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PREDICTION OF RESPONSE TO RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEpo) IN ANEMIA OF MALIGNANCY

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ABSTRACT

Background. **Since only a portion of anemic patients outside the uremia setting benefit from erythropoietin treatment, a reliable means of predicting potential responders and nonresponders would be very useful.**

Materials and Methods. **We retrospectively reviewed the clinical records of 58 patients with refractory anemia associated with various malignant disorders who had been treated with subcutaneous rHuEpo. The starting rHuEpo dose was 375 U/kg/week for 4 weeks, and was increased to 750 U/kg/week for another 4 weeks if no response was observed. Response was defined as a Hb increase ≥ 2 g/dL with no need for blood transfusion. We examined the value of various laboratory parameters (baseline levels, 2-week and 4-week changes) as predictors of response. Endogenous erythropoietin production was evaluated by its serum level and erythroid activity was assessed through reticulocyte count and circulating transferrin receptor.**

Results. **Forty-eight individuals were evaluable, 58% of whom responded to rHuEpo within 8 weeks. Multiple regression analysis showed that 53% of the variation in the 8-week Hb concentration was explained by variations in baseline serum erythropoietin and the 2-week change in serum transferrin receptor ($p < 0.001$). Based on these two parameters, response prediction in individual patients would have resulted in a sensitivity of 96%, a specificity of 79% and an overall accuracy of 88%. In addition, 58% of the variation in the 8-week Hb was explained by variations in the 4-week changes in Hb and reticulocyte count ($p < 0.001$). Utilizing these latter parameters and baseline serum erythropoietin, response prediction in individual patients would have resulted in a sensitivity of 92%, a specificity of 82% and an overall accuracy of 88%.**

Conclusions. **This retrospective analysis suggests that response to rHuEpo can be reasonably predicted by pretreatment serum erythropoietin together with early changes in simple laboratory parameters.**

Key words: anemia, erythropoietin, prediction, reticulocyte, transferrin receptor

Over 300,000 patients throughout the world are now receiving recombinant human erythropoietin (rHuEpo) for the treatment of anemia of renal failure, and more than 95% of them respond to rHuEpo if adequate doses are given.[1] Treatment is cost-effective to the extent that a standard maintenance dose of ≤ 100 U/kg/week abolishes a transfusion requirement of 2-3 units of blood per month and improves the quality of life in severely anemic patients.

Outside the uremia setting only a portion of anemic patients benefit from rHuEpo treatment.[2,3] Responsive individuals range from about 16% in myelodysplastic syndromes to nearly 70% in multiple myeloma, and there is a

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relationship between defective endogenous erythropoietin production and the likelihood of response to rHuEpo.[3]

Recombinant erythropoietin is an expensive drug and cost containment is a major issue in health care policy today. A reliable means of predicting potential responders and nonresponders would be clinically useful in nonrenal conditions. Therefore we retrospectively reviewed the clinical records of 58 neoplastic patients treated with rHuEpo. The aim of this work was to identify predictors of response and define a patient-oriented rather than a disease-oriented approach to rHuEpo treatment of nonrenal anemia.

Patients and Methods

Patients

The use of rHuEpo in the treatment of refractory anemia associated with hematological disorders and solid tumors was approved by the ethical committee of the Department of Internal Medicine and Medical Therapy, University of Pavia Medical School, Pavia, Italy. All patients gave written informed consent.

We delivered rHuEpo to 58 consecutive subjects with refractory anemia associated with various disorders and Hb levels < 10 g/dL. Nine patients suffered from myelodysplastic syndrome (MDS), 18 from multiple myeloma, 11 from non-Hodgkin's lymphoma, and 20 from solid tumors.

The rHuEpo used in the study was provided by Boehringer Mannheim GmbH, Mannheim, Germany. The treatment protocol was a modification of that previously adopted in a phase I-II clinical trial.[4,5] The drug was administered subcutaneously once a day, 5 d per week (Monday to Friday) on an outpatient basis; it was self-administered or given by relatives or private nurses. The starting rHuEpo dose was 375 U/kg/week for 4 weeks, and was increased to 750 U/kg/week for another 4 weeks if no response was observed. In each case dosage was adjusted so as to allow the optimal utilization of each single vial in order not to waste the product. Response was defined as a Hb increase ≥ 2 g/dL with no need for blood transfusion.

