Original Article

Increased preoperative collection of autologous blood with recombinant human erythropoietin therapy in tertiary care hospitals of Jammu

Kumkum Sharma, Sumit B. Sharma[1], Imran A. Pukhta, Amit B. Sharma[2], Abdul Q. Salaria[3]

[1]Department of Orthopedics, Duchess of Kent, Hospital, Hong Kong, [2]Department of Interventional Cardiology, Mount Sinai Hospital, New York, USA, Department of Transfusion Medicine, [3]Orthopedics, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu, India

Abstract:

Introduction: To study whether the administration of recombinant human erythropoietin increases the amount of autologous blood that can be collected before orthopaedic surgery. **Materials and Methods:** We conducted a randomized controlled trial of recombinant human erythropoietin in 68 adults scheduled for elective orthopedic procedures. The patients received either erythropoietin 600 units/kg of body weight or placebo intravenously every 5th day prior to each phlebotomy for 21 days during which time up to 5 units of blood was collected. Patients were excluded from donation when their hematocrit values were less than 33%. All patients received iron sulphate 325mg orally 3 times daily. The mean number of units collected per patient was 4.33 ± 0.4 for erythropoietin group and 3.05± 0.71 for the placebo group. **Results:** The mean packed red cell volume donated by patients who received erythropoietin was 32% greater than that donated by patients who received placebo (196.3 vs. 169.4 ml, $p<0.05$). 68% in the placebo group and 9% of patients treated with erythropoietin were unable to donate ≥4 units. No adverse effects were attributed to erythropoietin. While participating in the study, complications developed in 2 patients one in each group necessitating their removal from the study. **Conclusion:** We conclude that recombinant human erythropoietin increases the ability of the patients about to undergo elective surgery to donate autologous blood units.

Key words:

Autologous blood, preoperative, recombinant human erythropoietin

Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.106733

Quick Response Code:

Correspondence to:
Prof. Kumkum Sharma,
Department of Transfusion Medicine,
Acharya Shri Chander College of Medical Sciences and Hospital,
Sidhra,
Jammu – 180 017, India.
E-mail: drkumtandon@rediffmail.com

Introduction

The blood loss that accompanies a total hip or knee arthroplasty can be substantial, and many patients need perioperative transfusion with allogeneic or autologous blood.[1,2] Allogeneic blood transfusions carry some risk of complications, such as transmission of viral infections, transfusion reactions, alloimmunization and immune suppression.[3] Various methods have been used to reduce allogeneic blood transfusion requirements such as preoperative autologous blood donation (PABD),[4] acute normovolemic haemodilution,[5] use of pharmacological agents ,iron or recombinant human erythropoietin (rHuEPO).[6] One unit of autologous blood can be donated every 72 hours provided that the hematocrit (Hct) remains higher than 33 percent. The main limitations to the pre-donation of required amount of blood are iron restricted erythropoiesis[7,8] and an unadapted endogenous erythropoietin response to serial phlebotomy.[9] Studies have shown that endogenous erythropoietin response to autologous blood donation is inadequate to stimulate maximal marrow erythropoiesis.[10] While basal RBC production in response to phlebotomy induced anemia is doubled,[11] iron availability becomes the limiting factor for efficient erythropoiesis. Previous studies have shown that storage, circulation and total body iron were lower in patients unable to donate the required amount of blood even with an oral iron supplementation of 375 mg of iron sulfate three times a day.[11] A randomized controlled study comparing the efficacy of oral (100 mg iron three times a day) and intravenous (200mg) iron supplementation, however, did not show any improvement in the success of autologous blood donation with either of the applied regimens.[12] The use of rHuEPO in autologous blood donation programs results from these observations. Goodnough and coworkers[13] in a prospective, randomized, double blind study have shown that patients treated with 600 U per kilogram rHuEPO for 21 days of autologous blood donation (6 units collected) were able to procure more units and a higher RBC volume than patients receiving placebo.[14] The present study was undertaken to determine whether recombinant erythropoietin increases the amount of preoperative collection of blood for autologous use. In adults scheduled for elective orthopedic surgery for total hip replacement and total knee replacement which are the most common orthopedic procedures for which preoperative autologous blood is collected,[15] we conducted a

randomized placebo controlled multicentric study at tertiary care hospitals of Jammu [J & K] India.

