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# Effect of human recombinant erythropoietin on bleeding time, platelet number and function in children with end-stage renal disease maintained by haemodialysis

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**Abstract.** We studied platelet number and function in nine anaemic children with end-stage renal disease during a clinical trial with recombinant human erythropoietin (rHu-EPO). All the children showed a correction in both haematocrit and haemoglobin levels which was followed by a significant reduction in bleeding time. We also observed a significant increase in platelet count after both 6 and 12 weeks of therapy; at the same time mean platelet volume decreased and a normal platelet mass was maintained. The mean baseline platelet aggregation response to ADP was normal, but was decreased to collagen ($P < 0.05$ vs normal control). Platelet production of thromboxane $B_2$ in serum was also lower than normal controls. After correction of anaemia with rHu-EPO, platelet aggregation improved in patients with a decreased baseline response, and mean levels of thromboxane $B_2$ became normal. In conclusion, the treatment with rHu-EPO improved haemostatic balance not only by correcting anaemia, but also by increasing platelet count and function.

**Key words:** Recombinant human erythropoietin – Chronic renal failure – bleeding time – Platelet aggregation – Thromboxane $B_2$ – platelet volume

## Introduction

Anaemia is a predictable and severe complication of end-stage renal disease requiring regular red cell transfusions with the consequent risks of viral infection, iron overload and sensitization to HLA antigens [1]. However, recent trials have shown the efficacy and safety of recombinant human erythropoietin (rHu-EPO) in improving the uraemic anaemia [2]. Red blood cell number seems to have an important role in the pathogenesis of haemorrhage in uraemic patients [3, 4]. In fact an inverse relationship was demonstrated between haematocrit and prolongation of bleeding time [3], which seems to be the only haemostatic factor correlating with clinical bleeding [5]. Moreover, bleeding time is affected by platelet number, platelet function and by von Willebrand factor, all of which have often been described as abnormal in uraemic patients [6–8].

We studied haemostasis in nine children with end-stage renal disease and severe chronic anaemia treated with rHu-EPO. Bleeding time, platelet count, platelet aggregation and thromboxane production were related to rHu-EPO-induced improvement of anaemia.

## Patients and methods

*Patients.* Nine patients (4 males, 5 females, mean age 13 ± 3.7 years) entered the study after parental or, when appropriate, personal informed consent. All patients were on regular, haemodialysis 2–3 times/week for an average time of 24.7 months (range 2–85 months); their main clinical features are summarized in Table 1.

The criteria for rHu-EPO administration were: (1) regular hospital haemodialysis for at least 2 months; (2) post-dialysis haematocrit level below 25%; (3) age between 1 and 18 years; and (4) no other causes of anaemia

rHu-EPO was injected i.v. after each dialysis session in an increasing dose regimen at 2-week intervals, with the following schedule: 75–150, 300–450 IU/per week. The target haemoglobin (Hb) level was set for each patient on the basis of the lower end of the normal range for age and sex (mean-2SD). After reaching the target Hb value, the amount of rHu-EPO was halved to maintain the desired Hb concentration. Coagulation studies and haematocrit readings were carried out at the start of the study, and after 6 (300 IU/kg per week) and 12–16 weeks of rHu-EPO therapy (300 IU/kg per week in 6 patients and 450 IU in 2 patients). One patient (no. 6) received a transplant during the 7th week of treatment. Ferritin levels were low or normal (range 25–100 µg/l) in six patients (nos. 3–6, 8, 9) who were all treated with oral iron throughout the study; in these patients no change in ferritin was observed during treatment. The other three patients (nos. 1, 2, 7) had high basal values of serum ferritin (3300, 330, 250 µg/l) which normalized during the study.

*Methods.* Venous blood was collected pre-dialysis by clean venipuncture in polyethylene tubes containing sodium citrate 3.8% (1 : 9); the blood/citrate ratio was corrected for the haematocrit level.