Laboratory investigations

Complete blood counts were performed weekly. Reticulocyte count was corrected to account for anemia,[4] and body iron status was evaluated by measuring serum iron, transferrin saturation and serum ferritin.[5] Oral iron supplementation (ferrous iron sulphate, 100 mg/day) was given routinely except to patients whose baseline serum iron was > 150 µg/dL and/or transferrin saturation > 60%.

Circulating erythropoietin levels were measured by a commercially available radioimmunoassay.[6] To define erythropoietin levels as appropriate or inappropriate for a given degree of anemia, an exponential regression of serum erythropoietin versus Hct was determined in reference subjects and the 95% confidence limits were defined.[6] Based on this regression, the observed/predicted log(epo) ratio (O/P ratio) was derived for each sample. The O/P ratio averaged 1.00±0.11 in reference subjects (95% confidence interval: 0.80-1.19).

Serum transferrin receptor (TfR), the level of which provides an estimate of erythroid marrow activity, was measured by an enzyme-linked polyclonal antibody assay.[6] TfR levels in 165 normal control subjects were 5.0±1.1 mg/L, with 95% confidence limits ranging from 2.9 to 7.1 mg/L.

Statistical analysis

Data were stored, analyzed and reported with the packages Statistica/Mac (StatSoft.™, Tulsa, OK), Exstatix™ (Select Micro Systems Inc., Yorktown Heights, NY) and DeltaGraph™ Pro 3 (DeltaPoint Inc., Monterey, CA), all run on a Macintosh Quadra 650 (Apple Computer Inc., Cupertino, CA) personal computer. Results were expressed as mean±1 standard deviation (SD) unless otherwise stated. The Student's t-test and/or the F test (analysis of variance) were used to evaluate the probability of any significant difference between groups. Chi-square analysis was utilized to determine whether classifications of two different parameters were related or not. Multiple regression analysis was used to study the value of various laboratory parameters (baseline levels, 2-week and 4-week changes) as predictors of response to rHuEpo.

Definitions

The following definitions were adopted in relation to response prediction:

$$\textit{sensitivity} = \frac{\text{true-positive predictions}}{\text{all patients who would have been treated}}$$

$$\textit{specificity} = \frac{\text{true-negative predictions}}{\text{all patients who would not have been treated}}$$

$$\textit{overall accuracy} = \frac{\text{true-positive + true-negative predictions}}{\text{all patients}}$$

$$\textit{positive predictive value} = \frac{\text{true-positive predictions}}{\text{true + false positive predictions}}$$

$$\textit{negative predictive value} = \frac{\text{true-negative predictions}}{\text{true + false negative predictions}}$$

Results

There were ten withdrawals, so that a total of 48 subjects were evaluable. Fifty-eight percent of patients (28/48) achieved the defined response within 8 weeks of treatment. Their clinical and laboratory characteristics are summarized in Table 1.

Endogenous erythropoietin production and response to rHuEpo

There was an inverse relationship between the 8-week ΔHb (where Δ indicates the change with respect to the baseline value) and the baseline serum erythropoietin O/P ratio ($r = -0.57$; $p < 0.001$) (Figure 1).

In addition, 25/31 patients with serum erythropoietin < 100 mU/mL responded, whereas only 3/17 with values ≥ 100 mU/mL did ($\chi^2 = 10.1$; $p < 0.01$).

Relationship between response and changes in Hb, reticulocyte count and serum transferrin receptor after 2 and 4 weeks

The Hb increment after 8 weeks was directly related to the 2-week change in TfR ($r = 0.68$; $p < 0.001$) (Figure 2); 24/26 patients showing a 2-week TfR increment ≥ 25% responded to rHuEpo within 8 weeks, whereas only 4/22 with a ΔTfR < 25% did ($\chi^2 = 16.3$; $p < 0.001$). Hb changes after 2 weeks were weakly related to the Hb increment after 8 weeks ($r = 0.38$; $p < 0.01$), whereas there was no relationship between this latter parameter and changes in reticulocyte count after 2 weeks.

The Hb increment after 8 weeks was very closely related not only to the 4-week change in TfR ($r = 0.70$; $p < 0.001$) but also to the 4-week change in reticulocyte count ($r = 0.70$; $p < 0.001$) and in Hb level ($r = 0.68$; $p < 0.001$) (Figure 3).

