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Original Article

The haematopoietic effect of recombinant human erythropoietin in haemodialysis is independent of the mode of administration (i.v. or s.c.)

Gert De Schoenmakere[1], Norbert Lameire[1], Annemieke Dhondt[1], Anne Van Loo[1], Jan Van der Goten[2], Philippe Duym[1] and Raymond Vanholder[1]

[1]Renal Division, Department of Internal Medicine, University Hospital, Gent, and [2]Janssen/Cilag, Berchem, Belgium

Abstract

Background. Previous studies comparing intravenous (i.v.) and subcutaneous (s.c.) administration of recombinant human erythropoietin (rHuEpo) often did not achieve optimal iron reserve, were restricted to a limited follow-up period (not allowing equilibration) and/or did not exclude the role of other confounding factors. In addition all papers focused on the conversion from i.v. to s.c.

Methods. In this study, 30 equilibrated patients on s.c. rHuEpo were randomized into two groups, one converting to i.v. after 6 months of follow-up and one remaining on s.c. rHuEpo. In both groups rHuEpo was administered three times weekly. Only patients completing a further 6 months follow-up were considered for statistical evaluation. Serum ferritin was targeted at 200 ng/ml and haematocrits between 28 and 36% were pursued.

Results. The average haematocrit levels before conversion were $31.9 \pm 1.1\%$ in the conversion group and $31.4 \pm 1.6\%$ at the same time point in the non-conversion group ($P = \text{NS}$). After 6 months haematocrits were $31.5 \pm 0.5\%$ in the conversion group and $31.1 \pm 0.9\%$ in the non-conversion group ($P = \text{NS}$). Ferritin concentration in the conversion group was 219 ± 49 ng/ml before and 230 ± 83 ng/ml after the conversion. For the non-conversion group ferritin was 224 ± 25 ng/ml and 236 ± 52 ng/ml respectively ($P = \text{NS}$). The weight-standardized average rHuEpo dose per injection remained the same in the conversion group before and after conversion (44.0 ± 1.8 U/kg/injection *vs* 45.4 ± 4.7 U/kg/injection) ($P = \text{NS}$). In the non-conversion group the corresponding rHuEpo doses were 32.9 ± 4.2 U/kg/injection and 39.6 ± 7.0 U/kg/injection respectively ($P = \text{NS}$). There were no differences in serum PTH, aluminium, vitamin B_{12}, folic-acid levels, and intake of co-trimoxazole, ACE inhibitors or theophylline.

Conclusion. No changes in rHuEpo dose were observed after conversion from s.c. to i.v. There were no significant differences between the conversion and non-conversion group. These results are in contrast to some earlier studies suggesting lower rHuEpo requirements in case of s.c. administration.

Key words: anaemia; erythropoietin; intravenous erythropoietin; iron; subcutaneous erythropoietin

Introduction

A frequent and hazardous complication of end-stage renal disease (ESRD) is anaemia. The responsible mechanisms are varied and complex. First, the red cell life span is shorter in uraemic patients compared to individuals with normal renal function [1]. Second, the decrease in renal mass and the subsequent deterioration of endogenous erythropoietin production inhibits virtually all steps of erythropoiesis [1,2]. Finally, increased haemorrhagic diathesis (due to coagulation disturbances and anticoagulation) and decreased bone marrow response to endogenous erythropoietin might contribute in the generation of anaemia [1,3,4].

The administration of recombinant human erythropoietin (rHuEpo) in pharmacological quantities enabled a more efficient therapy of ESRD-induced anaemia, significantly reducing the need for blood transfusions [5]. Moreover, adequate rHuEpo substitution improved quality of life and patient well-being [6–8].

Until now, numerous publications have addressed the question whether the subcutaneous (s.c.) or intravenous (i.v.) administration route of rHuEpo affected the adequacy of the treatment [9–21]. All of these studies, however, focused on the switch from i.v. to s.c. administration and in some of them the target ferritin was 100 µg/l [9,16,19], whereas in other studies no target iron level was given. Furthermore, some studies were conducted over a limited period [12,16,18].

