Clinical usefulness of the reticulocyte hemoglobin equivalent in children on hemodialysis

Diana Di Pinto, M.D.[a], Marcos Paz, M.D.[a], Marta Adragna, M.D.[a] and Laura López, M.D.[a]

ABSTRACT

Introduction. Iron-deficiency anemia is common in hemodialysis patients. Serum ferritin and transferrin saturation (TS) are used for its detection. The reticulocyte hemoglobin equivalent (RET-He) is a marker that is not altered by inflammatory conditions and directly reflects iron availability in the bone marrow.

Objective. To explore the diagnostic capability of RET-He to detect absolute iron deficiency and assess its correlation with traditional markers of iron deficiency.

Population and methods. Retrospective study comparing RET-He with ferritin and TS in children on hemodialysis seen at Hospital Garrahan between July 2016 and July 2019.

Results. In 164 observations carried out in 40 children, a weak positive correlation was found between hemoglobin and RET-He (r = 0.35, $p < 0.001$), a significant positive correlation between TS and RET-He (r = 0.52, $p < 0.001$), a low negative correlation between hemoglobin and ferritin (r = -0.19, $p = 0.02$), and a lack of correlation between hemoglobin and TS (r = 0.05, $p = 0.5$). Anemia was observed in 50 %; iron-deficiency anemia was detected by traditional markers in 13 % and by RET-He in 44 %. RET-He showed a sensitivity of 90.9 % (95 % CI: 57.1-99.5 %), a specificity of 74.5 % (95 % CI: 66.7-81 %), a negative predictive value of 99.1 % (95 % CI: 94.5-99.9 %), and a positive predictive value of 20.4 % (95 % CI: 10.7-34.7 %) to detect iron-deficiency anemia with a cut-off value of 29 pg.

Conclusions. Despite its limited capability, the use of RET-He as a biomarker of iron deficiency increases the detection of iron-deficiency anemia in children on hemodialysis.

Key words*: anemia, reticulocyte hemoglobin equivalent, hemodialysis, child.*

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a. Department of Nephrology, Hospital Nacional de Pediatría "Prof. Dr. Juan P. Garrahan," Autonomous City of Buenos Aires, Argentina.

E-mail address:
Diana Di Pinto, M.D.: dianadipinto@yahoo.com

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INTRODUCTION

Anemia is common in patients with chronic kidney disease (CKD) and may be attributed to several factors: inadequate erythropoietin production, iron, vitamin B12 and/or folic acid deficiency, inflammation, blood loss, hyperparathyroidism, among others.[1] It is a major complication for children on hemodialysis and is associated with increased morbidity and mortality and reduced quality of life.[2-4]

After erythropoietin insufficiency, iron deficiency is the main cause of anemia in this population.[2] Hemodialysis patients have absolute iron deficiency due to dietary restrictions and reduced appetite, reduced intestinal absorption because of gastric acid inhibitors and phosphate binders use, and blood loss from the extra-corporeal circuit.[2,3] Chronic inflammation, associated to dialysis, results in elevated hepcidin, which reduces intestinal iron absorption further and inhibits the release from reticuloendothelial stores, limiting iron available for erythropoiesis.[2-4] Both absolute and functional iron deficiency limit the effectiveness of erythropoietin therapy, resulting in higher doses and, therefore, higher costs.[2,5]

For many years, the gold standard to assess iron metabolism in patients with renal anemia has been a combination of serum ferritin and transferrin saturation (TS); nevertheless, these markers have limitations.[2,5-7] Serum ferritin reflects total body iron stores, but it is also an acute phase reactant that may increase with chronic inflammation, making its interpretation difficult in patients with CKD;[1,2,8] whereas the levels of

transferrin -the serum protein that mediates iron transport from blood plasma to tissues- display diurnal fluctuation and can be falsely elevated in the setting of malnutrition or chronic disease.[1,2,6,9]