Multiple regression analysis for estimating the value of various laboratory parameters (baseline levels, 2-week and 4-week changes) as predictors of response to rHuEpo

Multiple regression analysis using baseline values and 2-week changes showed that the baseline O/P ratio and the 2-week change in TfR were independent predictors of response to

Condition (pt no.)	*Hb g/dL*	*Retic %*	*Serum iron µg/dL*	*Serum TfR mg/L*	*Serum Epo mU/mL*	*O/P ratio*	*Responders*
Myelodysplastic syndromes (n=9)	8.2± 1.3	0.9± 0.4	161± 52	3.39± 1.31	350± 423	1.00± 0.23	3/9
Multiplemyeloma (n=17)	8.7± 0.7	1.2± 0.6	99± 49	4.6± 2.7	95± 85	0.81± 0.18	11/17
Non-Hodgkin's lymphoma (n=8)	8.5± 1.1	1.5± 1.0	78± 33	6.0± 2.7	142± 104	0.90± 0.16	3/8
Solid tumors (n=14)	9.1± 0.8	1.5± 0.7	71± 36	4.8± 2.0	87± 94	0.82± 0.19	11/14

Table 1. Patient characteristics and response to rHuEpo (mean value ± 1 SD).

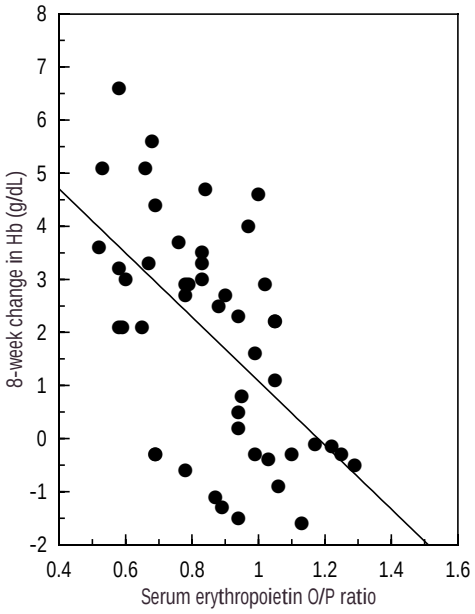

Figure 1. Relationship between the change in Hb after 8 weeks of rHuEpo treatment and the baseline serum erythropoietin O/P ratio.

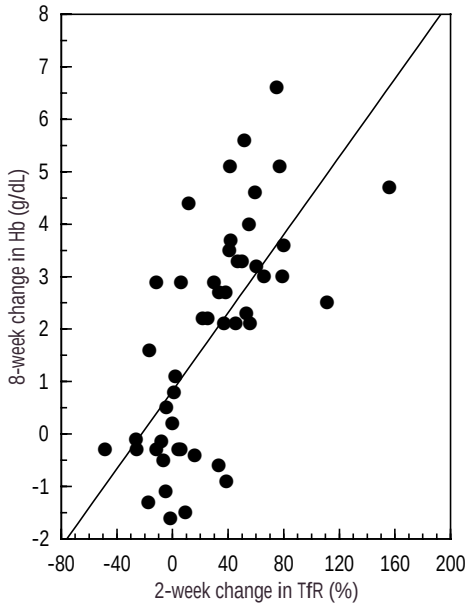

Figure 2. Relationship between the change in Hb after 8 weeks of rHuEpo treatment and the change in serum transferrin receptor (TfR) after 2 weeks.

