Ratio of Baseline Erythropoietin (EPO) Level and Corrected Reticulocyte Count as an Indicator for a Favourable Response to Recombinant Human Erythropoietin (rhEPO) Therapy in Anaemic Cancer Patients

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Purpose: Recently, recombinant human erythropoietin (rhEPO) was introduced for the management of anemia in malignancy. To identify an indicator for a favourable response to rhEPO, 28 anaemic cancer patients undergoing chemotherapy and treated with rhEPO were evaluated. **Methods:** Patients were classified into responder (16 of 28, 57%) and nonresponder (12 of 28, 43%) groups according to their responses to rhEPO therapy (response being defined as an increase in Hb level of >2 g/dl from baseline without blood transfusion). **Results:** Treatment with rhEPO showed significant improvements in the red blood cell (RBC) count, haemoglobin (Hb),

packed cell volume (PCV), and reticulocyte count (ret. count) after 4 weeks. Upon analysing the baseline value of the EPO level and the corrected ret.count in these two groups, we found that the ratio of the EPO level and the corrected ret.count (EPO/ret.count) demonstrated a statistical significance ($P = 0.03$) in the prediction of response to rhEPO therapy. This ratio showed a sensitivity of 87.5%, specificity of 66.7%, and overall accuracy of 78.6%. **Conclusion:** Our study suggested that the baseline ratio of EPO/ret.count should be used as an indicator for a favourable response to rhEPO therapy. *J. Clin. Lab. Anal.* 15:260–266, 2001. © 2001 Wiley-Liss, Inc.

Key words: baseline EPO level; corrected reticulocyte count; erythropoietin therapy; anaemia in malignancy

INTRODUCTION

Anaemia is a common condition in cancer patients. It has been reported that as many as one-half of all cancer patients are anaemic (1). In general, it is usually mild to moderate in severity, with haemoglobin (Hb) values in the 8–10 g/dl range (1,2). The pathophysiology of anaemia in malignancy is complex and is not completely understood. It was reported to be similar to the anaemia of chronic diseases (ACD) (3), which is considered to be a part of the haematologic stress syndrome induced by the activation of mononuclear macrophages and T-cells (4). The condition is typically normochromic and normocytic, with decreased ret.count, erythroplasia in the bone marrow, low serum iron, low to normal serum iron binding capacity, normal to high serum ferritin, reduced number of sideroblasts, and low serum level of erythropoietin (EPO). The EPO level is inappropriately low in anaemic conditions (5–7). There are three important mechanisms with respect to these anaemias (8,9): 1) a mild decrease in RBC survival, 2) a decreased reutilization of bone marrow iron stores, and 3) an inadequate

EPO response to anaemia. Regarding the treatment of anaemia in cancer patients, reports have shown that the percentage of patients requiring transfusion ranges from 20% to 50% (10,11). Unfortunately, the transfusion of allogeneic blood exposed the recipient to immunological and infectious risks (12,13). These risk factors triggered the search for a clinically safer treatment. With the recent advances in recombinant DNA technology, a large-scale production of recombinant human EPO (rhEPO) has become available. An in vitro study demonstrated a normal response of the erythroid progenitor cells from anaemic cancer patients to EPO (14).

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To evaluate and identify the indicator for a response to rhEPO in anaemic cancer patients undergoing chemotherapy, 28 cancer patients with anaemia treated with rhEPO were evaluated in our study.

MATERIAL AND METHODS

Protocol Design and Patient Selection

The inclusion criteria of anaemic cancer patients in our study were: 1) nonleukemic malignancy; 2) haemoglobin ≤ 10.5 g/dl; 3) patients were still undergoing chemotherapy; 4) no previous history of rhEPO administration; 5) life expectancy of 6 months or more; and 6) informed consent. The exclusion criteria were: 1) uncontrolled hypertension; 2) hypersensitivity to mammalian cell-derived products; and 3) anaemia due to factors other than cancer/chemotherapy, i.e., iron deficiency, folate deficiency, B12 deficiency, haemolysis, and gastrointestinal bleeding. Those recruited into the study were treated with subcutaneous rhEPO, 150 units/kg, three times a week for 16 weeks. Oral iron supplementation (ferrous sulphate, 100 mg/day) was given routinely to guarantee a sufficient body iron store. Furthermore, to rule out iron deficiency we analysed serum concentrations of iron, ferritin, and iron-binding capacity weekly. If the response to rhEPO was not satisfactory in terms of reducing transfusion requirements or increasing packed cell volume (PCV) after 8 weeks of therapy, the dosage of rhEPO was increased up to 300 units/kg, three times a week. The objective response was defined as an increase in haemoglobin concentration of at least 2 g/dl within 12 weeks, or an increase in PCV by more than 0.06 in 12 weeks. The patients were then followed up weekly with examinations of haemoglobin, PCV, and reticulocytes. The administration of the rhEPO was maintained to sustain the PCV value of 0.36–0.40, i.e., if the PCV reached 0.40, the rhEPO was withdrawn until the PCV fell below 0.36. If the initial dos-

age of the rhEPO resulted in a very rapid PCV response (e.g., an increase of more than 0.04 points in any 2-week period), the dosage of the rhEPO was reduced. Side effects were recorded accordingly.

