Red Cell Disorders

Impact of erythropoietic activity on red cell parameters in chronic renal failure patients

We measured red cell parameters during recombinant human erythropoietin (rHuEPO) therapy associated with appropriate iron supplementation in chronic hemodialysis patients. Increased erythropoietic activity led to a bias in red cell parameter determination. The percentage of hypochromic red blood cells, usually used as the most effective predictor of response to iron suplementation, increased following the appearance of a younger red cell population since the same Hb content in these younger, larger cells gives a lower Hb concentration.

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The percentage of hypochromic red blood cells (%HYPO) is the most sensitive and specific predictor of response to iron supplementation when erythropoietin (rHuEPO) is administered.[1] However, we have previously shown that %HYPO is positively influenced by the reticulocyte count.[2] Initiation of rHuEPO leads to reticulocytosis and the red blood cell (RBC) population is renewed by younger cells. Both phenomenona have been shown to increase %HYPO.[3]

In order to assess the influence of erythropoietic activity on red cell parameters, we studied the evolution of RBC and reticulocyte volume (MCV; MCVr), Hb content (MCH; MCHr), Hb concentration (MCHC; MCHCr) and %HYPO from the initiation of rHuEPO in 6 chronic hemodialysis patients, free of inflammation and transfusion. Erythropoietin was started intravenously at the dose of 200 IU/kg/week in three doses at the end of each dialysis session. To prevent iron deficiency, 100 mg iron sucrose (Venofer®) was administered once weekly from the start of rHuEPO therapy. Parameters were determined weekly for 11 weeks following initiation of rHuEPO treatment. Table 1 shows the comparison of parameters measured at the start and at the end of the follow-up. The Hb level rose from 8.3±1.2 to 12.1±0.6 g/dL. Serum transferrin receptors (sTfR) increased significantly (6.4±2.2 vs 3.4±1.5; p=0.003). Despite intravenous iron supplementation, ferritin (FRT) decreased by 50% (112.0±30.1 vs 214.2±130.5; p=NS) whereas transferrin saturation (TSAT) only decreased from 18 to 15% (p=NS). Whereas CHr remained constant and within the normal range, %HYPO was significantly higher at the end of the follow-up (7.2±4.1 vs 1.7 ± 0.8 %; p<0.05). An increased red cell volume (MCV) was observed (p<0.001), with a stable Hb content (MCH). The same evolution was noted for the reticulocyte parameters, i.e. a stable Hb content (MCHr), a non-significant trend towards an increased volume (MCVr) and a lower Hb concentration (MCHCr) (p<0.05). Figure 1 depicts the evolution of RBC and iron parameters over the whole period of observation. The changes in MCV and MCHC predominantly occurred during the first 4 weeks of rHuEPO therapy stabilizing thereafter.

Table 1. Comparison of parameters measured at baseline and the end of the follow-up.

Parameter	*Baseline (m±sd)*	*Week 11 (m±sd)*	*p value*
Hb (g/dL)	8.3±1.2	12.1±0.6	0.008
MCV (fL)	85.7±2.4	90.9±2.5	< 0.001
MCH (pg)	28.7±0.8	28.6± 1.8	0.89
MCHC (pg/mL)	33.7±0.6	32.3±0.5	0.14
%HYPO (%)	1.7±0.8	7.2±4.1	0.02
Retics (x1000/µL)	49.3±18.1	69.0±32.6	0.49
MCVr (fL)	107.8±2.5	112.3 ± 6.1	0.13
MCHr (pg)	32.4±0.7	31.8±2.1	0.56
MCHCr (pg/mL)	30.0±1.0	28.5±1.5	0.03
Ferritin (ng/mL)	214.2±130.5	112.0±30.1	0.13
TfSAT* (%)	18±3	15±3	0.20
sTfR°(mg/L)	3.4±1.5	6.6±2.2	0.003

