Evaluation of Recombinant Human Erythropoietin Responsiveness by Erythrocyte Creatine in Haemodialysis Patients

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Abstract

Background: One of the main causes of anaemia in patients with end-stage renal disease is relative deficiency in erythropoietin production. Recombinant human erythropoietin (rHuEpo), a potent haematopoietic growth factor, is used to treat anaemia in haemodialysis patients. The effect of rHuEpo is usually assessed by haematological indices such as red blood cell count, haemoglobin concentration and haematocrit, but erythrocyte indices do not provide information of the rapid change in erythropoietic activity. As erythrocyte creatine directly assess erythropoiesis, the aim of this study was to evaluate the effect of rHuEpo in haemodialysis patients by measuring erythrocyte creatine.

Methods: rHuEpo dose was fixed 3 months prior to the enrollment and was maintained throughout the entire study period. Eerythrocyte creatine was measured with haematologic indices in 83 haemodialysis patients. Haemoglobin was also measured 3 months after.

Results: rHuEpo dose (152.4±62.9 vs. 82.2±45.5 units/kg/week, P=0.0001) and erythrocyte creatine (2.07±0.73 vs. 1.60±0.41 μmol/gHb, p=0.0003) were significantly higher in 27 patients with haemoglobin <10g/dL compared to 56 patients with haemoglobin ≥10g/dL. There was a fair correlation between rHuEpo dose and erythrocyte creatine (r=0.55, P <0.0001). Increase in haemoglobin (>0.1g/dL) was observed in 37 patients, whereas haemoglobin did not increase in 46 patients. Erythrocyte creatine was significantly higher in patients with increase in haemoglobin compared to those without (2.04±0.64 vs. 1.52±0.39 μmol/gHb, p <0.0001). When 8 variables (rHuEpo dose, erythropoietin resistance index, C-reactive protein, intact parathyroid hormone, incidence of iron deficiency, presence of anaemia, erythrocyte creatine and reticulocyte) were used in the multivariate logistic analysis, erythrocyte creatine emerged as the most important variable associated with increase in haemoglobin (Chi-square=6.19, P=0.01).

Conclusion: Erythrocyte creatine, a useful marker of erythropoietic capacity, is a reliable marker to estimate ameliorative effectiveness of rHuEpo in haemodialysis patients.

Background

The primary cause of anaemia in patients with end-stage renal disease is inadequate erythropoietin production [1]. Recombinant human erythropoietin (rHuEpo), a potent haematopoietic growth factor, has been shown to be effective in correcting anaemia in chronic renal failure [2–5], but the response rate is variable because resistant factors of erythropoiesis such as iron deficiency, inflammation, aluminum intoxication, hyperparathyroidism, blood loss or bone marrow dysfunction are involved [5, 6]. Early detection of poor rHuEpo response is clinically important to increase the dose of rHuEpo and eliminate transfusion requirements [7]. On the other hand, early detection of favorable response to a relatively low dose of rHuEpo could save costs and avoid increased incidence of side effects. In contrast to haemoglobin levels which take a relatively long time to increase, total reticulocyte count can assess rapid change in erythropoiesis [8, 9]. However, total reticulocyte count has seldom been used in clinical practice,

because it is generally considered to be inaccurate and imprecise [10, 11]. Since creatine content in the erythrocyte rapidly declines with erythrocyte aging, it is used to measure erythrocyte life span and erythropoietic capacity [12–14], but the clinical usefulness of erythrocyte creatine in patients receiving rHuEpo is not yet fully elucidated. Accordingly, we attempted to evaluate the ameliorative effectiveness of rHuEpo in patients undergoing maintenance haemodialysis by measuring erythrocyte creatine.

Methods

Study patients

We evaluated 83 patients (57 men and 26 women, mean age of 74 years) aged ≥20 years who had been receiving maintenance haemodialysis 3 times a week for at least 6 months in the Dialysis Unit of Takarazuka Hospital. The exclusion criteria were as follows: bleeding event within 3 months (n=1), infection requiring parenteral antibiotics (n=1) and mechanical heart valves (n=2). None of the patients had concurrent malignancy, haemolytic disease or blood transfusion within 3 months.