Clinical and Laboratory Studies

A complete history was taken, and physical and laboratory examinations were performed. The laboratory examination included a complete blood count (CBC), RBC count, platelet count, ret.count, EPO level, serum iron, total iron binding capacity, serum ferritin, folic acid, B12, Coomb's test, stool haematest, and prothrombin time prior to the rhEPO administration. EPO levels were measured by enzyme-linked immunosorbent assay (ELISA) (Quantikine IVDTM human EPO immunoassay, R&D Systems, Inc., Minneapolis, MN). After the rhEPO therapy, a CBC and reticulocyte study were followed up weekly for 16 weeks.

Statistical Analysis

Group data are expressed as mean $\pm$ standard deviation (SD). Paired Student's *t*-tests were used to compare data within each group, and unpaired Student's *t*-tests were used to compare data between groups. A *P* value ≤ 0.05 was considered to be statistically significant.

The definitions below were adopted in relation to response prediction.

RESULTS

We recruited 39 anaemic cancer patients who were receiving chemotherapy at King Chulalongkorn Memorial Hospital. Eleven of the 39 patients were excluded from the study (seven patients were lost from follow-up, three patients requested transfers to other hospitals, and one patient refused further chemotherapy). Twenty-eight patients (Table 1) were

$$\text{Sensitivity} = \frac{\text{true positive predictions}}{\text{All responded patients}}$$

$$\text{Specificity} = \frac{\text{true negative predictions}}{\text{All non-succeeded patients}}$$

$$\text{Overall accuracy} = \frac{\text{true positive predictions} + \text{true negative predictions}}{\text{All patients}}$$

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

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

TABLE 1. Characteristics of the patients

	Responder group		Non-responder group	
Number	16		12	
Age (year); average (range)	52.2 (25–70)		54.8 (38–69)	
Sex; male:female	7:9		6:6	
Diagnosis	NSCLC	4	NSCLC	4
	SCLC	1	NPC	2
	NPC	3	CA colon	1
	CA colon	3	CA breast	1
	CA breast	1	Hepatoma	1
	CA trachea	1	CA kidney	1
	Hepatoma	1	CA stomach	1
	CA kidney	1	Angiosarcoma	1
	Embryonal carcinoma	1		
Chemotherapy	Cisplatin/5FU	2	Cisplatin/5FU	1
	Cisplatin/VP-16	2	Cisplatin/UFT	1
	Cisplatin/etoposide	2	Taxol/carboplatin	2
	Cisplatin/taxol	1	Taxol	1
	Cisplatin/taxol/ ifosfamide	1	Ifosfamide/ VP-16/TOCE	1
	Cisplatin/etoposide/ ifosfamide	1	UFT/5FU/intron A	1
	Cisplatin/mitomycin/ vinblastin	1	5FU/intron A/aredia	2
	5FU/intron A	1	5FU/intron A	1
	UFT/leucovarin	1	UFT/leucovarin	1
	CPT 11	1	Radiation/VP-16	1
	5FU/leucovarin	1		
	Ifosfamide	1		
	Gemzar	1		

NSCLC, non small cell lung carcinoma; SCLC, small cell lung carcinoma; NPC, naso-pharyngeal cancer.

included in the study, and any symptom occurring during rhEPO treatment was considered to be potentially related to the administration of the drug. Among these 28 patients, 12 were not able to receive the complete regimen and rhEPO treatment was discontinued (one patient developed hypertension, one had bone pain, two had infections, one required blood transfusion, four did not respond to the rhEPO treatment, and three succumbed to their diseases). Sixteen patients responded to the rhEPO therapy without any complication.

Haematological Parameters in Response to Administration of rhEPO

We followed the pattern of the haematological parameters (RBC, Hb, PCV, percentage and absolute ret.count, and EPO level) in these patients from the first visit before and after the weekly rhEPO administration up to the fourth week. There were changes in all of the haematological parameters in response to the administration of rhEPO. The results demonstrated the direct effect of rhEPO on all the parameters, except the percentage of the ret.count. Owing to the small number of data, we could not demonstrate the correlation between the EPO level and the haematological parameters.

