Reticulocyte Parameters: Markers of Early Response to Oral Treatment in Children With Severe Iron-deficiency Anemia

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Reticulocyte parameters: markers of early response to oral treatment in children with severe iron deficiency anemia

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Summary

The aim of the present study was to determine the effects of exclusive oral iron supplementation (iron sulphate 2 mg/kg/die) in asymptomatic children with severe iron deficiency anemia (median Hb level before treatment 6.3 g/dl; range 4.5-7) and to investigate the accuracy of hemoglobin (Hb), reticulocyte hemoglobin content (CHr) and absolute reticulocyte count (ARC) as markers for monitoring early response to treatment.

The rise in ARC and CHr was statistically significant at day + 3 (**p<0.005 in both cases**) .

There was a significant association between a suitable logarithmic function of the percentage increase in ARC and CHr at day +3 and the fraction of required hemoglobin increase compared to baseline to reach the mean reference value for age and gender at day +14 (**p < 0.1 and p<0.05 respectively**).

If these results are confirmed in a larger population, ARC and CHr could be considered accurate, affordable, and widely available markers to detect early responders to oral iron therapy, and to switch unresponsive children to parenteral iron supplementation or transfusion.

Introduction

Iron deficiency anemia (IDA) is the most common hematological disease in infancy and childhood. According to World Health Organization (WHO) statistics, 43% of children worldwide are iron deficient; in industrialized countries, 17% of children under 5 years of age suffer from IDA [1]**.** Factors leading to IDA in children include inadequate dietary iron intake, intestinal malabsorption, and blood loss.

The goal of therapy for IDA is to supply sufficient iron to repair the hemoglobin (Hb) deficit and replenish storage iron. Oral iron administration is a well estabilished, effective and widely accepted treatment for anemia secondary to inadequate dietary iron intake because of its efficacy, safety, and cost-effectiveness. Parenteral iron therapy should be reserved for those patients who have chronic uncontrollable bleeding, malabsorption, or remain intolerant to oral iron despite repeated modifications in the dosage regimen [2]**.** Recent publications highlighted the efficacy of parenteral iron administration in patients with IDA suggesting its use as first-line treatment [3, 4,5]**.**

Front-line use of i.v. iron in patients whose parameters must be raised rapidly has been suggested, in the hypotesis that parenteral administration would lead to a more rapid increase in hemoglobin levels [6].

In our Pediatric Hematology Unit, oral iron treatment is the first-line therapy for all children with IDA with anamnestic suspicion of inadequate iron intake, whatever their baseline Hb level, if they are clinically asymptomatic.

The aim of the present study was to evaluate the efficacy of exclusive oral iron supplementation in children with severe IDA. We also investigated whether hemoglobin and reticulocyte parameters (reticulocyte hemoglobin content [CHr] and absolute reticulocyte count [ARC]) might be accurate markers in monitoring early response to iron supplementation [7].

Methods

Patients

We retrospectively analyzed the records of patients with IDA (defined as an Hb level more than two standard deviations below the mean reference value for age and gender and transferrin saturation <15%) admitted between January 2007 and December 2010 to our Pediatric Hematology Unit.

Among these, we identified the patients affected by severe IDA (defined as Hb below 7.0 g/dL) who were treated with exclusive oral supplementation.

Analytical methods

Hb and reticulocyte parameters were measured with an automated flow cytometer (Advia 120 Bayer®) with optic measure; reticulocytes were stained with dye oxazine 750. Approximately 50,000 cells were counted for each red blood and reticulocyte determinations.

Statistical Analysis

Median and range values were calculated for full samples. To assess the effect of changes in Hb level, ARC, and CHr related to therapy, **a non-parametric repeated measure ANOVA was employed**.

The cases analyzed in the present study were characterized by an extended range of ages and of baseline Hb levels, too (range 4,5-7). As a consequence, the deficit in Hb (i.e. the difference between target Hb and baseline Hb levels) that had to be replenished varied broadly within the patients. To evaluate the percentage of Hb deficit replenished by the single patient at day + 14, taking into account both the mean expected Hb value for age and sex and the baseline Hb value, we used the percentage fraction of Hb increase required to reach the mean reference value of age and gender covered at day +14, expressed for each patient by the formula *(Hb at day +14 – baseline Hb) / (target Hb – baseline Hb) * 100.*

To investigate the relationship between the percentage increase in ARC and CHr after 3 days of therapy and the percentage fraction of Hb increase at day +14, linear regression methods with were employed **after suitably transforming the variables involved**. Data analysis was performed using the R software package (release 2.14.1).