Study protocol

Study protocol is shown in Fig. 1. rHuEpo treatment was fixed 3 months prior to the enrollment and was maintained throughout the study period. Baseline blood examination including the erythrocyte creatine and other laboratory examination (red blood cell count, haemoglobin, haematocrit, reticulocyte, haptoglobin, transferrin saturation, ferritin, intact parathyroid hormone, serum calcium, serum phosphorus, serum albumin and C-reactive protein) were performed. Intravenous iron treatment with 40mg ferric saccharate 3 times a week was administered at the end of each haemodialysis session and followed by 100mg of oral iron supplements in patients with absolute iron deficiency, defined as transferrin saturation <20% and serum ferritin <100 ng/ml [15,16]. Patients were divided into 2 groups; patients with haemoglobin <10g/dL and those with haemoglobin >10g/dL according to the Guidelines of Japanese Society for Dialysis therapy [16] and clinical characteristics, haemodialysis conditions, treatments and laboratory indices were compared between the 2 groups. Haemoglobin was measured 3 months later and percent change in haemoglobin; (3 months minus baseline)/ baseline x 100% was calculated and expressed as percentage. The study protocol was approved by the Takarazuka Hospital ethical committee on human research. All the patients provided written informed consent, and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Haemodialysis

All patients were dialysed for 4.0-8.0 hours, using a single-use dialyser with a 1.3-2.1 m^2 effective surface area of cellulose, poly-sulfone, polyethersulfone or polymethylmethacrylate membrane, and blood flow of 200 ml/min with dialysate flow of 500 ml/min. Haemodialysis was performed via native arteriovenous fistulas with a dual plastic needle and 16-gauge cannula. The patients in the haemodialysis group uniformly received a dialysate (D-dry, Nikkiso Co., Ltd., Tokyo, Japan) and an anticoagulant with heparin sodium. Bolus of heparin sodium 1,000 units was intravenously administrated at the start of

haemodialysis followed by continuous administration of 500 to 750 units/hour. The dialysate temperature of extracorporeal circulation was strictly maintained at 36–38 °C. rHuEpo and iron therapy were prescribed in accordance with the Kidney Disease: Improving Global Outcomes Clinical Practice Guideline 2012 [15]. Erythropoietin therapy with epoetin beta pegol was administrated intravenously at the end of haemodialysis. Haemodialysis time (hours/week), intradialytic ultrafiltration rates (ml/hour/kg) were measured and Kt/V, as an index of urea clearance was calculated. These indices were calculated as the average of the values from 3 consecutive haemodialysis sessions. One of the following dialysis membranes was used: cellulose FB, Nipro Corporation, Osaka, Japan), poly-sulfone, (PN, Nikkiso Co., Ltd., Tokyo, Japan), polyethersulfone (PES, Nipro Corporation, Osaka, Japan), or polymethylmethacrylate (NF-H, Toray Medical Co., Ltd., Tokyo, Japan).

Laboratory measurements

Blood samples were drawn immediately before the haemodialysis. Haematologic examinations and reticulocyte counts were carried out with a Sysmex XN 1000 (Sysmex, Kobe, Japan). Haptoglobin was measured by a TIA method with JCA-BM 6010 (JEOL, Tokyo, Japan). Serum iron was measured by a Nitroso-PSAP method with AU 5840 (Beckman Coulter; Tokyo, Japan), unsaturated iron binding capacity (UIBC) was by a Nitroso-PSAP method with BM-6050 (JEOL, Tokyo, Japan) and ferritin by radioimmunoassay with AU-5840 (Beckman Coulter, Tokyo, Japan) and transferrin saturation was calculated as [Serum iron/ (Serum iron + UIBC)] x100 (%). Intact parathyroid hormone was measured by the ECLIA method with Cobas 8000 (Roche Diagnostics, Tokyo, Japan). The other biochemical laboratory measurements were performed by TBA-120 FR automated biochemical analyzer (Canon, Osaka, Japan).

Erythrocyte creatine

Erythrocyte creatine was assayed enzymatically in accordance with previous report [17]. Measured date are expressed as micromole per gram of haemoglobin (μmol/g Hb). Briefly, blood samples were collected in ethylenediaminetetraacetic acid-containing tubes and centrifuged to remove the plasma and buffy coat. After lysis and deproteinisation of packed erythrocytes, the supernatant was obtained by centrifugation and filtration. The creatine concentration in the supernatant was measured using the enzymatic assay method. Erythrocyte creatine represents the average or cumulative erythropoiesis up to the present [18,19].