Materials and Methods

Prospective study conducted in Govt. Medical College, Jammu and Acharya Shri Chander College of Medical Sciences and Hospital, Jammu from September 2008 to September 2011 after approval from institutional ethical committee of each participating institution. During this period, all adults scheduled for elective orthopedic surgery for total hip replacement, total knee replacement, were motivated to participate in the autologous blood donation with educational material circulated in the form of posters, pamphlets and through verbal communication with patients and discussion with surgeons.[16]

Patients Eligibility

The study patients were adults for which ≥2 units of blood was requested. All eligible patients were in good general health (except for condition for which surgery was scheduled), as evidenced by medical history, physical examination, clinical laboratory test and 12-lead electrocardiography. Patients were ineligible if they had a hemoglobin concentration less than 11g/dl, Hct less than 33%,[17] history of hematological disease, seizures or uncontrolled hypertension (i.e. diastolic blood pressure ≥ 100 mm Hg), had blood loss (e.g. gastrointestinal) or active inflammatory, infectious or neoplastic diseases that could compromise the response to erythropoietin therapy, or had received blood within six month of study entry, androgens, cytotoxic agents, immunosuppressive agents, or other agents known to affect erythropoiesis, or patients who had a history of drug or alcohol abuse within the previous two years, unstable angina, recent myocardial infarction, congestive heart failure or severe aortic stenosis.[18] Women were eligible if they had been post menopausal for at least one year, were surgically sterile or had been taking oral contraceptives for at least one month before study entry or had been using barrier and spermicide methods of contraception. Pregnant females and till 12 months after delivery lactating females, history of abortion less than six months were exempted from participation.[19]

Study Design

A prescription or order form from patient's physician[20] and informed consent signed by patient was obtained before enrollment in the study. The patients received iron sulphate tablet(325 mg orally three times daily), 7-10 days prior to first PABD and were advised to continue the same during the study period and for two months after the surgery as has been suggested by Guinea *et al*.[21] An autologous unit of blood was collected according to Morse formula[22] (10 % of estimated blood volume) at a time in 450 ml CPDA-1 double blood bags from each patient once every 5 days for three to four weeks, for a maximal collection of 5 units[23] as an outpatient regimen. Last unit to be collected was scheduled 3-6 days prior to proposed date of surgery. At each visit for blood collection, the patients received either erythropoietin (600 units per kg of body weight) or placebo (diluents) intravenously; whether or not blood was actually collected. Patients were excluded from donation if the Hct was less than 33%. The erythropoietin used in these studies was manufactured by Amgen (thousand oaks', Calif.) and Genova Biopharmaceuticals Ltd. marketed as rHuEPO injection (VIntor).

Laboratory Studies

Laboratory test performed in all patients initially during the study included complete hemogram, liver function tests, renal function tests, coagulation parameters, prothrombin time, partial throboplastin time, measurement of serum ferritin, iron levels, total iron binding capacity, transferrin saturation and reticulocyte count. An enzyme linked immunosorbant assay (ELISA) performed was used to detect antibodies in serum to Human immunodeficiency virus, hepatitis C virus, hepatitis B surface antigen, malarial parasite antigen detection by immunochromatographic technology and syphilis serology by detection of reaginic antibodies. ABO blood group, Rh D phenotype and presence of red cell antibodies was determined in all patients taking part in the predeposit programme on all first units collected.[17] An ELISA performed was used to detect antibodies to rHuEPO in serum. Hemoglobin and Hct values were checked before and after each donation and daily after the operation until discharged.[24] Reticulocyte count and iron studies were repeated at visits 1 through 5.

The level of total mobilzable iron was calculated as a sum of mobilizable circulating iron level (1mg in 1 ml of red cells) and the iron stored. The amount of stored iron (in milligrams) was calculated as 400 (log serum feritin (in milligrams per litre)-log 12),[25] the level of mobilizable circulating iron was calculated as the whole blood volume (60 ml per kg) x body weight in kg) x [(initial Hct (as a %) – 34%].[26] RBC volume donated=blood volume donated (ml) x same day Hct (%). Blood volume [BV.mL]=body weight [kg]×60 ml/kg.

Statistical Analysis

A unpaired t-test was used to compare the difference between two groups namely erythropoietin and placebo.

Mean, standard deviation, range and median have been used to represent the different characteristics of the two study groups.

Value in graphs are shown as mean of two groups at visits 1 through 5 for comparisons between the groups.

Results

84 patients were enrolled, motivated and educated but only 68 fullfiled the criteria and completed the study. The clinical characteristics of the 35 patients in the placebo group and the 33

Table 1: Characteristics of the Erythropoietin and Placebo groups

Characteristics	Erythropoietin N = 33	Placebo N = 35
Age (Yr)		
Median	55	52
Sex		
Male	23	24
Female	10	11
Surgical Procedures		
Hip replacement	20	20
Knee replacement	13	15
Mean days of hospitalization (± SD)	4.9 ± 0.8	5.2 ± 1.0
Mean Hct (± SD)		
Admission	41.02	42.07
Men	42.2 ± 1.6	43.98 ± 1.5
Women	38.3 ± 1.2	37.9 ± 1.4
Discharge	33.0	28.81
Men	33.1 ± 0.7	29.6 ± 0.5
Women	32.8 ± 0.6	27.1 ± 0.4

in the erythropoietin group are shown in Table 1. There are no differences between groups in the mean age, sex, type of surgical procedure or duration of hospitalization.