The aim of the present study was to examine prospectively whether the conversion from s.c. to i.v. rHuEpo administration influences the haematological

Correspondence and offprint requests to: R. Vanholder Renal Division, Department of Internal Medicine, University Hospital, De Pintelaan 185, B-9000 Gent, Belgium.

parameters and rHuEpo dosage, provided an adequate iron status was maintained.

Subjects and methods

Patient selection and treatment

Patients, older than 18 years, on a regular haemodialysis schedule, treated with s.c. rHuEpo and with a stable haematocrit between 28 and 36% for at least 3 months, were enrolled. Exclusion criteria were patients with polycystic kidney disease, chronic inflammatory disease, bleeding disorders and chronic drug therapy interfering with coagulation (e.g. coumarin derivatives, ticlopidine). From all patients under treatment in the haemodialysis unit of the University Hospital Gent ($n=60$), 30 patients were found to conform with the above-mentioned inclusion criteria. The selected patients were randomized into two groups at the start of the study: a conversion (C) group ($n=15$) received a 6-month rHuEpo therapy using the s.c. pathway, followed by a 6 month period of i.v. rHuEpo treatment; a non-conversion group ($n=15$) received a 12-month course of s.c. rHuEpo. Only patients that completed an entire 1-year follow-up period were considered for statistical evaluation. Drop-out criteria were defined as death, renal transplantation, transition to continuous ambulatory peritoneal dialysis (CAPD), major surgery necessitating hospitalization for >1 week, and transfer to another dialysis centre. Acute infectious episodes were not considered as a reason to withdraw from follow-up. In the conversion group, four patients were excluded during the study period, due to death ($n=2$), major surgery ($n=1$), and transfer to another dialysis centre ($n=1$). Eight drop-outs were noted in the non-conversion group for reasons of renal transplantation ($n=3$), death ($n=2$), transfer to another centre ($n=2$) and major surgery ($n=1$). Consequently, 11 patients in the conversion group and 7 patients in the non-conversion group were submitted to analysis.

The time of conversion in the conversion group was arbitrarily determined as time point 0. Months preceding the switch were labelled -6 to -1, months after the switch were labelled $+1$ to $+6$. For reasons of similarity, the same definitions were used in the non-conversion group.

Both s.c. and i.v. rHuEpo were administered three times weekly, at the end of the dialysis session. In the conversion group, the dosage of the first i.v. administration was the same as that of the last s.c. injection. Eprex® (Epoetin Alfa, Janssen-Cilag, Schaffhausen, Switzerland) was used for both treatment modalities.

Doses of rHuEpo in each patient were revised monthly, or whenever the clinician in charge of the dialysis department considered it necessary, based on the targeted haematological parameters. Haematocrit levels between 28 and 36% were pursued.

Serum ferritin was taken as the main indicative parameter for iron status [23–24]. Throughout the entire study period, target serum ferritin levels were 200 ng/ml. Intravenous iron (Venoferrum®, Vifor Int. Inc., St Gallen, Switzerland) was administered whenever the iron status was deficient or when at least two successive haematocrit levels $<25\%$ were registered, in spite of ferritin values between 200 and 400 ng/ml (the latter situation reflecting functional iron deficiency). In addition, medication other than rHuEpo was kept as constant as possible and the patient medication lists were examined on possible drug interference with erythropoiesis (e.g. ACE-inhibitors, co-trimoxazole, theophylline [25]).

Haemodialysis characteristics

All patients were dialysed three times weekly for 3–4 h by pressure–pressure monitored single-needle bicarbonate haemodialysis. Blood flows and dialysate flows of 220–270 ml/min and 500 ml/min respectively were pursued.

Patient characteristics

The following characteristics were registered: gender, age, time since start of haemodialysis, protein catabolic rate (PCR), ratio of total urea clearance over its distribution volume (Kt/V), residual creatinine clearance (Ccrea), and the primary renal diagnosis.

Therapeutic and biochemical parameters

The mean dose of rHuEpo per injection was registered monthly and normalized for body weight. The effect of rHuEpo on erythropoiesis was quantified by monthly measurements of haematocrit levels and red blood cell counts.