Statistical analyses

Results are expressed as mean ± standard deviation. Statistical analyses between the 2 groups were performed by one-way layout analysis of variance or chi-square analysis followed by Scheffe type multiple comparison method. Change in the haemoglobin from baseline to 3 months after was estimated by 2-way repeated measures ANOVA. Multivariate logistic regression analysis was performed to evaluate the important variables related to change in haemoglobin at 3 months. A probability value of <0.05 was

considered significant. Parameters were compared with the use of commercially available statistical software (STATVIEW, Abacus Concepts, Berkeley, CA).

Results

Clinical characteristics are shown in Table 1.

Table 1
Clinical characteristics

Number of patients	**83**
Age (years)	73.7 ± 14.5
Gender (male/female)	56/27
Diabetes mellitus (present/absent)	34/49
Height (cm)	162.0 ± 9.2
Weight (kg)	56.8 ± 14.0
Body surface area (m^2)	1.60 ± 0.21
Haemodialysis time (hours/week)	16.0 ± 4.2
Intradialytic ultrafiltration rate (ml/hour/kg)	8.8 ± 3.0
Kt/v	1.72 ± 0.48
Data are represented as mean ± SD. *Kt/v* urea clearance	

Anaemia

Twenty-seven patients (33%) had haemoglobin < 10g/dL and 56 patients had haemoglobin > 10g/dL. Patients with haemoglobin < 10g/dL received significantly higher rHuEpo dose compared to those with haemoglobin < 10g/dL (Table 2). There were no significant differences in reticulocyte, haptoglobin, transferrin saturation, ferritin, incidence of iron deficiency, intact parathyroid hormone, serum calcium, serum phosphorus, albumin, C-reactive protein and frequency of iron supplementation between the 2 groups. However, erythrocyte creatine, rHuEpo dose and erythropoietin resistance index were significantly higher in patients with haemoglobin < 10g/dL compared to those with haemoglobin > 10g/dL. A fair correlation was observed between rHuEpo dose and erythrocyte creatine ($r = 0.55$, $P < 0.0001$) (Fig. 2).

Table 2
Comparison between patients with and without anaemia

	Haemoglobin		p Value
	< 10mg/dL (n = 27)	≥ 10mg/dL (n = 56)	
Age (years)	71.4 ± 13.5	71.9 ± 15.1	0.87
Gender (male/female)	14/13	42/14	0.05
Haemodialysis time (hours/week)	16.4 ± 4.1	15.7 ± 4.2	0.45
Intradialytic ultrafiltration rate (ml/hour/kg)	9.3 ± 3.1	8.6 ± 2.9	0.35
Kt/v	1.8 ± 0.5	1.7 ± 0.5	0.5
Erythrocyte creatine (μmol/gHb)	2.07 ± 0.73	1.60 ± 0.41	0.0003
rHuEpo dose (units/kg/week)	152.4 ± 62.9	82.2 ± 45.5	0.0001
Erythropoietin resistance index (units/kg/week/g/dL)	17.6 ± 7.9	7.6 ± 4.4	0.0001
Red blood cell (x 10^4μL)	288.0 ± 35.4	358.8 ± 33.1	0.0001
Haematocrit (%)	27.2 ± 4.4	34.5 ± 2.4	0.0001
Haemoglobin (g/dL)	8.9 ± 0.9	11.0 ± 0.7	0.0001
MCV	98.6 ± 6.7	97.2 ± 5.6	0.32
MCH	31.2 ± 2.4	30.9 ± 2.0	0.54
MCHC	31.6 ± 0.9	31.8 ± 0.7	0.42
Reticulocyte (%)	16.2 ± 5.1	15.1 ± 4.8	0.31
Haptoglobin (g/dL)	88.5 ± 51.1	97.3 ± 50.4	0.46
Transferrin saturation (%)	24.4 ± 12.9	29.5 ± 17.5	0.19
Ferritin (ng/mL)	110.4 ± 88.0	126.6 ± 114.8	0.52
Iron supplement (present/ absent)	18/9 (67%)	29/27 (52%)	0.2
Iron deficiency (present/ absent)	9/18 (33%)	13/43 (23%)	0.43
Intact parathyroid hormone (pg/mL)	146.7 ± 99.0	155.1 ± 104.2	0.73
Serum calcium (mg/dL)	9.5 ± 0.5	9.5 ± 0.5	0.98
Serum phosphorus (mg/dL)	4.5 ± 1.2	5.1 ± 1.4	0.085

