CLINICAL INDICATIONS FOR MEASURING ENDogenous ERYTHROPOIETIN LEVELS

Critical Review
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Spring 1990

Introduction

Human erythropoietin (EPO) is an acidic 165 amino acid glycoprotein, weighing 30,400 daltons which induces red blood cell production by stimulating proliferation, differentiation, and maturation of erythroid precursors (1). It is produced predominantly in the kidney, probably in peritubular interstitial cells and/or endothelial cells in the renal cortex, and to a lesser extent in the liver (2). Hypoxia is the usual stimulus for the production of EPO. EPO reacts with receptors on BFU-E and CFU-E, stimulates terminal differentiation, increases hemoglobin synthesis, and shifts marrow reticulocytes into the circulation.

Normal, non-anemic individuals will have serum EPO levels generally ranging from about 5 to 20 mU/ml (2,3,4). Anemic patients may have either elevated or depressed levels of EPO. In conditions of marrow hyporesponsiveness, as in iron deficiency anemia or aplastic anemia, EPO levels will be elevated. In conditions such as chronic renal failure where there is an inability to produce EPO secondary to lack of renal tissue, EPO levels will be decreased. In conditions such as AIDS (5) and anemias of chronic disease (6) the EPO levels may be low or elevated, but are often inadequately elevated for the degree of anemia.

Large quantities of human erythropoietin, suitable for therapeutic interventions, have recently become available because of the successful introduction of the EPO gene into mammalian cells (Chinese hamster ovary or CHO cells). This recombinant human erythropoietin (r-HuEPO) is produced and marketed by Amgen as "Epogen" and was recently approved by the Food and Drug Administration (FDA) for the treatment of anemia associated with chronic renal failure (7). A chemically identical product, "Eprex" is available from the Biotech Division of Ortho Pharmaceuticals in the U.S. on an investigational basis for the treatment of HIV-1 associated anemia.

Methodology for Measuring Erythropoietin Levels

Evaluation of erythropoietin levels in human plasma or serum had previously been used primarily as an aid in the diagnosis and classification of polycythemias and, to a much lesser degree, for classification of anemias (1). In polycythemia vera, for example, EPO levels are very low while in most other polycythemias, the EPO levels will be elevated. With the recent
availability of recombinant EPO for therapeutic use in anemias, the role of evaluation of endogenous EPO levels has become an issue. A variety of assays have been used over the years and the major ones will be briefly discussed, including the in vivo polycythemic mouse assay, in vitro cultures, radioimmunoassays, and enzyme linked immunosorbent assays (ELISA).

The activity of EPO has been standardized with reference to the First and Second International Reference Preparations (1,8). These standards are based on the in vivo erythropoietic activity of the cobaltous ion, CoC12, in the starved rat assay (9). One unit of EPO activity is defined as the equivalent erythropoietic effect of a 5uM dose of CoC12 in the starved rat assay.

Until about 1980, the most commonly used (1) and widely recognized reference EPO assay was the polycythemic mouse assay (1,10,11). In this assay, mice were made polycythemic by long term exposure to reduced oxygen tensions, resulting in compensatory polycythemia. When these mice were subsequently returned to normal atmospheric oxygen levels, EPO production was greatly reduced. The administration of exogenous EPO from human or other test samples then resulted in erythropoiesis, the activity of which could be measured by the uptake of radioactive iron (Fe). Although this assay is highly specific for EPO activity, it is relatively insensitive to normal or low levels of EPO, with a minimum detection level of about 50mU/ml (1,10,11). Additionally, it is cumbersome, expensive, time consuming, and not well suited to the needs of the clinical laboratory (10).

Various in vitro culture assays measuring erythroid colony formation, heme or hemoglobin synthetic rates, or RNA/protein synthesis have been tried but most suffer from relatively low sensitivity (in the range of 20-30 mU/ml) and poor specificity. The results can be influenced by various factors in the culture medium such as iron/transferrin, source of serum, or the presence of other growth promoters (1).