Iron status was evaluated by monthly determinations of serum Fe^{2+}, total iron binding capacity (TIBC), serum ferritin, and transferrin saturation (TS).

As additional indicators of possible interference with rHuEpo stimulated erythropoiesis were considered: C-reactive protein (CRP), serum protein, albumin, parathormone (PTH), vitamin B_{12}, extracellular and intracellular folic acid and serum aluminium. These data were collected bi-monthly according to standard techniques.

Aluminium accumulation was evaluated by performing a desferrioxamine test (Desferal®, Ciba-Geigy, Basel, Switzerland), 3 months before and after the switch. Desferrioxamine (1500 mg) was administered i.v. through the arteriovenous fistula or central venous access catheter during the last 30 min of the haemodialysis session [26]. Serum aluminium levels were measured immediately before and 44 h after the desferrioxamine administration. The test was considered to be indicative of aluminium accumulation when serum aluminium rose to >100 µg/l (normal values 0–14 µg/l) or if a fourfold increase was observed. No i.v. iron was administered when the desferrioxamine-test was performed.

Statistical analysis

Data were stored and processed using the Unistat® Statistical Package version 3.0a, © Unistat Ltd. 1984–1995, and analyses were performed by the Wilcoxon signed rank test, the Mann–Whitney U test and variance analysis where appropriate. Significance was accepted for $P\leqslant 0.05$.

Results

There were no significant differences in gender, age, time since start of haemodialysis, PCR, Kt/V and Ccrea between both groups when considering only the patients completing the study (Table 1).

The overall mean doses per injection, when averaging all data collected, before and after time point 0 in the conversion group were 44.0 ± 1.8 and 45.4 ± 4.7 U/kg/injection respectively($P=$NS). In the non-conversion group, these averages were 32.9 ± 4.2

Table 1. Patient characteristics at the time of the study start

	Conversion group	Non-conversion group
Patients studied	11	7
Male/female	5/6	3/4
Age	61 ± 17.4 years	65 ± 11.5 years
Time on haemodialysis	1.1 ± 0.87 years	2.1 ± 2.96 years
Protein catabolic rate (PCR)	0.9 ± 0.25 mg/(kg.24 h)	1.0 ± 0.24 mg/(kg.24 h)
Ratio of total urea clearance over its distribution volume (Kt/V)	1.1 ± 0.21	1.3 ± 0.22
Residual creatinine clearance	0.2 ± 0.31 ml/min	2.2 ± 2.72 ml/min
Primary diagnosis of studied patients	Chronic interstitial nephritis (5) Renovascular disease (3) IgA nephropathy (2) Chronic glomerulonephritis (1)	Chronic interstitial nephritis (3) Renovascular disease (3) Chronic glomerulonephritis (1)

and 39.6 ± 7.0 U/kg/injection respectively (P = NS). The weight-standardized rHuEpo doses at monthly intervals in both study groups are illustrated in Figure 1. There were no differences between the conversion group and the non-conversion group at similar time points. When compared to time 0, all other time points in the conversion group showed no significant differences (Figure 1a). In the non-conversion group (Figure 1b), the dose was significantly higher at 3 and 4 months after time point 0 ($P \leqslant 0.05$). The cumulative rHuEpo doses over the whole study period for both groups are compared in Figure 2. No significant differences were observed.

The overall mean haematocrits when averaging all data collected were similar between conversion and non-conversion groups in the period preceding time point 0 (32.9 ± 1.1% *vs* 31.4 ± 1.6%) and in the 6 months after time point 0 (31.5 ± 0.5% *vs* 31.1 ± 0.9%). Haematocrits were similar at each time point for both groups ($P > 0.05$) (Figure 1). For the conversion group (Figure 1a), haematocrit values at all time points compared to the moment of switch showed no difference. In the non-conversion group, haematocrits were lower at time points −6, −5, 1 and 2 ($P \leqslant 0.05$) than at time point 0 (Figure 1b). A similar pattern was seen for red blood cell counts (data not shown).