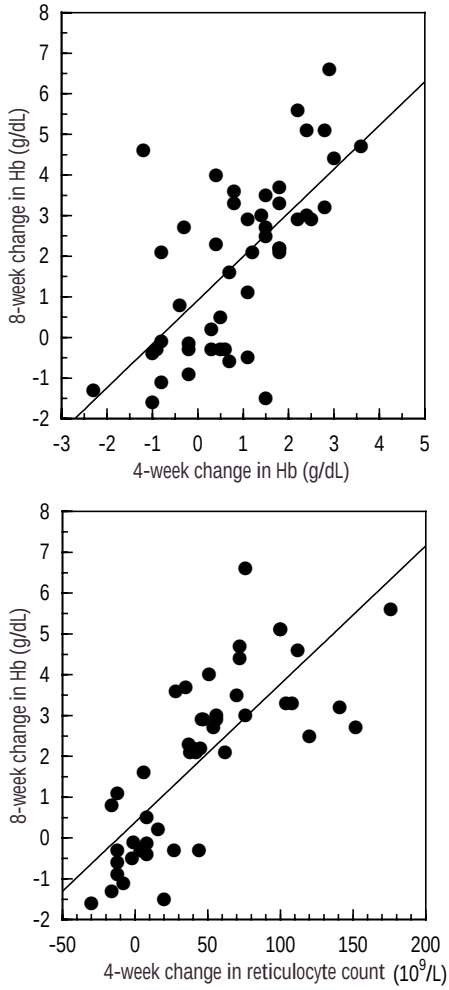

Figure 3. Relationship between the change in Hb after 8 weeks of rHuEpo treatment and the 4-week change in Hb level (top) or reticulocyte count (bottom).

rHuEpo. In fact, 53% of the variation in the 8-week change in Hb was explained by variations in these two parameters (adjusted multiple-r = 0.73; $p < 0.0001$).

Regression of 8-week ΔHb versus baseline values and 4-week changes showed that 58% of the variation in the 8-week change in Hb was explained by variations in the 4-week changes in reticulocyte count and Hb level (adjusted multiple-*r* = 0.75; $p < 0.0001$).

Algorithms for predicting response to rHuEpo

Two algorithms for predicting response to rHuEpo treatment in individual patients could

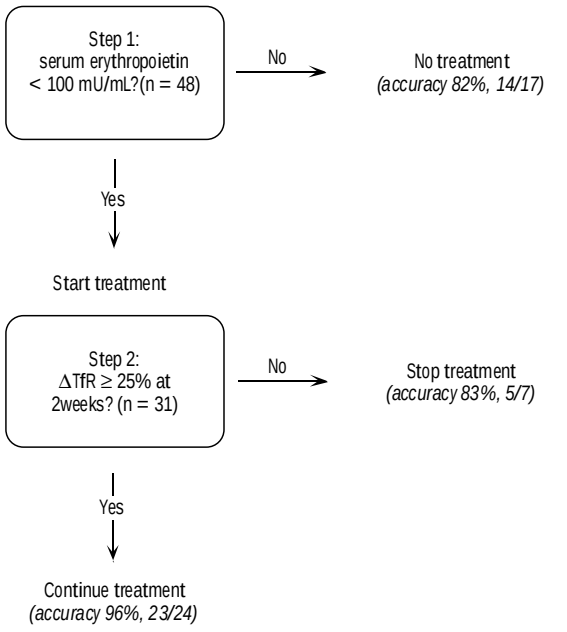

Figure 4. Algorithm to predict response to rHuEpo treatment in patients with anemia of malignancy based on serum erythropoietin and 2-week change in serum transferrin receptor (TfR).
Utilizing this algorithm would have resulted in a sensitivity of 96%, a specificity of 79% and an overall accuracy of 88%. Positive predictive value would have been 82% and negative predictive value 95%.

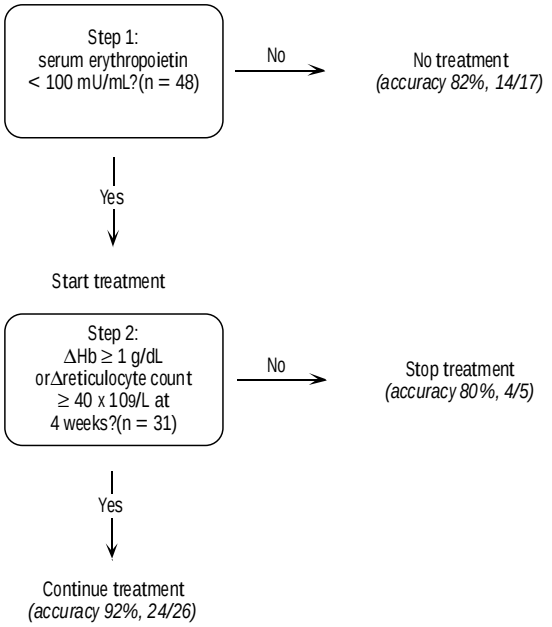

Figure 5. Algorithm to predict response to rHuEpo treatment in patients with anemia of malignancy based on serum erythropoietin and 4-week changes in Hb level and reticulocyte count.
Cutoffs for ΔHb and Δreticulocyte count were derived from analysis of data reported in Figure 3. Utilizing this algorithm would have resulted in a sensitivity of 92%, a specificity of 82% and an overall accuracy of 88%. Positive predictive value would have been 86% and negative predictive value 90%.

be elaborated.