Effect of rhEPO on Haematological Parameters in the Responder Group

Effect of rhEPO on RBC, Hb, and PCV

In our study, there were statistically significant increments of RBC, Hb, and PCV before, during, and after the first week and also before, during, and after the 16th week of rhEPO therapy (Fig. 1). However, we could not demonstrate statistically significant increments of RBC, Hb, and PCV at any other time point of therapy. This could be explained by the variation in the rhEPO dosages required by each patient. There were only five patients in whom the dosage could be maintained. The dosage needed to be reduced by 25% at the 12th week in one patient. In another patient some dosages needed to be withdrawn during the 4th week. Some dosages of rhEPO were withdrawn at the 8th, 12th, and 16th week in another five patients. However, the dosages of rhEPO were increased to 300 units/kg body weight at the 8th week in two patients.

Effect on MCV, MCH, MCHC, and RDW

The four RBC parameters—mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and RBC distribution width (RDW)—were studied. There were no significant changes at any time in terms of MCHC and RDW before, during, and after rhEPO therapy (Fig. 1). In contrast, there were significant differences in MCV and MCH before, during, and after the first week of rhEPO therapy. These results could represent the premature stimulation of the erythropoiesis with early release of the reticulocytes from the bone marrow. These results also showed that the rhEPO could effectively stimulate the bone marrow to produce RBC without any morphological defect.

Effect on the ret.count

Our study showed there was a trend toward increments in the corrected percentage ret.count and the absolute ret.count but not in the percentage ret.count per se (Fig. 1) in every patient, especially during the first week of therapy. These results suggested that there was a tendency toward a positive response in the beginning of the therapy, and this positive effect continued even though a different dosage of the rhEPO was required to maintain the response.

Effect of rhEPO on Haematological Parameters in the Nonresponder Group

The effects on the haematological parameters (RBC, Hb, PCV, MCV, MCHC, RDW, and ret.count) were all the same as in the responder group (Fig. 1). These data suggested that there was no difference in the effect of rhEPO between the responder and nonresponder groups, as demonstrated by the improvement in all of the haematological parameters. How-

Fig. 1. Change of hematological parameters (mean) at different times before and after the rhEPO treatment at the 4th, 8th, 12th, and 16th weeks in the responder (closed symbols and lines) and nonresponder (open symbols and

lines) groups. **a:** RBC, PCV, ret.count, and corrected ret.count. **b:** Hb, RDW, MCH, and MCHC. **c:** MCV and absolute ret.count.

ever, in this group there was no significant difference before and after the rhEPO therapy.

Comparative Study Between the Responder and Nonresponder Groups

We found that the RBC, Hb, PCV, and corrected and absolute ret.count were higher in the responder group compared

to the nonresponder group. Conversely, the baseline EPO level was higher in the nonresponder group (Table 2). These findings indicated a poorer outcome if the baseline EPO level was high. These parameters might be used as criteria for the selection of anaemic cancer patients to receive the rhEPO treatment. However, our study did not demonstrate that any single parameter could be used as an indicator of a positive

Fig. 1. Continued.

response to the rhEPO therapy. We did find that the ratio between the EPO level and the corrected ret.count or the absolute ret.count could be used as a positive indicator for rhEPO therapy ($P = 0.03$, Table 2 and Fig. 2).

DISCUSSION

In this study, the effect of rhEPO on haematological parameters has been examined in patients with solid tumours

who developed anaemia induced by chemotherapy, radiation therapy, or a combination of both. From the results, the patients were classified into two groups, responder and nonresponder, according to their responses to the rhEPO therapy ("response" being defined as an increase in Hb level of >2 g/dl from baseline without blood transfusion). There were 16 patients in the responder group and 12 patients in the nonresponder group.

In the responder group, we found the average RBC, Hb, and PCV improved significantly at the 4th, 8th, and 16th weeks after starting the rhEPO therapy. For the corrected and absolute ret.count, the improvement was significant at the 4th and 16th weeks of rhEPO therapy. In the nonresponder group, the average RBC, Hb, and PCV improved at the 4th and 16th weeks. For the corrected and absolute ret.counts, the improvement was significant at the 4th week. These results showed that the haematological parameters were improved significantly after the 4th week of rhEPO therapy. At any time earlier, there was no significant improvement (data not shown).