The overall mean ferritin, when averaging all data collected, in the conversion group was 219 ± 49 ng/ml and 224 ± 25 ng/ml, before and after conversion (P = NS). In the non-conversion group, values of 230 ± 83 ng/ml before and 236 ± 52 ng/ml after time

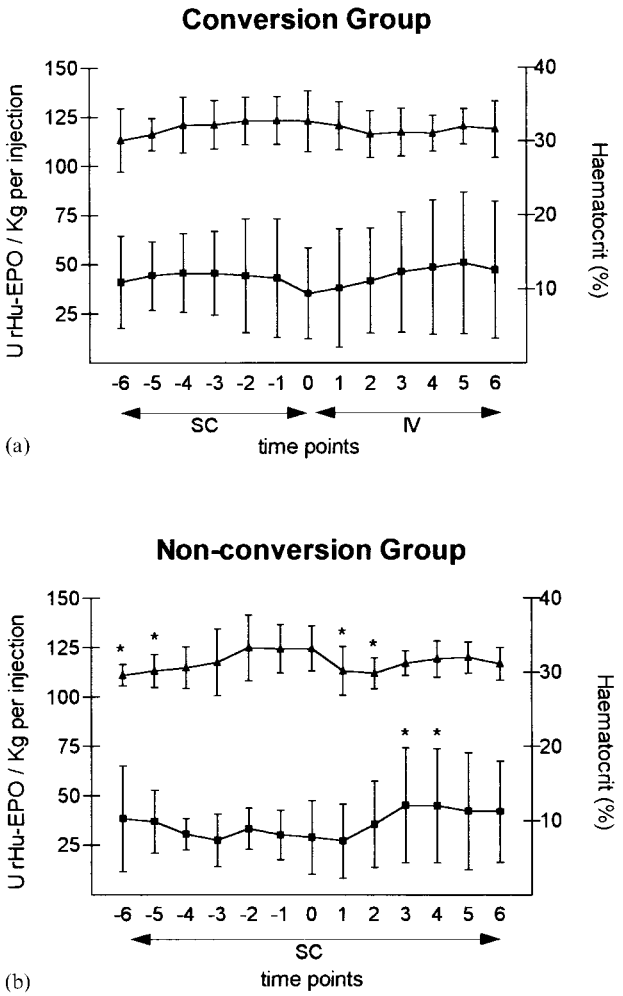

Fig. 1a,b. Evolution of rHuEpo/kg per injection (squares; left axis) and of haematocrit (triangles; right axis) (**a**) in the conversion group and (**b**) in the non-conversion group. *Statistical differences ($P < 0.05$) for both parameters *vs* time 0. The doses per injection of rHuEpo at each time were not significantly different between groups.

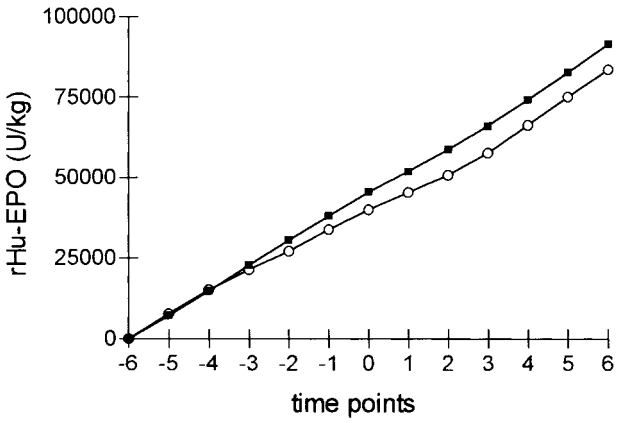

Fig. 2. Cumulative dose of rHuEpo/kg from time point −6 to 6 for the conversion group (squares) and the non-conversion group (open circles); no differences were observed between groups.

point 0 were observed. Monthly registered iron status parameters (Table 2) were not different between groups, except for an isolated higher serum ferritin in the conversion group, 4 months after the conversion. No differences in iron status parameters were observed in the conversion group as compared to time 0. In the non-conversion group, significance was observed for TIBC at time points -1, 2 and 5 and for ferritin at time points -2 and -1 ($P<0.05$).