The first one was developed by using the baseline serum erythropoietin, taken as an indicator of the adequacy of endogenous erythropoietin production, and the 2-week TfR increment, taken as an indicator of early response (Figure 4). Utilizing this algorithm would have resulted in a sensitivity of 96%, a specificity of 79% and an overall accuracy of 88%. Positive predictive value would have been 82% and negative predictive value 95%.

The second algorithm was developed by using the baseline serum erythropoietin, taken as an indicator of the adequacy of endogenous erythropoietin production, and the 4-week changes in Hb and reticulocytes, taken as indicators of early response (Figure 5). Utilizing this algorithm would have resulted in a sensitivity of 92%, a specificity of 82% and an overall accuracy of 88%. Positive predictive value would have been 86% and negative predictive value 90%.

It should be noted, however, that intensive chemotherapy may transiently elevate serum erythropoietin.[7] The predictive power of the above algorithms could therefore be altered in patients receiving chemotherapy immediately before and/or during the initial phase of rHuEpo treatment. Excluding individuals under cyclic chemotherapy would have raised both specificity and overall accuracy. For example, the accuracy of the first step in both predictive algorithms would have increased from 82 to 100%.

Discussion

In the present study, as well as in previous reports,[8-10] defective endogenous erythropoietin production was found to be a major predictor of response to rHuEpo. This observation is in keeping with the biology of erythropoietin, which expands erythropoiesis mainly by pre-

venting apoptosis of late erythroid progenitor cells and proerythroblasts.[11,12] The erythroid hormone is therefore unlikely to increase red cell production when endogenous production is physiologically elevated and nearly all available progenitors are already surviving and differentiating to immature red cells. By contrast, when endogenous erythropoietin levels are inappropriately low for the degree of anemia,[6] administration of pharmacological doses of erythropoietin may prevent apoptosis of considerable numbers of erythroid progenitors and increase red cell production.

Several reports point to the use of a serum erythropoietin threshold of ≤ 100 mU/mL to predict response to rHuEpo. In the study by Stenke *et al.*[8] on the use of rHuEpo in the treatment of myelodysplastic syndromes, all responders but one had serum erythropoietin levels lower than 100 mU/mL. Rose *et al.*[13] treated 100 MDS patients, 28% of whom responded to rHuEpo. Overall, 86% (24/28) of responders had baseline erythropoietin levels ≤100 mU/mL. Hellström-Lindberg[14] analyzed 205 MDS patients from 17 published reports; her meta-analysis confirms that baseline serum erythropoietin is crucial for response to rHuEpo, since no patient with serum levels > 200 mU/mL benefited from rHuEpo. Cazzola *et al.*[10] used rHuEpo in the treatment of anemia associated with multiple myeloma and non-Hodgkin's lymphoma. In patients receiving 500 U/kg/week or more, baseline serum erythropoietin proved to be the best predictor of response; about three quarters of subjects with levels ≤ 100 mU/mL responded to treatment, whereas less than one fifth of those with levels > 100 mU/mL did so.

Although the use of the O/P ratio as a measure of the adequacy of endogenous erythropoietin production would have been more rigorous, adopting a cutoff for serum erythropoietin of 100 mU/mL in the first step of the predictive algorithm proved to be clinically useful, at least for patients with Hb levels < 10 g/dL (Figures 4 and 5). The positive predictive value of this simple parameter was 82%, but increased to 100% when those patients who had received chemotherapy immediately before measurement were excluded. This implies that serum erythropoietin should not be assayed immediately after administration of intensive chemotherapy. There are only a few exceptions to the rule of a low endogenous erythropoietin level as a prerequisite for response to rHuEpo, one being represented by patients suffering from pure red cell aplasia.[15]