In erythropoiesis, human burst-forming unit erythroid (BFU-E) requires at least 2 weeks to develop into clusters of hundreds to thousands of mature erythroblasts, then another 1–2 weeks are required for the erythroblasts to develop into mature erythrocytes (15). This could be the reason for the 4-week delay in response after starting the rhEPO therapy. An increase in the Hb level of >10 g/dl was seen in 13 of the 16 patients (81%) in the responder group, and in five of the 12 patients (42%) in the nonresponder group.

In patients with malignancy, treatment of the underlying neoplasms is the priority. Anaemia once developed can be treated by rhEPO to increase the circulating RBCs. The dosage of 150–300 units/kg body weight, subcutaneously, three times a week, is appropriate. This dosage is somewhat larger

TABLE 2. Comparison of haematological parameters and EPO level (mean $\pm$ SD) at baselines between the responder and the non-responder group

Parameters	Responder (n=16)	Non-responder (n=12)	P-value
RBC ($\times 10^{12}/L$)	3.30 (± 0.46)	3.08 (± 0.47)	0.23
Hb (g/dl)	9.2 (± 0.81)	8.7 (± 1.28)	0.25
PCV (decimal fraction)	0.28 (± 0.02)	0.26 (± 0.04)	0.16
Reticulocyte count (%)	3.1 (± 1.31)	0.1 (± 2.00)	0.33
Corrected reticulocyte count (%)	1.9 (± 0.75)	1.4 (± 0.92)	0.09
Absolute reiculocyte count ($\times 10^9/L$)	99.70 (± 37.34)	69.96 (± 46.87)	0.09
EPO level (mIU/ml)	42.80 (± 59.02)	97.26 (± 87.41)	0.08
Absolute reticulocyte count/EPO level	7.10 (± 10.87)	1.29 (± 1.30)	0.05 ^a
Corrected reticulocyte count/EPO level	0.13 (± 0.19)	0.02 (± 0.03)	0.04 ^a
EPO level/Absolute reticulocyte count	0.42 (± 0.43)	2.38 (± 2.74)	0.03 ^a
EPO level/Corrected reticulocyte count	21.73 (± 19.78)	127.18 (± 141.02)	0.03 ^a

^aStatistical significance ≤ 0.05 .

Fig. 2. Comparison of the ratio of baseline EPO level and ret.count. **a:** absolute ret.count/EPO level. **b:** corrected ret.count/EPO level. **c:** EPO level/

absolute ret.count. **d:** EPO level/corrected ret.count (each data point is represented by an open circle; mean is represented by a closed square).

than for anaemia in renal disease because of the decrease in bone marrow responsiveness to rhEPO in cancer patients (16). Our results, as well as those of several other reports (17–19), demonstrate that the defective production of endogenous EPO is a major factor in predicting response to rhEPO therapy. EPO expands erythropoiesis mainly by preventing apoptosis of the late erythroid progenitor cells and proerythroblasts (20,21). This hormone is therefore unlikely to increase RBC production when the endogenous production is physiologically elevated and nearly all the available progenitors are already surviving and differentiating into mature RBCs. In contrast, when the endogenous EPO level is inappropriately low for the degree of anaemia (22), administration of a phar-

macological dosage of EPO will prevent the apoptosis of a considerable number of erythroid progenitors, resulting in an increase in RBC production. In agreement with this, our data showed that the response to rhEPO therapy among patients with higher baseline EPO levels (97.26 mIU/mL) was less dramatic than among patients with lower baseline EPO levels (42.80 mIU/mL). Several reports suggested the use of a serum EPO threshold of <100 mIU/mL to indicate a good response to the rhEPO therapy (23). In our patients with solid tumours, the endogenous EPO levels were generally lower than the levels in patients suffering from haematologic problems, including myelodysplastic syndrome (MSD) and multiple myeloma (23). This could explain a better response to

rhEPO therapy in our study compared to the study in haematologic malignancies. While elevated baseline EPO levels of >100 mIU/mL have been regarded as an indicator of poor response to rhEPO (18,23), our findings showed that this particular threshold might not be appropriate in patients with solid tumours.

Our data showed there was a significant difference ($P = 0.03$) regarding the baseline values of EPO level in relation to the corrected ret.count (EPO/ret.count) between the responder and nonresponder groups. This ratio could be used to predict a favourable response in patients with a sensitivity of 87.5%, specificity of 66.7%, overall accuracy of 78.6%, positive predictive value of 77.8%, and negative predictive value of 80.0%.

In conclusion, we propose the use of the EPO/ret.count as an indicator for a favourable response to rhEPO therapy. Since our study includes only a small number of patients, a multicenter study is required to verify our findings.

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