Soon after erythropoietin was purified in the late 1970's, a number of radioimmune assays (RIAs) were developed by different investigators. One of the first was developed by Sherwood and Goldwasser (12). This test utilized an I-labeled erythropoietin preparation, anti-EPO antibodies raised in rabbits, and a goat anti-rabbit gamma globulin to aid in separation of bound from unbound radiolabeled EPO. This assay was able to detect 20-30 mU/ml of EPO. Subsequent refinements in RIAs by other investigators (3,4) were able to measure levels of EPO as low as 0.4mU/ml and, with dilutions, up to at least 20,000 mU/ml (4). Although this method was time consuming (it required incubations of up to 4 days), and involved working with radioactive substances, high levels of sensitivity and good correlation with bioassays were obtained.

In 1987 an RIA based on recombinant human erythropoietin as tracer and as immunogen was developed in conjunction with Amgen, one of the producers of the r-HuEPO (13). This RIA was found to be "appropriately sensitive and specific" for clinical specimens from anemic patients. Results corresponded well with previously developed RIAs in the range from 4 to 120 mU/ml and to over 40,000 with dilutions (15). Several commercial labs, including Smith-Kline in Van Nuys, have until recently used RIAs developed with Amgen reagents (14).
Recently Amgen Diagnostics began marketing an ELISA assay for erythropoietin called the "Clinigen Erythropoietin EIA" (15). In this test, microtiter wells are coated with a monoclonal antibody specific for EPO and incubated for 3 hours at room temperature with a serum sample or EPO standard. Thus EPO becomes immobilized on the microtiter well. Excess specimen is removed and the wells are then incubated with an anti-EPO rabbit antibody conjugated to horseradish peroxidase. After washing, a 3,3',5,5'-tetramethylbenzidine chromogen is added. After sulfuric acid is added, the absorbance is read on a spectrophotometer at 450 nm and the EPO concentration determined from a standard curve. The detection limit of the test is 2 mU/ml and specimens with EPO levels above 200 mU/ml must be diluted (15). The cost of this assay varies with the number of tests run, but the standard list price charged by Smith-Kline to its clients is $73.50 for an individual test as of July 11, 1990, while Mayo Clinic Laboratories' list price is $58.00 (16).

Clinical Use of Erythropoietin in HIV-1 Infected Patients

Anemia is a common feature of infection with the Human Immunodeficiency Virus, type 1 (HIV-1). It is estimated that 20% of asymptomatic HIV-1 infected patients, 50-60% of patients with AIDS related complex (ARC) and 75% of patients with documented AIDS are affected (5,17). This anemia in AIDS may be due to decreased production of red blood cells, increased peripheral destruction, and/or blood loss. Jacobsen et al (18) found a variety of factors contributing to the anemia of AIDS including antimicrobial therapy in 38%, disseminated Mycobacterium avium complex infection in 24%, Zidovudine (AZT) therapy in 20%, G.I. bleeding in 12%, and antineoplastic therapy in 9%. Other contributing factors included lymphoma, splenomegaly, and hemolysis; however 11% had no definitive etiologic factors other than HIV infection. Other investigators have shown that i) serum from HIV-1 infected individuals impairs proliferation of RBC precursors, ii) the elaboration of certain cytokines is altered (increased tumor necrosis factor and decreased gamma interferon), and iii) reduced production of erythropoietin may contribute to the anemia (17).

Spivak et al (5) showed that 75% of 73 AIDS patients not taking AZT were anemic. Furthermore, when compared with 23 adults with uncomplicated iron deficiency anemia, the EPO levels were significantly lower in AIDS patients. Thus untreated AIDS patients may have inappropriately low levels of EPO for their degree of anemia.