Data are represented as mean ± SD. *Kt/v* urea clearance, *MCH* Mean cell haemoglobin, *MCHC* Mean cell haemoglobin concentration, *MCV* Mean cell volume, *rHuEpo* recombinant human erythropoietin

	Haemoglobin		p Value
Albumin (g/dL)	3.6 ± 0.4	3.6 ± 0.3	0.39
C-reactive protein (mg/dL)	0.35 ± 0.29	0.31 ± 0.37	0.61

Data are represented as mean ± SD. *Kt/v* urea clearance, *MCH* Mean cell haemoglobin, *MCHC* Mean cell haemoglobin concentration, *MCV* Mean cell volume, *rHuEpo* recombinant human erythropoietin

Change In Haemoglobin

Thirty-seven (45%) patients showed increase in haemoglobin (> 0.1g/dL) at 3 months, whereas haemoglobin did not increase in 46 patients. There were no significant differences in haptoglobin, transferrin saturation, ferritin, incidence of iron deficiency and intact parathyroid hormone between the 2 groups, but presence of anaemia, reticulocyte, erythropoietin resistance index, rHuEpo dose, frequency of iron supplementation and erythrocyte creatine were significantly higher in patients with increase in haemoglobin compared to those without (Table 3). Erythrocyte creatine had a fair correlation with percent change in haemoglobin ($r = 0.54$, $p < 0.001$)(Fig. 3), but reticulocyte was not related to percent change in haemoglobin ($r = 0.26$, $p = 0.02$). To determine the important variables related to increase in haemoglobin, 8 variables (rHuEpo dose, erythropoietin resistance index, C-reactive protein, intact parathyroid hormone, incidence of iron deficiency, presence of anaemia, erythrocyte creatine and reticulocyte) were used in the multivariate logistic analysis. As a result, erythrocyte creatine emerged as the most independent variable associated with increase in haemoglobin (Chi-square = 6.19, P = 0.01) (Table 4).

Table 3
Comparison between patients with and without increase in haemoglobin

	Increase in haemoglobin		p Value
	yes (n = 37)	no (n = 46)	
Age (years)	73.7 ± 14.5	70.2 ± 14.5	0.28
Gender (male/female)	22/15	34/12	0.24
Haemodialysis time (hours/week)	15.6 ± 3.6	16.2 ± 4.6	0.57
Intradialytic ultrafiltration rate (ml/hour/kg)	8.7 ± 2.7	8.9 ± 3.2	0.78
Kt/v	2.0 ± 2.0	1.7 ± 0.5	0.34
Erythrocyte creatine (μmol/gHb)	2.04 ± 0.64	1.52 ± 0.39	0.0001
rHuEpo dose (units/kg/week)	125.3 ± 67.8	88.8 ± 50.2	0.006
Erythropoietin resistance index (units/kg/week/g/dL)	13.8 ± 8.7	8.4 ± 5.2	0.0008
Anaemia (present/absent)	19/18 (51%)	8/38 (17%)	0.001
Haemoglobin (g/dL)	9.73 ± 1.34	10.79 ± 0.31	0.0001
Haemoglobin at 3 months (g/dL)	10.47 ± 1.35	10.17 ± 1.03	0.25
Reticulocyte (%)	16.7 ± 4.4	14.4 ± 5.0	0.02
Haptoglobin (g/dL)	88.5 ± 51.1	97.3 ± 50.4	0.46
Transferrin saturation (%)	27.8 ± 17.1	27.8 ± 15.6	0.99
Ferritin (ng/mL)	113.6 ± 101.3	127.5 ± 111.2	0.56
Iron supplement (present/ absent)	26/11 (70%)	21/25 (45%)	0.02
Iron deficiency (present/ absent)	12/25 (32%)	10/36 (22%)	0.27
Intact parathyroid hormone (pg/mL)	160.86 ± 109.4	145.50 ± 96.27	0.49
C-reactive protein (mg/dL)	0.32 ± 0.37	0.32 ± 0.31	0.96

Data are represented as mean ± SD. Increase in haemoglobin; haemoglobin from baseline to 3 months > 0.1g/dL, *Kt/v* urea clearance, *rHuEpo* recombinant human erythropoietin