CRP, serum protein, albumin, PTH, vitamin B_{12}, intracellular and extracellular folic acid and aluminium were not different in both groups. Comparison of drug intake demonstrated a similar number of patients treated by ACE-inhibitors (3 *vs* 2); there were no patients treated with co-trimoxazole and theophylline. There was no significant difference in the number of infectious episodes between conversion and non-conversion groups during the entire study period (4 and 2 respectively).

Aluminium mobilization tests with desferrioxamine were negative in all patients of the two groups. Mean aluminium levels before and 44 h after desferrioxamine were comparable (data not shown).

Discussion

This prospective study was performed to evaluate whether the transition from s.c. to i.v. rHuEpo was feasible without adaptations in rHuEpo dose, provided an adequate iron status was maintained. Under these conditions, equivalence of both the s.c. and the i.v. route was demonstrated (Figures 1 and 2).

The question of whether s.c. rHuEpo necessitates dose modification has been considered in various original or review papers [9–21]. The conclusions were, however, inconsistent. Some authors indicate a superiority of the s.c. route [14–15,17,21], allowing dose-reduction varying from 14.5 to 70%, thus resulting in a considerable improvement of the cost-effectiveness ratio.

An essential element, underestimated in some of these earlier studies, is the fact that increased haemoglobin synthesis under rHuEpo administration results in an enhanced iron demand [27]. Thus absolute or functional iron deficiency, the latter characterized by a defective response of haematocrit in spite op serum ferritin >200 ng/ml, can arise in the course of the therapy. It is of interest to note that Hörl first demonstrated that s.c. administration of rHuEpo allowed a considerable dose reduction in comparison to the i.v. route [20], confirming data reported earlier by Bommer *et al.* [9]; afterwards this viewpoint was tempered, because further investigations by the same group indicated an equivalence of the two administration modes, provided i.v. iron supplements were given [22]. It remains unclear whether the observation of differences between s.c. and i.v. administration of rHuEpo in the earlier studies should be attributed to a differential effect of the route of administration rather than to alterations in iron status during the follow-up period, as in several studies relatively low target iron levels were pursued, whereas in other studies these target values are not mentioned (Table 3). The stringent limitations imposed on ferritin levels and the absence of significant changes in TS and serum iron in the present study (Table 2) combined with the timely supply with i.v. iron guaranteed a steady and sufficient iron reserve; therefore no capital role can be attributed to this confounding factor.

In some studies, follow-up periods may have been too short to allow equilibration of the response to rHuEpo [12,16,18]. It has been demonstrated that rHuEpo induces both a stimulation of red blood cell precursor maturation [28,29] and an increased survival of erythrocytes [29,30]. As a consequence, it can be estimated that a new stabilization point is only reached after several weeks, upon equilibration of these two

Table 2. Iron parameters (serum iron, TIBC, ferritin, and TS)

Time	Serum Fe^{2+} (μg/dl)		TIBC (μg/dl)		Ferritin (ng/ml)		TS (%)	
	Conv	Non-conv	Conv	Non-conv	Conv	Non-conv	Conv	Non-conv
−6	44±19	57±16	221±64	227±39	180±102	236±114	19.8±7.0	26.0±9.3
−5	48±18	50±21	219±45	216±32	192±120	179±102	22.1±7.4	23.2±9.8
−4	47±17	57±15	225±43	229±34	193±100	175±95	20.8±6.8	25.9±10.0
−3	58±20	61±12	238±54	248±29	273±101	277±114	24.4±7.3	25.2±7.2
−2	64±20	60±20	237±47	248±27	187±86	143±65*	27.6±7.4	24.2±8.0
−1	53±15	52±17	222±37	224±27*	290.1±91	369±132*	24.1±6.4	23.9±8.6
0	63±40	61±31	235±29	237±28	216±111	242±81	27.7±18.0	25.7±12.0
1	59±2.9	57±23	234±41	239±26	248±208	271±32	24.8±8.7	24.1±9.5
2	55±15	55±18	245±44	250±32*	211±107	318±218	23.1±7.2	21.8±6.5
3	55±20	55±16	246±43	246±33	254±100	199±110	22.8±8.7	22.5±4.8
4	57±22	51±14	242±48	243±28	233±58	181±51°	23.6±8.0	20.9±4.0
5	49±13	60±18	240±44	249±26*	206±75	243±79	20.9±6.7	24.3±6.3
6	51±12	52±17	252±41	245±20	194±68	205±44	20.6±5.0	21.3±6.2

Significance of time points *vs* time 0 is indicated by *($P<0.05$); significance between groups is indicated by °($P<0.05$). Conv, conversion group; Non-conv, non-conversion group.

