrumental measurements and hospital services) should be further added to the cost of drug prescriptions. Finally, indirect costs such as those of morbidity and mortality should be not overlooked (18).

Medical errors is the third leading cause of death in the United States, after heart diseases and cancer, according to recently published findings (19), burdened by skyrocketing health care costs. Medication errors can occur in deciding which medicine and dosage regimen to use. Errors in prescribing include ineffective under-prescribing and inappropriate over-prescribing (20).

A limitation of this study could be the relatively small sample size, although a prolonged time of observation should be considered a strength.

In conclusion, the use of erythrocyte indexes and their related parameters allows avoidance of over-dosage of IV iron, which can potentially damage organs, and reduction in health care direct and indirect costs.

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