Table 4
Factors related to increase in haemoglobin

	Chi-square	p Value
rHuEpo dose	4.96	0.03
Erythropoietin resistance index	5.63	0.02
C-reactive protein	0.40	0.53
Intact parathyroid hormone	0.08	0.78
Iron deficiency	0.09	0.76
Anaemia	0.02	0.89
Erythrocyte creatine	6.19	0.01
Reticulocyte	3.13	0.08

rHuEpo recombinant human erythropoietin

Discussion

Primary mechanism of renal anaemia is inadequate erythropoietin production by the kidney [1]. Treatment of rHuEpo is effective in correcting anaemia and improves quality of life, exercise tolerance and left ventricular hypertrophy, which leads to better prognosis [20–25]. Therefore, it is important to establish a monitoring system to evaluate the ameliorative effectiveness of rHuEpo in patients receiving rHuEpo treatment. In the present study, we measured erythrocyte creatine with other haematologic indices in patients receiving maintenance haemodialysis on rHuEpo treatment and found that patients with anaemia (haemoglobin < 10g/dL) had significantly higher erythrocyte creatine and were on higher dose of rHuEpo administration as compared without anaemia (haemoglobin > 10g/dL). Moreover, erythrocyte creatine was found to be the most important marker related to the ameliorative effectiveness of rHuEpo at 3 months follow-up.

An excellent correlation between erythrocyte creatine and direct measurement of red blood cell survival time by ^{51}Cr method has been reported [12, 13]. In patients with normal erythropoietic capacity such as intravascular haemolysis and haemolytic anaemia, red cell production is accelerated in proportion to the amount of erythrocyte destruction, which lead to an increase in the young erythrocytes containing higher erythrocyte creatine levels [26–29]. In contrast, renal anaemia is mainly caused by decreased erythoropoietic capacity due to inadequate erythropoietin production and/or hypoesponsiveness to rHuEpo treatment [6, 30]. In haemodialysis patients with renal anaemia, erythrocyte creatine is regarded as an index of erythropoiesis rather than erythrocyte age. On the basis of this concept, we measured erythrocyte creatine to evaluate erythropoietic capacity in patients undergoing chronic haemodialysis. Despite no significant differences in haptoglobin, transferrin saturation, ferritin, C-reactive protein and parathyroid hormone between patients with anaemia and those without anaemia, rHuEpo dose and

erythrocyte creatine were significantly higher in patients with anaemia compared to those without. Moreover, a significant positive relation was observed between rHuEpo dose and erythrocyte creatine. Therefore, erythropoiesis is accelerated in response to high dose of rHuEpo treatment in haemodialysis patients with renal anaemia due to inadequate erythropoietin production.

The optimal frequency of follow up testing in patients with anaemia is still not known. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommended to measure haemoglobin at least every 3 months in patients with anaemia. However, haemoglobin should be monthly checked in patients with erythropoiesis stimulating agent initiation for dose adjustments to assess its response [15]. Beguin et al. [30] reported 2 groups of responder to rHuEpo treatment; respond within 3 months after baseline examination (early responders) and respond 3–6 months after baseline examination (late responders). In clinical practice, it is proposed that at least 1–2 months is needed to observe a significant response to rHuEpo by monitoring the erythrocyte indices; haemoglobin or haematocrit [15, 16]. However, due to the long life-span of mature red blood cell, erythrocyte indices do not provide information of the rapid change in erythropoietic activity. Reticulocyte is the first form of red blood cell that enters circulation and reflects present erythropoiesis, but the measurement of total reticulocyte count has seldom been used in a clinical practice because erythrocyte has a rapid turn-over in the circulation (1–2 days) [9, 12]. Although reticulocyte was significantly higher in patients with increase in haemoglobin compared to those without in this study, reticulocyte was not related to percent change in haemoglobin. Moreover, reticulocyte was not an independent variable associated with increase in haemoglobin by the multivariate logistic analysis. Therefore, there is a need to seek a reliable marker to assess the effectiveness of rHuEpo treatment. Erythrocyte creatine with a single blood sample measurement reflects average or cumulative erythropoiesis up to the present [18, 19]. We measured erythrocyte creatine and observed the change in haemoglobin for 3 months by keeping the same dose of rHuEpo during this period. Despite no significant relation between reticulocyte and percent change in haemoglobin, erythrocyte creatine had a fair correlation with percent change in haemoglobin. Patients with a higher erythrocyte creatine exhibited a larger improvement of anaemia 3 months later, indicating that erythrocyte creatine could predict accelerated erythropoiesis by rHuEpo treatment.