Table 3. Review of literature data (original papers)

Author, year [ref]	Number (s.c./i.v.)	Time period (months)	Iron levels pursued
Grannoleras *et al.*, 1989 [10]	4/4	5	NM
Bommer *et al.*, 1991 [9]	16/16	12	100 μg/l***
Stockenhuber *et al.*, 1991 [18]	6/6	3	NM
Tomson *et al.*, 1992 [12]	9/9	3	150 μg/l*
Muirhead *et al.*, 1992 [14]	45/38	6	NM
Eidemak *et al.*, 1992 [19]	9/11	4	100 μg/l*
Besarab *et al.*, 1992 [17]	28/28	3	NM
Taylor *et al.*, 1994 [16]	16/16	2	100 μg/l*
Schaller *et al.*, 1994 [21]	44/46	8	NM
Paganini *et al.*, 1995 [15]	72/72	3	20%**

*Serum ferritin; **transferrin saturation; ***ferritin levels markedly different during s.c. and i.v. Epo period.

determining factors. In the present study, we respected a 12 month follow-up period equally divided before and after the switch. This provided enough time for the response to rHuEpo to reach a stabilization point. In addition, no differences among the two groups were found regarding other factors that potentially could interfere with the metabolism/incorporation of iron and/or the response to rHuEpo (aluminium overload, inflammatory status, secondary hyperparathyroidism, vitamin B_{12} and folic acid deficiency and ingestion of ACE-inhibitors, co-trimoxazole and theophylline).

In contrast to the present study, all papers already published examined the switch from the i.v. to the s.c. route. Undoubtedly, the rationale for this can be found in the fact that the s.c. administration route was initially applied in outpatients and CAPD patients, and only afterwards implemented in haemodialysed patients. Nevertheless the possible disadvantages associated with s.c. administration (e.g. difficulty in delivering higher doses, pain at the site of injection) and/or the conversion of outpatients and CAPD patients to haemodialysis might necessitate a switch from s.c. to i.v. This study suggests that such a conversion is possible without major adaptation in rHuEpo dose.

The question arises as to whether the sequence of conversion influences the response to rHuEpo. To rule out this possible bias, we performed an order of conversion that was the reverse of that in all other studies in this field. In addition, the possibility should be considered that when a s.c. study arm follows an initial period of i.v. rHuEpo, optimal equilibration of iron status is achieved only during the i.v. study arm. This would allow a reduction in dose during the s.c. period. Along these lines, a reverse order of conversion may result in equilibration near the end of the s.c. arm and subsequently lead to a relative decrease in i.v. rHuEpo. However, in our study design, iron dysequilibrium was excluded by enrolling only patients with stable iron status during the 3 months preceding the study start, and by closely monitoring the iron status parameters.

Several pharmacokinetic studies [17–18,27,31–32] have demonstrated a marked difference in bioavailability of rHuEpo depending on the route of administration: the area under the curve (AUC)—as measure for bioavailability—of the s.c. route shows a high inter-patient variability and is only 50% of the AUC of the i.v. pathway after a single administration of rHuEpo [17]. In spite of this difference, similar dose–response curves are observed for both administration modes. It is hypothesized that this effect originates from the absence in the s.c. route of peak levels (which are believed to be only partially effective) [17], combined with a higher utilization rate by target cells.

The results of this study need to be considered with the necessary care as it was undertaken on a limited number of patients. To allow definite conclusions, the present protocol should be reconsidered in a multicentre setting, in view of the possible β-error. Several earlier studies regarding this issue have, however, been undertaken on similar or only slightly larger patient groups (Table 3). The shift from i.v. to s.c. in the present study induced hardly any change in Epo dose or haematocrit. A change, if any, was observed in the non-conversion group. The question could be raised of whether the differences found, even if they would become statistically significant in a sufficiently large sample, would be of clinical relevance.