Units of blood collected and transfused

The 33 patients who received erythropoietin therapy donated 143 units [(mean ± SD) 4.33±0.4] as compared with the 107 units donated [(mean ± SD) 3.05±0.71] by 35 patients who received placebo (P < 0.05). When number of donations was analyzed according to sex, there was no difference observed between men and women in the erythropoietin group or placebo group. During the three to four weeks study period, the only reason to exclude patients from donation was a low Hct (33%).

24 of 35 (68%) patients who received placebo and 3 out of 33 (9%) patients treated with erythropoietin were unable to donate ≥ 4 units. In the subsequent preoperative period, the erythropoietin group underwent transfusion of 68 of 143 (48%) autologous units and the placebo group of 80 of 107 (75%) autologous units [Tables 2 and 3]. No patient in the erythropoietin group and 2 patients (5%) in the placebo group received two homologous units, each of these patients had donated 2 autologous units before the operation.

Volume of Packed Red cells collected

The analysis of the volume of red cells collected from the patients in each group are shown in Table 4. The volume was determined by multiplying the volume of blood donated (in ml) by the Hct of the donated blood (as a %). The mean red cell volume per autologous unit collected was significantly higher in the erythropoietin group than in the placebo group at 196.3 vs. 169.4 ml (P < 0.05). An analysis of the red cell volume by unit showed that 20 of the 107 unit (19%) obtained from the placebo group had less than 154 ml of red cells (minimal standard for homologous blood units, according to the American association of blood banks)[11] as compared with 1 of the 143 units (0.7%) obtained from the erythropoietin treated patients (P<0.05).

Statistical analysis

Total RBC volume donated in erythropoietin vs placebo group (P<0.05); Mean Red cell volume donated in erythropoietin vs placebo group (P<0.05)

Hematological changes

The Hct data for patients for each group on admission, discharge and at visits 1 through 5 are shown in the Table 1 and Figure 1. The mean values for men and women in the erythropoietin and placebo group were similar at the time of admission however at the time of discharge the patients who received erythropoietin had a smaller decline in Hct (men: 9.1%, women: 5.5% (P<0.05)) than the placebo group (men: 14.38%, women: 10.8%) which showed lower Hct values. Figure 2 shows the reticulocyte data corrected for the change in hematocrit. The patients who received erythropoietin had significantly greater increase in reticulolyte levels than the patients who received placebo (5.9% vs 4%, P<0.05) at visit 5.

Table 2: Units of blood donation by patients receiving erythropoietin or placebo

Group	Units collected (Whole blood)					Total units	Total patients
	1	2	3	4	5		
	No. of patients						
Placebo	0	10	14	10	1	107	35
Erythropoietin	0	0	3	16	14	143	33
Total	0	10	17	26	15	252	68

Table 3: Comparison of AB donation and perioperative Transfusion requirement in the erythropoietin and placebo group

Group	Blood Donation (Whole blood)	Autologous Transfusion	Homologous Transfusion
Erythropoietin			
Total Units Collected	143	68 (48%)	Nil
Mean no. of units collected per patient (SD per patient)	4.33 ± 0.33	2.06 ±0.5	
No. of patients	33	33	
No. of Collected Units Discarded	-	75 (52%)	-
Placebo			
Total Units	107	80 (75%)	4
Mean no. of units collected per patient (SD per patient)	3.05 ± 0.71	2.28 ± 0.6	0.1 ± 0.4
No. of patients	35	35	2 (5.7%)
No. of Collected Units Discarded	-	27 (25%)	

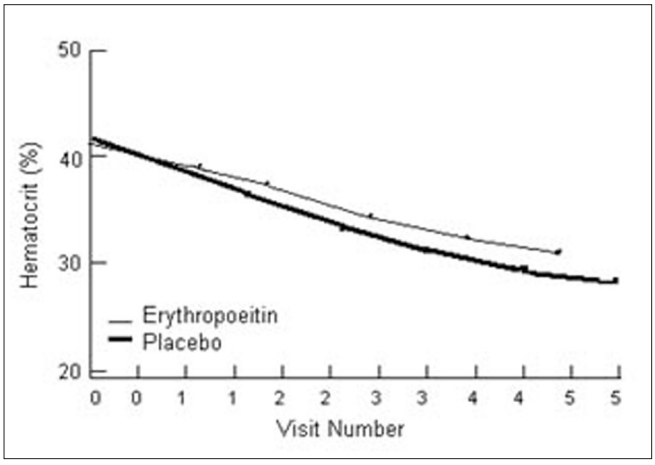

Figure 1: Mean Hematocrits for each Group before the study and at visits 1 through 5