Erythropoietin resistance index, a useful marker of increased mortality in patients with haemodialysis treatment, is reported to be an index of hyporesponsiveness to rHuEpo at the time of examination. The reasons for erythropoietin hyporesponsiveness other than inadequate erythropoietin production are still not known but several factors could be involved, such as iron deficiency, inflammation, hyperparathyroidism or bone marrow dysfunction [6]. Iron deficiency, one of the main causes of hyporesponsiveness to rHuEpo, has been widely investigated [30.31]. In this study, incidence of iron deficiency evaluated by transferrin saturation and serum ferritin was not different between patients with favorable response (increase in haemoglobin) and those with hyporesponsiveness (no increase in haemoglobin) to rHuEpo, because patients with absolute iron deficiency has been treated by iron supplements. Moreover, there were no differences in haptoglobin, C-reactive protein, serum ferritin and intact parathyroid hormone between patients with favorable response and those with hyporesponsiveness to rHuEpo. By taking into account of these factors in the multivariate analysis,

erythrocyte creatine emerged as the most important variable related to the increase in haemoglobin. Considering the report by Takemoto et al. [19], our data indicate that accelerated erythropoiesis by rHuEpo was responsible for the improvement of anaemia and hence, erythrocyte creatine was a useful marker reflecting ameliorative effectiveness of rHuEpo.

Clinical implications

As erythrocyte creatine is a good indicator of the erythropoietic response to rHuEpo, early detection of therapeutic efficacy of rHuEpo can be achieved. In patients with a low erythrocyte creatine value, dose of rHuEpo should be increased early in the course of treatment without waiting for 3 months.

Limitations

Two limitations of this study should be addressed. First, this study was limited by a small number of patients. Further studies in a large group of haemodialysis patients are warranted to confirm our data. Second, the effect of rHuEpo treatment is diminished by insufficient available iron supply [30,31]. Absolute iron deficiency is defined as a decrease in total body iron stores and functional deficiency exhibits defective iron mobilization/utilization that cannot keep pace with the demands for the accelerated erythropoiesis regardless of the degree of iron reserve [30]. Reticulocyte haemoglobin content and high-fluorescence reticulocyte count are shown to be accurate predictors of iron-deficient erythropoiesis in patients with rHuEpo treatment [31-33], but we did not measure reticulocyte haemoglobin content and high-fluorescence reticulocyte count to evaluate the functional iron-limited erythropoiesis. However, intravenous and oral iron supplements were given as needed to avoid possible functional iron deficiency.

Conclusion

Erythrocyte creatine, useful marker of erythropoietic capacity, is a reliable marker to estimate the ameliorative effectiveness of rHuEpo in patients undergoing chronic haemodialysis.

Abbreviations

rHuEpo, Recombinant human erythropoietin

Declarations

Ethics approval and consent to participate: This study was approved by the ethics committee of Takarazuka Hospital and permission to conduct the study was obtained from Takarazuka Hospital included in the study. All participants of the study were informed about the study, and they gave written informed consent to be included in the study. All methods were performed in accordance with the relevant guidelines and regulations by including a statement. Research involving human participants or human data was performed in accordance with the Declaration of Helsinki.

Consent for publication: The patients signed an informed consent from for the publication of their data.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: S.H., S.N.: data interpretation and manuscript writing. T.S.: study design and study protocol writing. Y.T.: data collection and statistical analyses. N.T. and K.M.: data interpretation and critical editing of the text and the figures. T.S.: measuring erythrocyte creatine, technical advice and providing the information to analyse collected data. M.B.: participant recruitment and patients' management. I.S.: conceptual and methodological support to the all of this work. All authors read and approval the final manuscript.