By contrast, the reticulocyte hemoglobin equivalent (RET-He), which estimates the amount of hemoglobin in reticulocytes that reflects the erythropoiesis index over the preceding 2-3 days (early predictor), is not altered by inflammatory conditions and provides real-time information on iron availability in the bone marrow for incorporation into reticulocytes.[2,5,6,10] Due to these characteristics, in recent years, international guidelines have included RET-He as an iron-deficiency marker, considering a cut-off value of > 29 pg.[5,7,8,11,12]

Given that there is limited experience of its usefulness in pediatrics, this retrospective study was conducted in order to explore the diagnostic capability of RET-He to detect absolute iron deficiency in children on hemodialysis seen at Hospital de Pediatría "Prof. Dr. Juan P. Garrahan." The correlation between RET-He and traditional markers of iron deficiency was also assessed.

MATERIAL AND METHODS

Retrospective study of diagnostic accuracy conducted in the Division of Hemodialysis of our hospital between July 2016 and July 2019. Inclusion criteria: patients younger than 18 years who were on hemodialysis at our hospital for more than 3 months and for whom the four biochemical measurements were taken, at least once, to assess iron deficiency (hemoglobin, RET-He, ferritin, and TS). Exclusion criteria: blood dyscrasias, known as hemoglobinopathies, hemolytic anemia and hemorrhagic disorders, myelofibrosis diagnosed by bone marrow biopsy, red blood cell transfusion or active bleeding in the last month.

Clinical data to be assessed: demographic variables, causes of CKD, history of renal transplant, dialysis withdrawal, erythropoietin administration, dialysis duration, and follow-up duration. Laboratory data: hemoglobin, RET-He, serum ferritin, and TS. Information was obtained retrospectively from the electronic medical records.

The following terms were defined:

- Anemia: hemoglobin level below 10 grams per deciliter (g/dL) in children younger than 2 years.
- Absolute iron deficiency: serum ferritin level below 100 micrograms per liter (ug/L) together with TS below 20 %.[4,7,12]

In our study, a Sysmex XN-1000 autoanalyzer (Roche) was used to measure RET-He and hemoglobin. TS was obtained based on the calculation of blood iron and total iron binding capacity, which were measured with a Cobas 500 analyzer (Roche), whereas ferritin was measured with an ARCHITECT i2000 analyzer (Abbott).

Statistical analysis

Qualitative variables were summarized using frequencies and percentages, and quantitative variables using median (interquartile range [IQR]) when exhibiting asymmetrical distribution (Kolmogorov-Smirnov test). Pearson's coefficient was used to estimate the correlation between hemoglobin, RET-He, and traditional markers of iron deficiency (ferritin and TS). The ability of the RET-He cut-off value (≤ 29) reported in the bibliography[5,7,11,12] to diagnose iron deficiency was assessed by estimating sensitivity, specificity, positive predictive value, and negative predictive value with their corresponding 95 % confidence intervals (95 % CI), using as gold standard the combination of serum ferritin below 100 ug/L and TS below 20 %. A value of $p < 0.05$ was considered significant. Data were analyzed using SPSS 25. This study was approved by the Research Ethics Committee of Hospital Garrahan.

RESULTS

Out of the 46 patients on hemodialysis during the study period, 40 met the inclusion criteria. The remaining 6 were excluded because they were older than 18 years or the four biochemical measurements were not available.

Their demographic and clinical characteristics are summarized in *Table 1*. The main causes of CKD were glomerulopathies (35 %) and congenital anomalies of the kidney and urinary tract (28 %). Twenty-three patients were males (57 %). Twenty-one children (53 %) were 12-18 years old at study initiation, and none of them was younger than 2 years. Thirteen children (32 %) had a history of renal transplant. The median dialysis duration was 17.25 months (IQR: 11.21-34.60), and the median follow-up duration, 15.83 months (IQR: 4.92-16.93). All patients were administered erythropoietin.