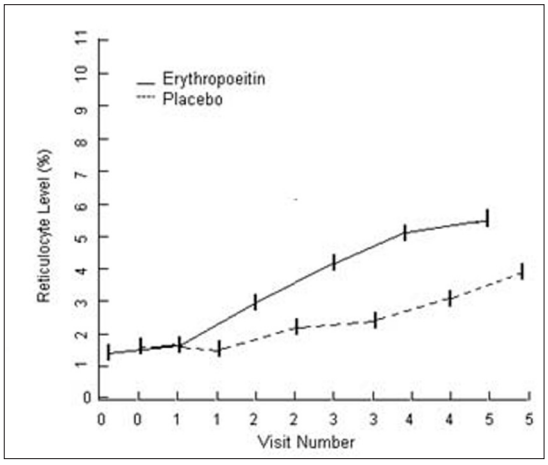

Figure 2: Mean Reticulocyte counts for each Group before the study and at visits 1 through 5

Table 4: Cumulative packed Red cell volume donated per patient

Total No. of patients	Placebo		Erythropoietin (600 UI/kg)	
68	35		33	
Cumulative interval between each donation	Total units (107)	RBC volume donated / patient (mL)	Total units (143)	RBC volume donated / patient (mL)
Day 0	35	200±12	33	199±10
Day 5	35	160±11	33	190±15
Day 10	25	140±15	33	185±22
Day 15	11	115±8	30	170±15
Day 20	1	65	14	166±13
Total RBC volume donated by group (mL)	18125		28033	
Mean Red cell volume	169.4		196.3	

Statistical analysis: Total RBC volume donated in erythropoietin vs placebo group (P<0.05); Mean Red cell volume donated in erythropoietin vs placebo group (P<0.05)

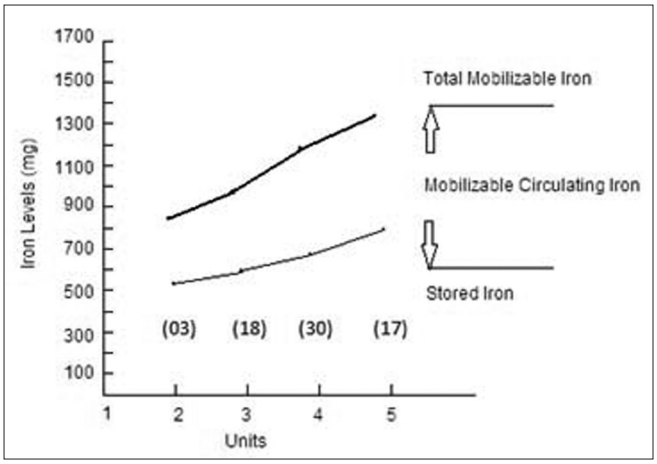

Figure 3: Relation between initial levels of stored iron, mobilizable circulating iron and total mobilizable iron; and total units of blood donated in the group as a whole
The number of patients in each group is shown in parentheses

The platelet count in erythropoietin and placebo groups were not different, 301±17 and 311±14×10³ per cubic mm initially and 387 ± 23 and 367±22×10³ per cubic mm after the study.

Adverse Effects

The frequency of adverse effects was similar in both groups. The most common adverse effect were mild vasovagal attack, fatigue (2 patients in erythropoietin group and 4 in placebo group), dizziness (3 and 7), nausea (3 and 4), diaphoresis (3 and 2), hyperventilation and hypotension occurring together one in each group.[24] Serious complication developed in two (2.9% of study group) after donating two units of blood each; one patient in placebo group had severe chest pain, the patient had no history of cardiac disease earlier and was diagnosed as myocardial infarction, one in erythropoietin had peripheral artery thrombosis and was not known to have had peripheral vascular disease, necessitating their removal from the study. Both donors were referred to the emergency unit for management. In a previous study, 5.1% patients participating in the PABD programme were suspended because of onset of complications.[24] No erythropoietin antibodies were detected in 68 patients before the study or one month after its completiton.[13] Further no adverse effects associated with rHuEPO were observed in the two groups.