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Figures

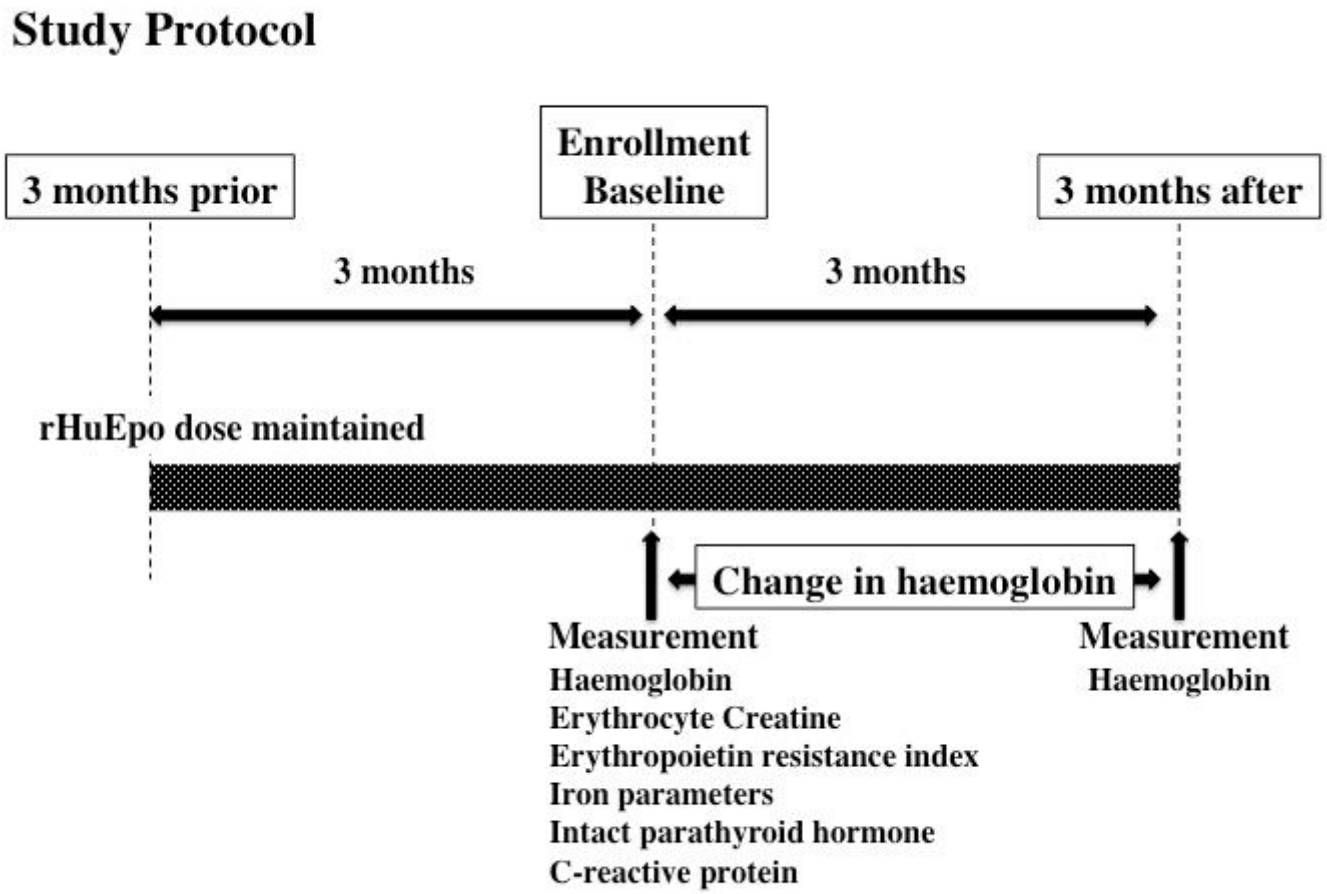

Figure 1

Study protocol. Recombinant human erythropoietin (rHuEpo) treatment was fixed 3 months prior to the enrollment and was maintained during 6 months period. Laboratory tests were performed at baseline and haemoglobin was measured 3 months later.