Since some patients with serum erythropoietin < 100 mU/mL may nonetheless be unresponsive to rHuEpo (e.g. 6/21 in the present study and 37/72 in the report by Rose *et al.*[13]) there is a need for indicators of early response which allow clinicians to decide whether to continue or terminate rHuEpo therapy. The second parameter we employed to develop the first predictive algorithm (Figure 4) was the 2-week change in circulating transferrin receptor, taken as an indicator of early response. Serum transferrin receptor is a measure of total marrow erythropoietic activity.[4,6] Beguin *et al.*[16] already demonstrated the usefulness of this parameter for early prediction of response to rHuEpo in patients with anemia of renal failure. In their study, when the 2-week TfR increment was greater than 20%, the response rate was 87%; when the TfR increment was less than 20%, the response rate was 48%. In the present study, the change in serum transferrin receptor after 2 weeks proved to be a valuable factor for predicting response to rHuEpo. Only one out of the 23 patients showing a 2-week $\Delta TfR \geq 25\%$ failed to achieve the defined response.

Previous studies had documented that the change in Hb after 2 weeks was a powerful predictor of responsiveness to rHuEpo treatment;[9,10] however, this parameter may be useless in patients receiving transfusions and/or concomitant chemotherapy. Changes in the reticulocyte count after 2 weeks may simply reflect the output of shift reticulocytes and not a true expansion of erythropoiesis.[17] By contrast, a 4-week increment in reticulocyte count of $\geq 40 \times 10^9$/L was found to correlate positively with response to rHuEpo in cancer anemia.[18] Increments in high-fluorescence reticulocytes were found to predict response to rHuEpo in myelodysplastic syndromes.[19] In the present study, 4-week changes in both Hb level and reticulocyte count proved to correlate with response (Figure 3) and

Table 2. Factors predicting response to rHuEpo in nonrenal anemia that may be employed in a predictive algorithm for a patient-oriented approach to treatment.

Factor	*References*
Serum Epo < 100 mU/mL	Stenke et al, 1993 (myelodysplastic syndromes) Rose et al, 1995 (myelodysplastic syndromes) Henry et al, 1995 (cancer anemia) Cazzola et al, 1995 (multiple myeloma, non-Hodgkin lymphoma) Present study on anemia of malignancy
2-week change in Hb level	≥ 0.5 g/dL, Ludwig et al, 1994 (cancer anemia) ≥ 0.5 g/dL, Henry et al, 1995 (cancer anemia) ≥ 0.3 g/dL Cazzola et al, 1995 (multiple myeloma, non-Hodgkin lymphoma)
2-week change in TfR	≥ 25% basal level, present study on nonrenal anemia
4-week change in Hb level	≥ 1.0 g/dL, Henry et al, 1995 (cancer anemia) ≥ 1.0 g/dL, present study on anemia of malignancy
4-week change in reticulocyte count	≥ 40 x 10^9/L, Henry et al, 1995 (cancer anemia)

to be useful for developing a simple predictive algorithm (Figure 5). Although changes in serum transferrin receptor assay appear to be the best indicator of response to rHuEpo after 2 weeks, changes in hemoglobin and reticulocyte count after 4 weeks seem to be equally useful. Unlike the TfR assay, these latter parameters are widely available and this may justify two additional weeks of treatment; one full month of rHuEpo treatment may also be more reasonable in the patient's eyes.

Erythropoietin is currently approved only for specific conditions, but one of the duties of a physician is to be able to move from public policy implemented for the average patient to one that serves the individual patient's needs.[3] We believe that rHuEpo should be provided to all patients who can benefit from it, regardless of whether the basic disorder is an approved condition or not. As summarized in Table 2, a number of factors predicting response to rHuEpo have now been identified. Utilizing predictive algorithms may allow a patient-oriented rather than a disease-oriented approach to rHuEpo treatment of nonrenal anemia. Clearly these algorithms need to be refined (for example, the timing of both treatment and laboratory investigations with respect to chemotherapy is critical) and validated in future prospective studies.

While discussing this work with colleagues, some of them looked at our algorithms with a certain disdain. We realize that many physicians will argue that these are just statistical values and that their own patients might be among the few who will respond despite all the negative predictions (Figures 4 and 5). Some of them will also state that they will not discontinue rHuEpo treatment in the individual patient until there is a definite lack of response. The question is: how long should treatment be continued before it can be decided with certainty that the individual patient is unresponsive? More generally, the problem can be seen from the patient's or the community's point of view. If the latter is to pay for the cost of treatment, it appears wise to adopt criteria that might penalize very few individuals but help many, many others.

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