Discussion

These results demonstrate the efficacy of therapy with rHuEPO in increasing the procurement of autologous blood.[13,27,28] The mean packed red cell volume donated by patients treated with erythropoietin and placebo group during the study was 196.3 vs 169.4 ml (P < 0.05), total red cell volume donated by erythropoietin group was 28033 ml and donated by placebo group was 18125.8 ml. The mean red cell volume donated by patients who received erythropoietin was 32% greater than that donated by patients receiving placebo. Similar findings were recorded by Christophe Bouy *et al* 2006[23] and Goodnough, *et al* 2007.[13] In the current study in which patients were enrolled in an autologous donation program designed to store up to 5 units of blood in three to four weeks, 68% patients who received placebo and 9% treated with erythropoietin were unable to donate ≥ 4 units [Table 2]. Subsequently, the erythropoietin group underwent transfusion of 48% of autologous units and the placebo group 75% of autologous units [Table 3]. No patient in the erythropoietin group and 5.7% patients in the placebo group received two homologous units; each of these patients had donated 2 autologous units before the operation. In a previous retrospective study designed to store autologous units, 29% in placebo group and 4% in the erythropoietin treated group were unable to donate ≥4 units. And further in the same study, placebo group in subsequent perioperative period underwent transfusion of 83% of autologous units collected and the erythropoitein group received 70% autologous blood donations,[13] where as study conducted by Cristophe Bouy *et al* 5% of patients treated with erythropoietin and 40% of the placebo group received allogenic transfusion.[23] The overall wastage of autologous units in the present study was 52% in the erythropoietin group and 25% in the placebo group. The discard rate of autologous units in elective orthopedic surgeries shows a wide variation from 6.9% to 56%.[13,24,29] Although the small number of patients in our study precluded a demonstration that the frequency of exposure to homologous blood in the two groups was significantly different, previous studies have indicated that patients scheduled for elective orthopaedic surgery who are able to donate the amount of blood requested have a 13% probability of subsequently receiving homologous blood, as compared with a 43% probability among patients unable to store the requested amount of blood because of Hct limitations.[30,31] Studies of more patients are needed to confirm that increased procurement of autologous blood results in reduced need for homologous transfusion. For example, patients who are scheduled for surgical

procedures requiring ≥4 units of blood may be one group that will particularly benefit from erythropoietin therapy. In addition, our analysis of the relation between mobilizable iron levels and the number of units donated indicates that the amount of iron stored is a less important determinant than the initial levels of mobilizable circulating iron [Figure 3]. The initial level of circulating hemoglobin iron is calculated on the basis of body size (i.e. blood volume) and hematocrit; thus these two values most closely predict the success of donation in autologous blood donors and explain why women are most often excluded from donation. The efficacy of erythropoietin in the autologous donation programme is due to increased red cell production [Figure 2] through accelerated conversion of stored iron into circulating iron. This analysis suggests that the patients who would most benefit from the ability of erythropoietin to enhance red cell production are those with smaller blood volumes (e.g. women and children) and those who are anemic. The frequency of adverse effects was comparable in the erythropoietin and placebo groups. Two patients were removed from this study because of major cardiovascular events although neither event was attributable directly to the treatment or to the autologous blood donation protocol.

Conclusion

Our study in addition to confirming the success rate of autologous donations with rHuEPO has shown that the erythropoietin group has greater number of autologous blood unit collection, higher mean red cell volume per donor unit, more stable red cell volume collection throughout successive autologous phlebotomies as compared to placebo group. Further, on basis of current study the MSBOS (Maximum Surgical Blood Ordering Schedule) of our tertiary care hospitals are being reviewed to minimize discard rate of Autologous units collected. Thus the present study suggests that a comprehensive program of pre-deposit autologous blood for transfusion is an effective method for reducing the need for allogeneic transfusion.

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Cite this article as: Sharma K, Sharma SB, Pukhta IA, Sharma AB, Salaria AQ. Increased preoperative collection of autologous blood with recombinant human erythropoietin therapy in tertiary care hospitals of Jammu. Asian J Transfus Sci 2013;7:42-7..

Source of Support: Nil, **Conflict of Interest:** None declared.

Forthcoming Events

1. **ISBTI 1st National Conference on Voluntary Blood Donation, Harayana, 2013.**
2. **23rd Regional Congress of the International Society Blood Transfusion (ISBT), Amsterdam, The Netherlands June 2 - 5, 2013.**
3. **Annual National conference of Indian Society Blood Transfusion and Immunohaematology (ISBTI), Surat, 2013.**
4. **2nd Annual conference if Indian Society of Transfusion Medicine (ISTM), Bangalore, 2013.**