TABLE 1. *Demographic and clinical characteristics of 40 children on hemodialysis*

Characteristics	n (%)
Males	23 (57)
History of renal transplant	13 (32)
Cause of renal disease	
Glomerulopathies	14 (35)
Kidney and urinary tract anomalies	11 (28)
Neurogenic bladder	8 (20)
Atypical hemolytic uremic syndrome	2 (5)
Other	5 (12)
Age at dialysis initiation	
1-6 years old	8 (20)
6-12 years old	15 (37)
12-18 years old	17 (43)
Age at study inclusion	
1-6 years old	6 (15)
6-12 years old	13 (32)
12-18 years old	21 (53)
Hemodialysis withdrawal	27 (67)
Renal transplant	8 (20)
Change of dialysis center	11 (27)
Change of dialysis modality (peritoneal dialysis)	3 (8)
Death	4 (10)
Recovery of renal function	1 (2)
Dialysis duration in months (median and interquartile range)	17.25 (11.21-34.60)
Follow-up duration in months (median and interquartile range)	15.83 (4.92-16.93)
Erythropoietin therapy	40 (100)

%: percent.

A total of 164 observations, were carried out. Anemia was detected in half of cases, and its cause was absolute iron deficiency using traditional markers in 13 % (11 observations) and RET-He in 44 % (36 observations) (*Table 2*).

Table 2 shows the median and IQR values of hemoglobin, RET-He, ferritin, and TS levels. The linear regression analysis showed a weak positive correlation between hemoglobin and RET-He ($r = 0.35$, $p < 0.001$) and a significant positive correlation between TS and RET-He ($r = 0.52$, $p < 0.001$), as observed in *Figures 1* and *2*, respectively. A low negative correlation was found between hemoglobin and ferritin ($r = -0.19$, $p = 0.02$), and there was no correlation between hemoglobin and TS ($r = 0.05$, $p = 0.5$) (*Figures 3* and *4*). Using the cut-off value of 29 pg, the diagnostic test showed a sensitivity of 90.9 % (95 % CI: 57.1-99.5 %), a specificity of 74.5 % (95 % CI: 66.7-81 %), a negative predictive value of 99.1 % (95 % CI: 94.5-99.9 %), and a positive predictive value of 20.4 % (95 % CI: 10.7-34.7 %) to detect absolute iron deficiency.

DISCUSSION

Anemia is common in CKD, particularly in patients on hemodialysis.[2,6] According to the 2006 Annual Report of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), the prevalence of anemia in children on hemodialysis treated with erythropoietin is

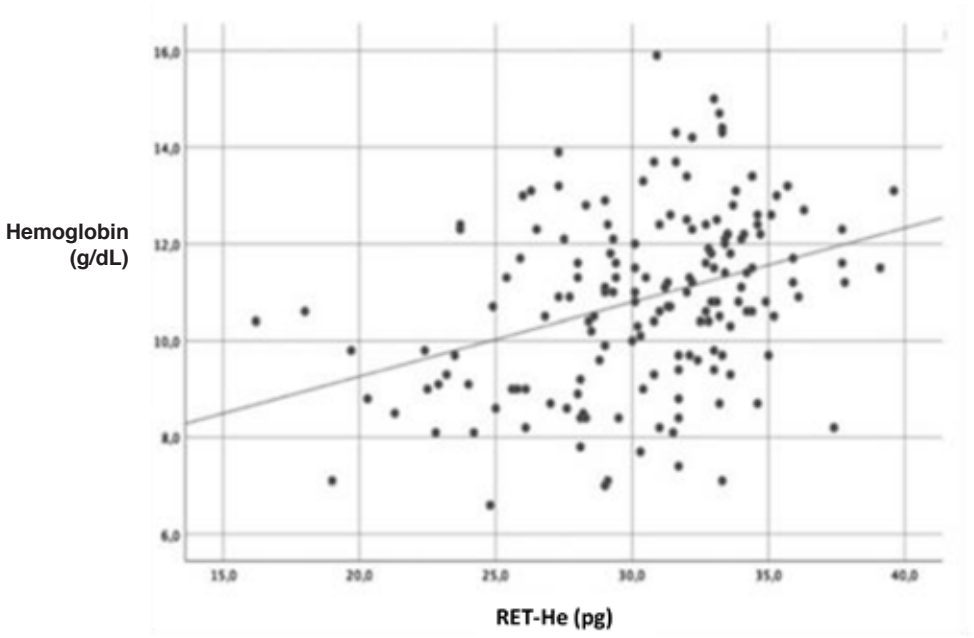
TABLE 2. *Hemoglobin, reticulocyte hemoglobin equivalent, ferritin, and transferrin saturation levels in 40 children on hemodialysis*