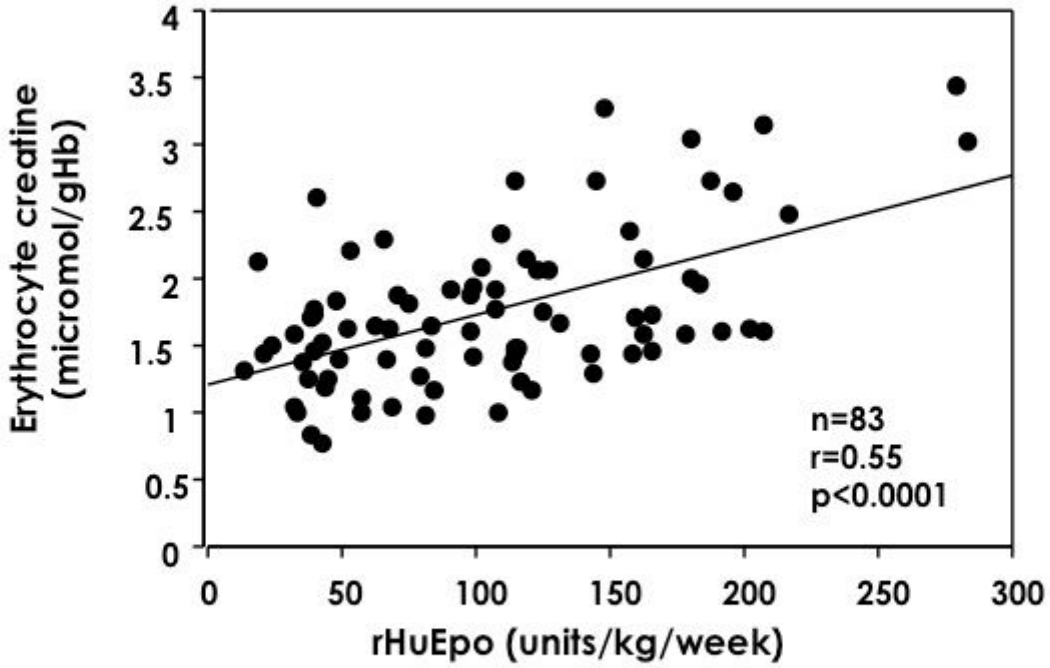

Figure 2

Relation between dose of recombinant human erythropoietin (rHuEpo) and erythrocyte creatine.

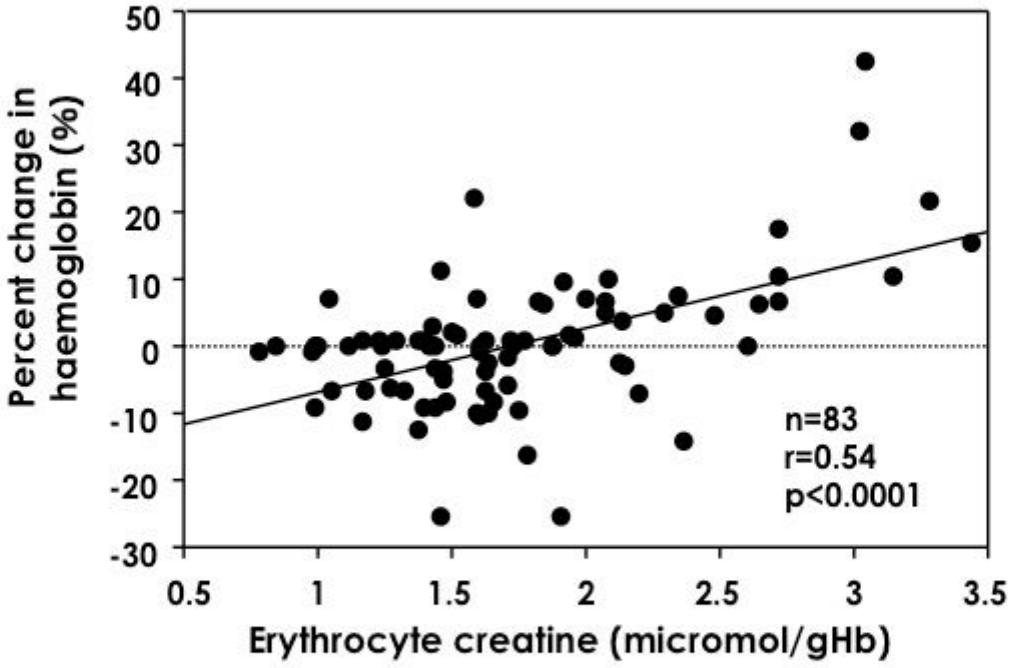

Figure 3

Relation between erythrocyte creatine and percent change in haemoglobin. Percent change in haemoglobin: 3 months minus baseline/ baseline x 100 (%).