Erythropoietin need was slightly but non-significantly higher in the non-conversion group, compared to the conversion group. We have no readily available explanation for this difference, as the most relevant confounding factors (aluminium, vitamin B and iron status, parathyroid hormone, indices of inflammation) were similar in both groups. It is of note that the most important difference presented in the period before month 0, whereas after conversion (i.e. the period of interest), the average difference only amounted to 5.8 U/kg per injection.

Although an optimal iron status was pursued in this study, one must be cautious to attribute the present results to the iron administration. To prove the role of iron, a group of patients receiving inadequate quantities of iron should have been included.

In conclusion, in the present trial no changes in rHuEpo demand were observed when conversion occurred from s.c. to i.v. There were no significant differences between the conversion and the non-conversion groups. These results are different from some earlier papers suggesting a lower rHuEpo need when administration is performed subcutaneously.

References

1. Lusvarghi E. Hematopoiesis in renal failure patients. *J Nephrol* 1995; 8: 79–86
2. Jensen JD, Madsen JK, Jensen LW, Pedersen EB. Reduced production, absorption, and elimination of erythropoietin in uremia compared with healthy volunteers. *J Am Soc Nephrol* 1994; 5: 177–185
3. Gawaz MP, Bogner C, Gurland HJ. Flow-cytometric analysis of mepacrine-labelled platelets in patients with end-stage renal failure. *Haemostasis* 1993; 5: 284–292
4. Erslev AJ, Besarab A. The rate and control of baseline red cell