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**Table 1.** Main clinical features of the patients

| Patient no. | Age (years) | Sex | Dialysis duration (months) | Bleeding diathesis | Nephropathy |
|---|---|---|---|---|---|
| 1 | 12.8 | F | 44 | None | MPGN |
| 2 | 16.2 | M | 2[a] | None | IgA |
| 3 | 18.9 | M | 85[a] | None | Reflux |
| 4 | 13.11 | F | 5[a] | None | HUS |
| 5 | 9.5 | M | 28[a] | None | Congenital/hypoplasia |
| 6 | 11.1 | F | 15 | None | Congenital/hypoplasia |
| 7 | 17.6 | F | 32[a] | Yes | Reflux |
| 8 | 9.11 | F | 10 | None | Reflux |
| 9 | 10 | M | 2[a] | None | Prune-belly |

HUS, Haemolytic-uraemic syndrome; MPGM, membrano-proliferative glomerulonephritis
[a] Haemodialfiltration

**Table 2.** Changes of main laboratory features during treatment with recombinant human erythropoietin (rHu-EPO)

| Patient no. | Haematocrit (%) | | | Bleeding time (min) | | Platelet count ($10^9$/l) | | |
|---|---|---|---|---|---|---|---|---|
| | Before | +6 weeks | +12 weeks | Before | +12 weeks | Before | +6 weeks | +12 weeks |
| 1 | 17 | 31 | 32 | 9.25 | 7.4 | 180 | 339 | 629 |
| 2 | 24 | 32 | 37 | 10 | 7.2 | 229 | 309 | 315 |
| 3 | 24 | 31 | 40 | 12 | 8 | 175 | 253 | 372 |
| 4 | 21 | 37 | 33 | 13.2 | 6.3 | 469 | 253 | 494 |
| 5 | 18 | 23 | 36 | 15 | 5.5 | 231 | 507 | 321 |
| 6 | 18 | 24 | – | 17 | – | 234 | 201 | – |
| 7 | 18 | 32 | 36 | 10.4 | 5.1 | 197 | 363 | 202 |
| 8 | 20 | 35 | 38 | 10.1 | 11.1 | 217 | 296 | 607 |
| 9 | 18 | 22 | 34 | 18.3 | 15.3 | 199 | 184 | 195 |
| Mean(SD) | 19.7 (2)** | 29.6 (5)* | 35.7 (2.4)* | 12.8 (3.1)** | 8.2 (3.2)* | 236 (84)** | 330 (112)* | 391 (157)* |
| Controls | 38.2 (2) | | | <8 | | 309 (65) | | |

* $P$ <0.05 vs before rHu-EPO values; ** $P$ <0.05 vs controls values

Platelet aggregation was performed on citrated platelet-rich plasma as previously described [9] using 2 μM ADP, 2 μg/ml collagen and 1.5 mg/ml ristocetin (Mascia Brunelli, Milan, Italy). Platelet production of thromboxane $B_2$ ($TxB_2$) was obtained by thrombin-induced clotting of citrated blood as reported elsewhere [10]. $TxB_2$ assay was performed using a commercially available radio-immunoassay (New England Nuclear Boston, Ma., USA) and results were expressed as nanograms per $10^8$ platelets. Normal values of platelet aggregation and $TxB_2$ were obtained in eight normal children age- and sex-matched to the patients (ADP 56.25 ± 9.4% as irreversible wave; collagen 59.7 ± 14% as tangent of the aggregation slope; ristocetin 57 ± 4.75% as irreversible wave; $TxB_2$ 44 ± 10 ng/$10^8$ platelets).

Bleeding time (normal range 2–8 min) was measured on the forearm by Ivy's method (Simplate II, General Diagnostic, Morris Blaiws, N.J., USA). Platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and platelet mass were electronically evaluated in blood treated with $K_3$-EDTA anticoagulant (5 μM final concentration) using a Technicon 6000 counter (Technicon Terrytown, N.Y., USA) [11].

Statistical significance was calculated using Student's $t$-test for paired data. Pearson's linear correlation coefficient $r$ was obtained by linear regression analysis.

## Results

Haematocrit and Hb levels rose significantly ($P$ <0.001) in all patients both after 6 and 12 weeks of treatment with rHu-EPO (Table 2). No transfusion regimen was necessary during rHu-EPO therapy. Six patients reached target Hb levels after the 8–9 weeks using 300 IU/kg per week of rHu-EPO; in two patients (nos. 5, 9) only a dose of 450 IU was efficacious.