Characteristics	Number of observations	Level (median and interquartile range)
Hemoglobin (g/dL)	164	10.9 (9.4-12.2)
1-6 years old	22	10.1 (8.2-11.3)
6-12 years old	52	10.5 (9.1-11.9)
12-18 years old	90	11.3 (9.9-12.5)
Hemoglobin < 11 g/dL	82 (50 %)	9.5 (8.6-10.4)
+ ferritin < 100 ug/L and TS < 20 %	11 (13 %)	
+ RET-He ≤ 29 pg	36 (44 %)	
RET-He (pg)	164	31.3 (28.1-33.4)
Ferritin (ug/L)	164	229 (123-424)
< 100	31 (19 %)	
100-499	99 (60 %)	
≥ 500	34 (21 %)	
TS (%)	164	26 (18-38)
< 20	45 (27 %)	
≥ 20	119 (73 %)	

RET-He: reticulocyte hemoglobin equivalent; TS: transferrin saturation; g/dL: grams per deciliter; ug/L: micrograms per liter; pg: picograms; %: percent.

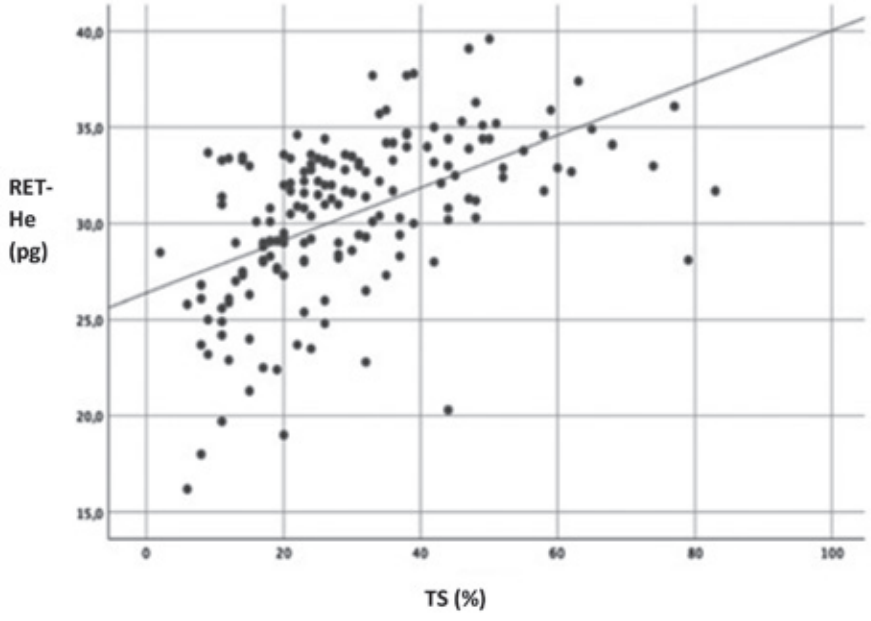
higher than 40 %.[13,14] In the study conducted by Davidkova et al., anemia was present in 45 % of 593 studied observations in 45 children older than 6 months on hemodialysis and peritoneal dialysis.[2] Our study showed a prevalence of 50 %, with a median hemoglobin level of 9.5 g/dL.