production in hematologically stable patients with uremia. *J Lab Clin Med* 1995; 3: 283–286
5. Eschbach JW, Abdulhadi MH, Browne JK. Recombinant human erythropoietin in anemic patients with end-stage renal disease. Results of a phase III multicenter clinical trial. *Ann Intern Med* 1989; 111: 992–1000
6. Lundin AP, Akerman MJH, Chesler RM *et al*. Exercise in haemodialysis patients after treatment with recombinant human erythropoietin. *Nephron* 1991; 58: 315–319
7. Park JS, Kim SB, Park SK, Lim TH, Lee DK, Hong CD. Effect of recombinant human erythropoietin on muscle energy metabolism in patients with end-stage renal disease: A [31]P-nuclear magnetic resonance spectroscopic study. *Am J Kidney Dis* 1993; 6: 612–618
8. Levin NW. Quality of life and haematocrit level. *Am J Kidney Dis* 1992; 20 [Suppl. 1]: 16–20
9. Bommer J, Barth H-P, Zeier M *et al*. Efficacy comparison of intravenous and subcutaneous recombinant human erythropoietin administration in haemodialysis patients. *Contrib Nephrol* 1991; 88: 136–143
10. Granolleras C, Branger B, Beau MC, Deschodt G, Alsabadini B, Shaldon S. Experience with daily self-administered subcutaneous erythropoietin. *Contrib Nephrol*; 1989: 143–148
11. Besarab A. Optimizing epoetin therapy in end-stage renal disease: the case for subcutaneous administration. *Am J Kidney Dis* 1993; 22 [Suppl. 1]: 13–22
12. Tomson CRV, Feehally J, Walls J. Crossover comparison of intravenous and subcutaneous erythropoietin in haemodialysis patients. *Nephrol Dial Transplant* 1992; 7: 129–132
13. Ashai NI, Paganini EP, Wilson JM. Intravenous versus subcutaneous dosing of epoetin: a review of the literature. *Am J Kidney Dis* 1993; 22 [Suppl. 1]: 23–31
14. Muirhead N, Churchill DN, Goldstein M *et al*. Comparison of subcutaneous and intravenous recombinant human erythropoietin for anemia in hemodialysis patients with significant comorbid disease. *Am J Nephrol* 1992; 12: 303–310
15. Paganini E, Eschbach J, Lazarus J *et al*. Intravenous versus subcutaneous dosing of Epoetin Alpha in haemodialysis patients. *Am J Kidney Dis* 1995; 26: 331–340
16. Taylor JE, Belch JJ, Fleming LW, Mactier RA, Henderson IS, Stewart WK. Erythropoietin response and route of administration. *Clin Nephrol* 1994; 5: 297–302
17. Besarab A, Flaharty K, Erslev A *et al*. Clinical pharmacology and economics of recombinant human erythropoietin in end-stage renal disease: the case for subcutaneous administration. *J Am Soc Nephrol* 1992; 2: 1405–1416
18. Stockenhuber F, Loibl U, Gottsauner-Wolf M *et al*. Pharmacokinetics and dose response after intravenous and subcutaneous administration of recombinant erythropoietin in patients on regular haemodialysis treatment or continuous ambulatory peritoneal dialysis. *Nephron* 1991; 59: 399–402
19. Eidemak I, Friedberg MO, Ladefoged SD, Lokkegaard H, Pedersen E, Skielboe M. Intravenous versus subcutaneous administration of recombinant human erythropoietin in patients on haemodialysis and CAPD. *Nephrol Dial Transplant* 1992; 6: 526–529
20. Hörl WH. Optimal route of administration of erythropoietin in chronic renal failure patients: intravenous versus subcutaneous. *Acta Haematol* 1992; 87 [Suppl 1]: 16–19
21. Schaller R, Sperschneider H, Thieler H *et al*. Differences in intravenous and subcutaneous application of recombinant human erythropoietin: a multicenter trial. *Artif Organs* 1994; 8: 552–558
22. Sunder-Plassmann G, Hörl WH. Importance of iron supply for erythropoietin therapy. *Nephrol Dial Transplant* 1995; 10: 2070–2076
23. Bergmann M, Grutzmacher P, Heuser J, Kaltwasser JP. Iron metabolism under rEPO therapy in patients on maintenance hemodialysis. *Int J Artif Organs* 1990; 13: 109–112
24. Anastassiades EG, Howarth D, Howarth J *et al*. Monitoring iron requirements in renal patients on erythropoietin. *Nephrol Dial Transplant* 1993; 8: 846–853
25. Dhondt AW, Vanholder RC, Ringoir SMG. Angiotensin-converting enzyme inhibitors and higher erythropoietin requirements in chronic haemodialysis patients. *Nephrol Dial Transplant* 1995; 10: 2107–2109
26. Coburn J, Slatopolsky E. Vitamin D, parathyroid hormone, and the renal osteodystrophies. In: Brenner BM, Rector FC, eds. *The Kidney*. Saunders, Philadelphia 1991; 2036–2120
27. Kühn K, Nonnast-Daniel B, Grützmacher P. Analysis of initial resistance of erythropoiesis to treatment with recombinant human erythropoietin. *Contrib Nephrol* 1988; 66: 94–103
28. Ricci G, Masotti M, De Paoli Vitali E, Vedovato M, Bedani PL, Gilli P. Effects of the recombinant human erythropoietin (rHuEPO) administration on haematologic parameters, red cell creatine and 2,3-diphosphoglycerate contents, in patients affected by end-stage renal disease. *Biomed Pharmacother* 1991; 8: 349–352
29. Koury MJ, Bondurant MC. The mechanism of erythropoietin action. *Am J Kidney Dis* 1991; 18 [Suppl.1]: 20–23
30. Schwartz AB, Orquiza CS. The effect of recombinant human erythropoietin on mean corpuscular volume in patients with the anaemia of chronic renal failure. *Clin Nephrol* 1995; 4: 256–259
31. MacDougall IC, Roberts DE, Neubert P, Dharmasena AD, Coles GA, Williams JD. Pharmacokinetics of intravenous, intraperitoneal, and subcutaneous recombinant erythropoietin in patients on CAPD. A rationale for treatment. *Contrib Nephrol* 1989; 76: 112–121
32. Hughes RT, Cotes PM, Oliver DO *et al*. Correction of the anaemia of chronic renal failure with erythropoietin: pharmacokinetic studies in patients on haemodialysis and CAPD. *Contrib Nephrol* 1989; 76: 122–133

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