P values <0.05 were considered to indicate statistical significance (two-tailed test).

Results

In the last four years, 72 children were referred to our Pediatric Hematology Unit for IDA.

Eighteen (25%) were affected by severe anemia. Fourteen of whom with severe IDA were asymptomatic (i.e. they did not present fatigue and/or tachycardia) at the time of diagnosis and presented with a high anamnestic suspicion of inadequate iron intake. Table I reports patient's characteristics.

All patients were initially treated with oral iron sulphate at a dosage of 2 mg/kg/die. One out of 14 patients was lost to follow up after the first week of treatment and was therefore excluded from the analysis. Hb, CHr, and ARC levels at diagnosis and at day +3 and day +14 from the beginning of iron supplementation were evaluated in 13 patients.

The median Hb level before treatment was 6.3 g/dl (range 4.5-7). Hb increased to 6.55 g/dl (range 4.5-8) and 9.1 g/dl (range 7.1-11.2) after 3 and 14 days of treatment, respectively. The rise in Hb level was statistically significant at day + 14 (p<0,0001).

The median ARC before treatment was 89,600 x 10^6/L (range 54,100-186,000). It increased to 164,150 x 10^6 /L (range 81,000-345,700) and 138,050 x 10^6/L (range 60,800-289,700) after 3 and 14 days of tretament, respectively. The rise in ARC was significant 3 days after the onset of iron supplementation (**p=0.0030**).

CHr could only be fully monitored in 7 patients. The median CHr before treatment was 15.8 fL (range 15.4-18.8). It increased to 19.9 fl (range 15-23.2) and 24.2 fl (range 19-30.5) after 3 and 14 days of treatment, respectively. The rise in CHr was significant 3 days after the onset of treatment (**p =0.0041**).

All patients (13/13) displayed a significant rise in Hb at day + 14 compared to the onset of iron supplementation.

A **barely significant** linear relationship was detected between the logarithm of the scaled percentage increase in ARC at day +3 and the fraction of required hemoglobin increase compared to baseline to reach the mean reference value for age and gender covered at day +14 (**p=0.0633**) (Figure 1). **An analogous significant** linear relationship was also present between the logarithm of the scaled percentage increase in CHr at day +3 and the fraction of required hemoglobin increase compared to the baseline to reach the mean reference value for age and gender covered at day +14 (**p=0.0297**) (Figure 1).

A significant linear relationship was also detected between a logarithmic function of the percentage increase in ARC at day +3 and the fraction of required hemoglobin at day +30, but the same relationship could not be found for the percentage increase in CHr at day +3 (results not shown).

Discussion

In the assumption that parenteral administration leads to a more rapid increase in hemoglobin levels, the effectiveness of front-line use of i.v. iron in patients with iron deficiency anemia has recently been highlighted in literature [3, 4,5, 6].

In particular, it has been suggested that intravenous iron therapy might replace oral therapy in patients whose blood parameters must be raised rapidly. Akarsu et al. [6] reported the efficacy of iron sucrose for rapid correction of anemia in 62 children who had received i.v. iron sucrose over 2–3 days to replenish their calculated iron deficit.

However, studies comparing oral and intravenous iron supplementation in children with IDA are lacking and there is no evidence regarding the fact that parenteral administration determines a faster increase in reticulocyte and hemoglobin levels than oral supplementation.

We report the efficacy of exclusive oral supplementation in children with very low baseline Hb levels (median Hb level before treatment 6.3 g/dl; range 4.5-7) and a high anamnestic suspicion of inadequate iron intake.

All patients showed a significant improvement in Hb levels at day +14 from the onset of iron supplementation. The hemoglobin rise in our patients was faster than previously observed. This might be due to the fact that this rise is not linear and is faster in correspondence to the lowest Hb values; the fast rise is probably due to the high iron absorption in subjects with severe deficiency.

Despite the limitations due to the small sample size, our data, according to literature [8], suggest that oral iron supplementation should be proposed as first-line treatment in all clinically asymptomatic patients, independently from the base Hb level, and transfusion should be reserved for symptomatic patients [2].