FIGURE 1. *Correlation between hemoglobin and reticulocyte hemoglobin equivalent levels in 40 children on hemodialysis*



RET-He: reticulocyte hemoglobin equivalent; g/dL: grams per deciliter; pg: picograms.

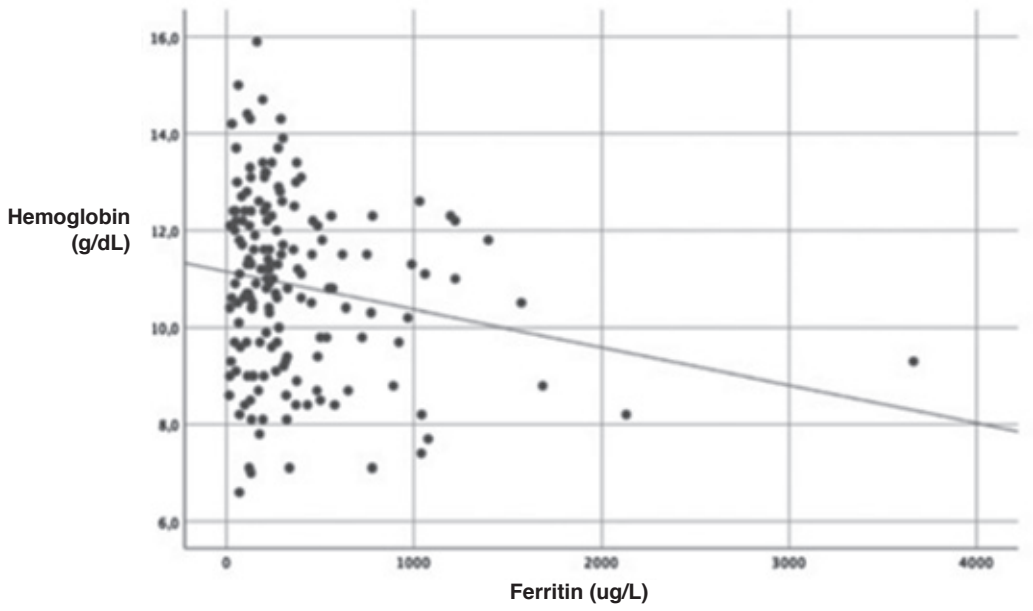
FIGURE 2. *Correlation between reticulocyte hemoglobin equivalent and transferrin saturation levels in 40 children on hemodialysis*



RET-He: reticulocyte hemoglobin equivalent; TS: transferrin saturation; %: percent; pg: picograms.

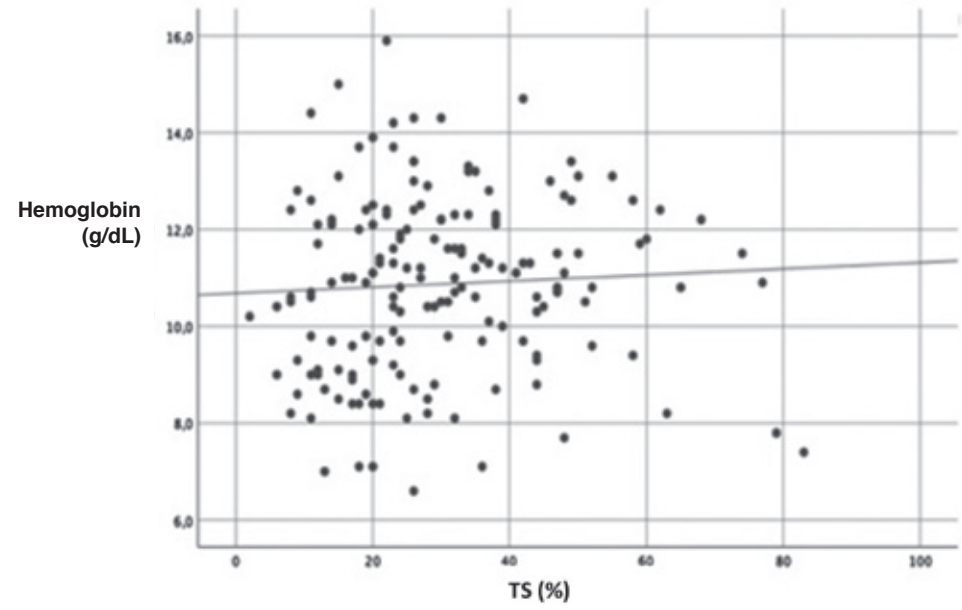
Before correcting anemia, accurate measurement of iron status is required to avoid unnecessary supplementation.[2] The currently accepted markers for such measurement in CKD are TS and serum ferritin, although they are known to have limited reliability in assessing iron availability in patients on dialysis.[1,2,4,5,11,12] Our study showed that, using the combination