**TfSAT: transferrin saturation; °sTfR: serum transferrin receptors.*

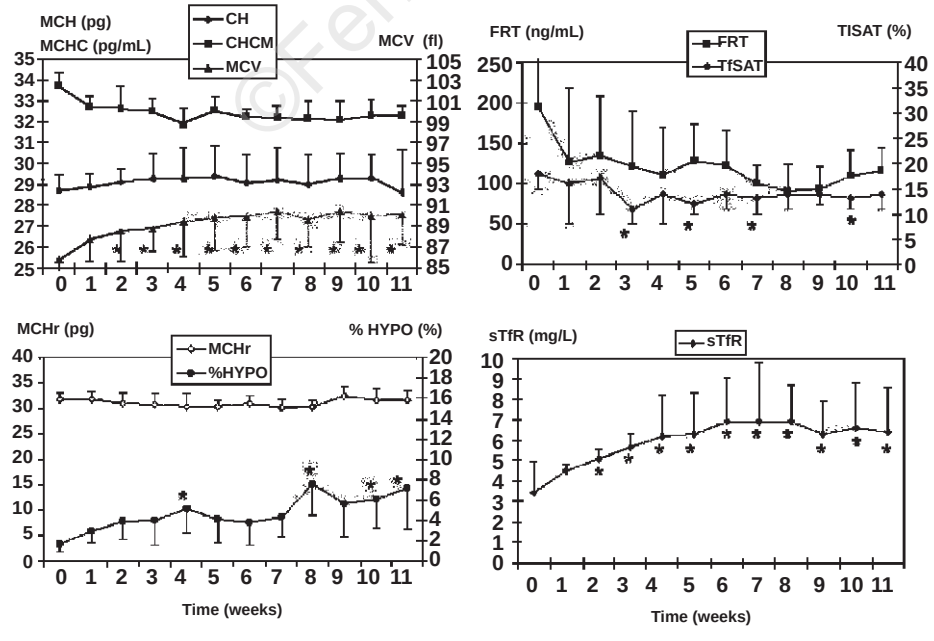

Figure 1. Evolution of red blood cell volume (MCV), hemoglobin content (MCH), hemoglobin concentration (MCHC), percentage of hypochromic red blood cells (% HYPO), reticulocyte hemoglobin content (MCHr), ferritin (FRT), transferrin saturation (TfSAT) and serum trasferrin receptors (sTfR) during the follow-up period. *p <0.05: versus baseline.

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The response to rHuEPO treatment is known to be dependent on adequate iron supplementation.[4,5] Intravenous iron administration can be associated with acute and long-term complications such as anaphylaxis, infection and oxidative damage. To provide the best risk-benefit ratio, reliable predictors of response to iron supplementation are needed. The %HYPO appears to fulfil this role[1] but is also influenced by both the reticulocyte count[2,3] and a younger age of the RBC population.[3] The specificity and sensitivity of other markers are not sufficient during rHuEPO therapy.[6] Ferritin and transferrin saturation usually decrease during rHuEPO treatment. However, these parameters have proven poor predictors of response to iron supplementation.[1] Discrepancies between ferritin and TSAT are usual during rHuEPO treatment.[7] sTfR increased progressively during the study, but this parameter is far more influenced by erythropoietic stimulation than by iron deficiency[8] With simultaneous administration of rHuEPO and iron, we observed that MCHr, an accurate early marker of iron-deficient anemia,[9,10] remained stable and normal, whereas %HYPO increased progressively. This cannot be explained by iron deficient erythropoiesis since MCHr and MCH remained normal throughout the follow-up. In fact, the explanation for this increase in %HYPO is a progressive decrease of erythrocyte Hb concentration due to increasing cell volume, mainly related to the appearance of a younger red cell population, including reticulocytes. This phenomenon was more important during the first 4 weeks of treatment since peak reticulocytosis occurred 1 week after rHuEPO initiation and the ratio between young and older RBC decreases as the anemia is corrected. A further increase in %HYPO was observed at the end of the follow-up. As transferrin saturation was below 20%, this could indicate a possible contribution from functional iron deficiency.

Acute erythropoietic stimulation, induced by rHuEPO, leads to reticulocytosis and renewal of the erythrocyte population leading to biased determination of red cell Hb concentration and percentage of hypochromic red blood cells. The specific determination of RBC parameters of mature erythrocytes, separately from those of reticulocytes, should provide a more accurate assessment of functional iron deficiency under conditions of increased erythropoietic activity.

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Chronic Myeloproliferative Disorders

Instability of PRV-1 mRNA: a factor to be considered in PRV-1 quantification for the diagnosis of polycythemia vera

High expression of PRV-1 mRNA in granulocytes has been proposed as a new diagnostic marker for polycythemia vera. We used real-time reverse transcription polymerase chain reaction (RT-PCR) to measure the levels of PRV-1 mRNA, GAPDH mRNA and 18S rRNA in granulocytes obtained from blood samples processed 2, 24 and 48 hours after collection and observed a significant decrease of PRV-1 levels after 24 and 48 hours. The instability of PRV-1 mRNA may affect the diagnostic value of the PRV-1 test in blood samples stored for extended periods.

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Polycythemia vera (PV) is the most common primary polycythemia[1] and is characterized by clonal expansion of myeloid cells from an acquired mutation of a single stem cell. The diagnosis of PV is based on the guidelines established by the Polycythemia Vera Study Group,[2] but atypical cases may not be discriminated readily by these criteria. Recently, it has been reported that levels of PRV-1 (polycythemia rubra vera-1) mRNA are increased in PV granulocytes from peripheral blood, but not in their progenitors.[3-5] While some investigators report a clear distinction between elevated PRV-1 mRNA in granulocytes of PV patients and low PRV-1 levels in normal controls,[6] we and others have found overlaps between the highest PRV-1 levels in healthy controls and the lowest levels in PV patients.[7-9] Further, the expression of PRV-1 mRNA could be modulated by cytokines, i.e. upregulated by granulocyte colony-stimulating factor[3] and downregulated by interferon-α treatment.[10] We hypothesized that PRV-1 mRNA in granulocytes might also be less stable than the RNA species used as internal references for PRV-1 quantification by real time RT-PCR and here we report on PRV-1 mRNA instability. We measured the levels of mRNA for PRV-1 in granulocytes from 10 normal controls and 3 PV patients. The study was approved by the Baylor College of Medicine (BCM) Istitutional Review Board; all studied subjects participated in the

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