Regarding the kinetics of early response using reticulocyte parameters, in our study a significant linear relationship was detected between the increase in ARC and CHr at day +3 from the onset of treatment and the hemoglobin increase at day +14.

The fact that reticulocyte indices may allow a real-time evaluation of iron deficient erythropoiesis and of the effectiveness of iron replacement therapy is well known [7, 9] .

CHr has been shown to be an accurate marker in monitoring response to iron therapy in adults and in pediatric patients receiving hemodialysis [10, 11, 12, 13]. Brugnara et al. [14] reported a significant increase of ARC and CHr in 10 iron-deficient adult females during oral iron replacement therapy 1 or 2 weeks after the onset of treatment.

To our knowelwdge, however, this is the first study on iron therapy response using reticulocyte parameters in children with IDA. Moreover, in our study the reticulocyte response to oral treatment was demonstrated well in advance of that previously reported (48 hours versus 1 or 2 weeks after the onset of treatment).

In conclusion, we underline the efficacy of oral iron in children with IDA and the usefulness of ARC and CHr as accurate, and inexpensive markers to detect responders to oral therapy.

References

1. World Health Organization. Iron Deficiency Anemia:Assessment, Prevention, and Control-A Guide for Program Managers. Geneva,Switzerland : World Health Organization; 2001. WHO/NHD/01.3

2. Zeng X, Wu T. Iron supplementation for iron deficiency anemia in children. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2007.

3. Auerbach M. Should intravenous iron be upfront therapy for iron deficiency anemia? Pediatr Blood Cancer. 2011; 56:511-512

4. Bansal G, Burton J, Win N, et al. Assessment of the Efficacy of Intravenous Iron Therapy (Venofer®) in the Treatment of Iron Deficiency Anemia (IDA). ASH Annual Meeting Abstracts. 2010; 116: 4229.

5. Poma A, Diana K, McLaughlin J, et al. A Head-to-Head Comparison of the Safety and Efficacy of Ferumoxytol to Iron Sucrose for the Treatment of Iron Deficiency Anemia. ASH Annual Meeting Abstracts. 2010; 116: 5157.

6. Akarsu S, Taskin E., Yilmaz E. et al. Treatment of iron deficiency anemia with intravenous iron preprations. Acta Haematol. 2006; 116:51-7

7. Piva E, Brugnara C, Chiandetti L, et al. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. Clinical Chemistry and Laboratory Medicine. 2010; 48:1369-1380.

8. Sandoval C, Berger E, Ozkaynak MF, et al. Severe iron deficiency anemia in 42 pediatric patients. Pediatr Hematol Oncol. 2002;19:157-161.

9. Brugnara C. Use of reticulocyte cellular indicators in the diagnosis and treatment of haematological disorders. Int J Clin Lab Res 1998;28(1):1-11.

10. Major A, Mathez-Loic F, Rohling R et al. The effect of intravenous iron on the reticulocyte response to recombinant human erythropoietin. Br J Hematol 1997: 98(2):292-4.

11. Mittman N, Sreedhara R, Mushnick R et al. Reticulocyte hemoglobin content predicts functional iron deficiency in haemodialysis patients receiving rHuEPO. Am J Kidney Dis 1997: 30(6):912-22.

12. Chuang CL, Liu RS, Wei YH et al. Early prediction of response to intravenous iron supplementation by reticulocyte hemoglobin content and high-fluorescence reticulocyte count in haemodialysis patients. Nephrol Dial Transplant 2003: 18(2):370-7.

13. Warady BA, Kausz A, Lerner G et al. Iron therapy in the pediatric hemodialysis population. Pediatr Nephrol 2004:19(6):655-61.

14. Brugnara C, Lauter MR, Friedman AJ, Bridges K, Platt O. Reticulocyte haemoglobin content (CHr): early indicator of iron deficiency and response to therapy. Blood 1994; 83:3100–3101.

Table I: Patient's characteristics.

Figure 1:
Relationships between the percentage increase in ARC (on the left, pARC) and CHr (on the right, pCHr) at day +3 (x-axis) and the fraction of the required Hb increase compared to baseline to reach the mean reference value for age and gender covered at day +14 (y-axis, Fraction).
The line for the ARC case corresponds to the formula: Fraction = 0.26591+0.34252*log(pARC+1), for the CHr case to the formula: Fraction = 0.8268+0.7947*log(pCHr+0.5).