AZT, currently the only FDA approved licensed therapy for HIV-1 infection, has been reported to cause severe anemia (Hgb< 7.5 g/dl) in 24% of recipients (19) vs 4% of placebo recipients. Walker et al (20) reported transfusion requiring anemia developing in 6 of 15 (40%) of patients on AZT, and also noted a marked increase in EPO levels in AZT treated patients from a mean of 21 mU/ml to 155 mU/ml. Spivak (5) noted similar changes with pre-AZT EPO levels averaging 26 and post AZT therapy EPO levels averaging 214 mU/ml (range 13 to 1940). It appears that the ability to produce EPO is intact in AIDS patients but the increased EPO levels stimulated by AZT therapy are insufficient to correct the anemia.
Fischl (21) studied 63 anemic AIDS patients treated with AZT to determine if high doses of exogenous r-HuEPO could reverse the AZT induced anemia. Twenty-nine patients received 100 U/kg of EPO intravenously three times a week, and 34 patients received placebo. At baseline, there were no significant differences between treatment groups in number of patients given transfusions, number of units of blood transfused per patient per month, dosage of AZT, or in reticulocyte count. The group was subsequently divided into those with endogenous EPO levels of <500 mU/ml versus those with >500 mU/ml. Transfusions were given when deemed necessary by the patients' physician. At the end of the 12 week therapy period, the EPO group with "low" (<500 mU/ml) endogenous levels had shown an average weekly increase in hematocrit (hct) of .00353, or .353%, versus a decrease of .00116 in the placebo group. No statistically significant changes in hct were noted in patients with high endogenous EPO levels. Additionally, the number of transfusions required per patient per month decreased from 1.31 to 0.84 in the low endogenous EPO r-HuEPO treatment group while the placebo group transfusion requirement increased from 1.68 to 2.74 units/patient/month. The high endogenous EPO r-HuEPO treatment group showed no significant change in the number of transfusions/patient/month. Also, the number of patients requiring transfusion per month decreased in the low endogenous r-HuEPO treatment group from 16 of 22 to 5 of 22, while the placebo group showed a decrease from 21 of 26 to 17 of 26. There were no significant changes in the transfusion requirements of r-HuEPO treated patients with high endogenous EPO levels vs placebo. The authors concluded that it was of crucial importance to measure the endogenous level of EPO in AZT treated patients in order to select out those patients who would benefit from r-HuEPO therapy (ie. <500 mU/ml).

Currently, Ortho Biotech is conducting a study to determine if its r-HuEPO "Eprex" may be useful in the treatment of HIV-1 related anemias (22). To be included in this study, a patient must have AIDS, a hct <30, and endogenous EPO levels ≤ 500 mU/ml. This study does not exclude patients not on AZT. Pre-therapy EPO levels must be determined in order to qualify for the study. The dosages of EPO for this protocol will be 4000 units/day for 6 consecutive days followed by a rest day, resulting in 24,000 units/week. This dose continues for 12 weeks. If the target hct of 38-40% is not reached, the dose is increased to 36,000 units/week. If the target is still not reached in another 12 weeks, the dose is increased to 48,000 units/week. The drug is currently provided free of charge by Ortho to SFGH as part of an Investigational New Drug treatment protocol. However, the cost of all laboratory testing associated with the study, including testing of EPO levels, must be borne by the patient or the individual investigator.

Costs vs Benefits of EPO Therapy in HIV-1 Associated Anemias

As stated earlier, a large percentage of HIV-1 infected patients are anemic and many are transfusion dependent. Jacobson et al (18) have estimated that by the year 1992, more than 150,000 units of packed red blood cells (PRBCs) will be required for transfusion to AIDS patients.

Transfusion of PRBCs, as with any blood product, carries inherent risks including transfusion reactions and infection. Additionally, there may be some degree of immune modulation or inhibition caused by repeated blood transfusions. The potential morbidity induced by this
immune suppression in the already immunocompromised AIDS patient is of uncertain significance and has not been studied. There are also direct costs involved in PRBC transfusion which include technologist, nursing, and physician time as well as physical materials. Currently, the charge at SFGH billed to the patient for a transfusion of a single PRBC unit is about $90. Fischl et al (21) have shown that in AIDS patients with low endogenous levels of EPO, the transfusion requirement can be reduced, on average, from 1.32 units/patient/month to 0.84 units/patient/month for a savings of about 6 units/patient/year (or $540/year).