FIGURE 3. *Correlation between hemoglobin and serum ferritin levels in 40 children on hemodialysis*



g/dL: grams per deciliter; ug/L: micrograms per liter.

Figure 4. *Correlation between hemoglobin and transferrin saturation levels in 40 children on hemodialysis*



TS: transferrin saturation; g/dL: grams per deciliter; %: percent.

of serum ferritin < 100 µg/L and TS < 20 %, 13 % (11 observations) of patients with anemia had absolute iron deficiency, which is higher than that observed in other studies, such as the one conducted by Davidkova et al. in children on dialysis, which showed a value of 3 % (7 observations).[2]

With the introduction of fourth-generation autoanalyzers, not only blood count accuracy improved, but also a new parameter started being measured: reticulocyte hemoglobin.[10] As mentioned above, the latter is useful as an indicator of iron deficiency; nevertheless, few studies have been conducted in the pediatric population.[5,6,11]

Using the cut-off value most widely accepted in the bibliography (29 pg), the prevalence of iron-deficiency anemia increased to 44 % (36 observations).[5,7,11,12] The study conducted by Davidkova et al., showed a prevalence of 38 % (84 observations), using a cut-off value of 28.9.[2]

Our results, like those of other studies, confirmed a very weak negative correlation between hemoglobin and ferritin.[2] Ferritin levels, instead of acting as markers of iron availability in patients on hemodialysis, might have reflected the degree of chronic inflammation over time, which would explain the negative correlation between them. The use of a negatively correlated variable in decision-making algorithms for iron management in dialysis is questionable and lacks evidence. In addition, there was a modest positive correlation between hemoglobin and RET-He, which was stronger than the lack of correlation between hemoglobin and TS, and is a more helpful biomarker, as also shown by Davidkova et al.[2] The good correlation between RET-He and TS observed in our study, as well as in those conducted in the population on hemodialysis, strengthens the hypothesis that this combination of biomarkers warrants further exploration independent of ferritin.[2,5,6]

The cut-off point of RET-He to diagnose absolute iron deficiency anemia in hemodialysis patients ranges from 27.2 pg to 33 pg, according to different studies, and 29 pg is considered to be the best cut-off value.[2,6,11,15] In the study we conducted in 40 children on hemodialysis, using 29 pg/ml as cut-off value for RET-He, the diagnostic test showed limited capability to detect absolute iron deficiency.

Although there are no local data regarding the usefulness of this parameter in the pediatric population, it is worth noting that a weakness of this study is its lack of adequate power due to the limited number of analyzed determinations. Therefore, studies with a higher number of patients are required to draw definite conclusions. In addition, the lack of a reliable gold standard diagnostic test engenders difficulty when assessing the usefulness of new biomarkers in the diagnosis of iron deficiency.

CONCLUSIONS

Despite its limited capability, the use of RET-He as a biomarker of iron deficiency increases the detection of iron-deficiency anemia in children on hemodialysis. ■

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