New Predictive Protocol for Therapeutic Treatment of Renal and Nonrenal Anemias with Recombinant Human Erythropoietin with a Simple Immunoenzymatic Dosage of Serum Burst-Promoting Activity (IL-3, IL-4, GM-CSF)

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Dear Sir,

It is known that some patients with anemia of end-stage renal disease (ESRD) have reduced response to recombinant human erythropoietin (rHu-EPO) not due to infections, increased occult blood losses, iron deficiency or other known causes of rHu-EPO resistance [1]. From the hematological point of view, colony-forming unit erythroids (CFU-E) are totally sensitive to rHu-EPO, but the more immature burst-forming unit erythroids (BFU-E) are responsive only to very high doses of rHu-EPO because they need other growth factors like Interleukin-3 (IL-3) [2, 3], Interleukin-4 (IL-4) and granulocyte macrophage colony-stimulating factor (GM-CSF) [4], a complex denominated burst-promoting activity (BPA).

So we can hypothesize that patients with low levels of BPA (low IL-3, IL-4 and GM-CSF) have deficient growth of BFU-E colonies which are not sufficiently mature to pass to the successive step of CFU-E and, consequently, need higher doses of rHu-EPO. The same problem may be involved in the mechanism of rHu-EPO resistance in patients with nonrenal anemias like aplastic anemias, myelodysplastic syndromes, refractory anemias, Fanconi anemia, Blackfan-Diamond anemia, anemias of myeloproliferative diseases. To confirm such hypothesis, 18 patients under treatment with rHu-EPO for ESRD

anemia were investigated before and after rHu-EPO therapy (Erytrogen, Boehringer Mannheim, Italy). All the laboratory data were collected before and after 6 months of rHu-EPO therapy. Hb, red blood cell count (RBC), white blood cell count (WBC), platelets (PT) and hematocrit (Hct) were determined with Techicon HI, USA.

IL-3, IL-4 and GM-CSF were determined with immunoenzymatic assays (IL-3 Quanti-kine Elisa Kit R and D Systems-USA, Inter-test-4 tm Human IL-4 Elisa Kit, Genzyme-USA,

Factor Test tm Human GM-CSF Elisa Kit, Genzyme-USA), EPO was detected with EPO-Elisa, Boehringer Mannheim.

At the beginning of the study, laboratory data showed the following values (mean $\pm$ SD): Hb = 6.8 ± 0.8 g/dl; Hct = $20.7 \pm 2.1\%$; Pt = 151.3 ± 510.0 fOVmm³; WBC = $4,108 \pm 1,301.3$ fOVmm³; RBC = $2,380 \pm 501.4$ 103/ mm³; IL-3 = 497.3 ± 292 pg/ml; IL-4 = 0.592 ± 0.88 ng/ml; GM-CSF = 53.72 ± 34.0 pg/ml.

After rHu-EPO therapy, at the end of the study, there were significant differences in mean values $\pm$ SD of Hb (9.67 ± 1.1 g/dl; $p = 0.001$), Hct ($32.1 \pm 2.6\%$; $p = 0.001$), RBC ($3,188 \pm 158$ fOVmm³; $p = 0.001$). There were no significant differences between the values of PT, WBC, IL-3, IL-4 and GM-CSF at the beginning and at the end of the study.

To reach target Hb (9.5-10.5 g/dl), patients needed different rHu-EPO doses (from 35 to 120 U/kg b.w. 3 times/week intravenously).

Laboratory levels of IL-3, IL-4 and GM-CSF were inversely correlated with rHu-EPO doses. So, patients with high levels of IL-3 (> 600 pg/ml), IL-4 (> 0.60 ng/ml) and GM-CSF (> 50 pg/ml) responded to lower doses (< 70 U/kg b.w. 3 times/week) of rHu-EPO to reach Hb peak; on the contrary, patients with lower levels of IL-3 (< 600 pg/ml), IL-4 (< 0.60 ng/ml) and GM-CSF (< 50 pg/ml) responded to higher doses (> 70 U/kg b.w. 3 times/week).

Only 2 patients did not show this correlation. IL-3 values were better correlated with our hypothesis.

Reported data suggest that in anemia of ESRD, moderate lack of BPA (IL-3, IL-4, GM-CSF) can produce moderate EPO resistance, because BFU-E colonies are not sufficiently promoted by these growth factors and consequently not so able to pass to the successive step of CFU-E colonies, the only ones totally rHu-EPO responsive. At the moment, there are only very few reports with experimental 'in vitro' data about lack of BPA, IL-3, GM-CSF in patients with anemia of ESRD [5-7]. However, in anemia of ESRD, the problem of EPO resistance

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seems to be relative and easily corrected by increasing rHu-EPO doses. Some authors prospect the use of IL-3 in such patients, but we remember that the actual use of IL-3 is problematic: so also in Blackfan-Diamond anemia (caused by a presumable lack of IL-3), it is preferable in the affected children to use blood transfusions, because IL-3-treated patients often get quite sick with fevers [8,9].

On the other side, we know that many nonrenal anemias, like aplastic anemias, myelodysplastic syndromes, Fanconi anemia, anemia of myeloproliferative syndromes, are rarely responsive to rHu-EPO.

Chemotherapy-induced anemia and anemia of malignancy (particularly of multiple myeloma) seem to respond better to rHu-EPO.

This very important clinical and therapeutical problem may be solved using also other growth factors like GM-CSF which seems to have a synergistic effect with rHu-EPO (there is not yet a big experience with IL-3 or IL-6).

In conclusion, our predictive protocol with immunoenzymatic Elisa dosage of the lacking growth factors, possibly using other laboratory techniques like soluble transfer-rin receptors together with EPO observed/ predicted ratio, could be useful also in the treatment of nonrenal

anemias for a better use of rHu-EPO and eventually of the other growth factors which can cooperate with rHu-EPO if used in the so selected patients.

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