What are the costs of administration of r-HuEPO to the AIDS patient? Currently Eprex is supplied free of charge on an investigational basis. The chemically identical Epogen is available to renal patients at approximately 1.2 cents/unit (29) at SFGH. For the purpose of this discussion I will assume that the eventual charges will be similar for Eprex as for Epogen. If the r-HuEPO dosage used in the Fischl AZT study (21) were utilized as a treatment protocol, this would require 21,000 units/patient/week, assuming an average patient weight of 70 kg. This would cost $252.00/week, or $13,104/year. If, however, the study protocol 188-083 (22) were used, the dose of EPO would begin at 24,000 units/week and could be increased to as much as 48,000 units/week. This translates to $14,976 to $29,952/patient/year for the drug alone. Although the current dosages of EPO in AIDS patients are experimental, and the exact dosages required are yet to be clearly defined, it appears that the required dosages will be quite high and therefore quite expensive.

Adverse reactions to r-HuEPO in AIDS patients were minimal; there was no significant difference between the treatment groups and placebo (21). This is in contrast to the significant number of renal failure patients (>25%) who develop hypertension as well as seizures and vascular access clotting (7).

Since it has been fairly clearly shown by Fischl (21) that only those AIDS patients with "low" endogenous levels of EPO will respond to exogenous r-HuEPO therapy, and the test for EPO levels costs in the range of $58 to $75, the potential savings in dollars achieved by performing this test, and thus eliminating even a few potential recipients, are substantial. The issue of whether or not the therapy itself is cost effective in this patient population is clearly another matter. Preliminary published studies quoted earlier (21) suggest that relatively high doses of this expensive drug are necessary in order to achieve relatively small benefit in reduction of transfusion requirements. In the Fischl study (21) $13,000/year in EPO costs would be required to reduce the cost of transfusion by about $540/year. Although direct dollar costs are not the only issue to be considered, this would clearly be an economically impractical therapy.

Clinical Use of Erythropoietin in Anemia Associated with Chronic Renal Disease

The use of erythropoietin in the treatment of the anemia associated with chronic renal disease is now well established. Gurney (23) in 1958 noted that the anemia of chronic renal failure did not result in elevated levels of EPO, and others (24) have noted that despite increasing anemia
in chronic renal failure, the serum EPO level fell with declining kidney function. Numerous studies (25-27) in dialysis and pre-dialysis patients have shown significant response to exogenous r-HuEPO as measured by reduction in transfusion requirements in these patients. In all of these studies, virtually all patients [17 of 17 (25), 10 of 10 (26), and 162 of 163 (27)] responded to various doses of EPO. In the Sundal study (25) a response corresponded to achievement of a hgb > 2 g/dl above baseline. In the Winearls study (26) the mean hemoglobin increased from 6.1 g/dl to 10.3 g/dl over 12 weeks, and in the Eschbach study (27) the median hct increased from 27 to 37. In all, over 2000 anemic renal failure patients have been studied and virtually all have responded by reaching a hct in the 33-38% range on EPO doses of 50-300 U/kg i.v. three times per week (28) with transfusion requirements being virtually eliminated. In none of the above cited papers were pre-protocol EPO levels considered. In the chronic renal failure population, since virtually all such patients respond well to r-HuEPO therapy, there is no need to perform pre-therapy EPO level testing.

In order to maximize the stimulatory effect of EPO, it is important that adequate iron stores be present. The National Kidney Foundation, [N.K.F.; (28)] recommends that serum ferritin be > 100 ng/ml and that transferrin saturation be > 20%. A relative or true iron deficiency is reported to develop in almost 50% of EPO treated renal patients and thus these patients require iron supplementation. The N.K.F. also recommends testing serum iron, TIBC, and ferritin before beginning therapy and every 3 months thereafter.

Among patients with chronic renal failure, significant side effects of r-HuEPO therapy include hypertension (developing or increasing in up to 25% of dialysis and pre-dialysis patients), seizures (6%), clotting of arterial-venous access sites (occasional) and significant myalgias (5%).

The role of EPO therapy in other anemias such as anemia of chronic disease, rheumatoid arthritis, malignancy, sickle cell anemia, or in patients making autologous blood donations is much less clear, and more investigation is needed and ongoing. The role of EPO testing in these cases is also not yet well defined.
REFERENCES


29) Richmond-Bloom, C., from "Drug Request- rHuEPO" presented to the Pharmacy and Therapeutics Committee, SFGH, Sept 14, 1989.