In the three patients (nos. 2, 3, 8) with the highest haematocrit values after 12 weeks of therapy, rHu-EPO was subsequently decreased to prevent thrombotic events. No systemic or vascular access thrombosis occurred during the study. Baseline bleeding time was prolonged in all patients (mean ± SD, 12.8 ± 3.1 min, Table 2); after 12 weeks of therapy this value was significantly reduced (8.2 ± 3.2, $P$ <0.01), and only in two patients (nos. 8, 9) was it still abnormal. A significant inverse correlation between rise in haematocrit levels and the shortening of bleeding time ($r$ = 0.73, $P$ <0.05) was observed. No acute or chronic bleeding occurred during the study. Mean platelet count (Table 2) increased above basal values (236 ± 84 × $10^9$/l) both after 6 (330 ± 112) and 12 (391 ± 157) weeks of therapy ($P$ <0.05). At the same time, we observed a significant decrease ($P$ <0.05 in MPV (Fig. 1) and a secondary increase in PDW (Fig. 2). Mean platelet mass (platelet count × platelet volume) was unchanged both after 6 and 12 weeks of therapy (before, 0.2 ± 0.07; at 6 weeks, 0.2 ± 0.06; at 12 weeks, 0.26 ± 0.13). Four patients (nos. 3–5, 7) had a decreased baseline aggregation response to ADP and three (nos. 3, 7, 9) to collagen (Table 3). Compared with normal, mean

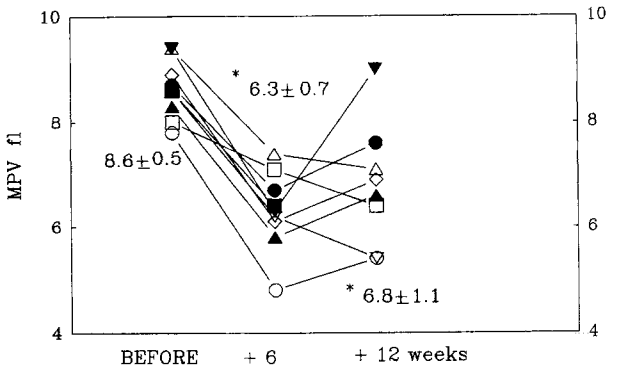


**Fig. 1.** Mean platelet volume (*MPV*) in the nine children with end-stage renal disease before and after 6 and 12 weeks of therapy with recombinant human erythropoietin (rHu-EPO). We observed a significant decrease in the mean ± SD values both after the 6th and 12th week of treatment ($P < 0.01$) with rHu-EPO in comparison with the baseline value

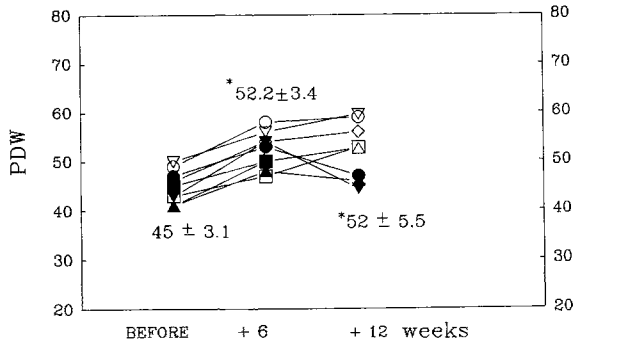


**Fig. 2.** Platelet distribution width (*PDW*) in the nine children with end-stage renal disease before and after 6 and 12 weeks of therapy with rHu-EPO. A significant increase in PDW was observed after correction of anaemia in comparison with the baseline value ($P < 0.05$)

aggregation values were significantly decreased only using collagen ($P < 0.05$). Normal aggregation values were obtained using ristocetin in all patients (data not shown).

During rHu-EPO treatment platelet aggregation improved in the four patients who were hypo-responsive to ADP. After 6 and 12 weeks of therapy, the mean value of platelet aggregation by collagen increased significantly ($P < 0.05$ and became normal (Table 3).

The mean baseline level of serum $TxB_2$ ($19.3 \pm 8.8$ ng/$10^8$ platelets) production in our patients was significantly ($P < 0.005$) lower than normal ($44 \pm 10$) and reduced in all but one patient (no. 3) (Table 3). We observed a very low production of serum $TxB_2$ (4 ng) in patient no. 7 who had a history of bleeding and the lowest aggregation response to collagen. In this patient, both collagen aggregation response and $TxB_2$ production improved only after 12 weeks of treatment.

An overall significant increase in $TxB_2$ production ($P < 0.005$) both after 6 ($31 \pm 22$) and 12 weeks ($27 \pm 13$) of rHu-EPO treatment was observed, and mean values of $TxB_2$ became normal.

## Discussion

Several haemostatic abnormalities may contribute to the abnormal bleeding often shown by uraemic patients [6, 7]. A qualitative platelet defect with a low in vitro aggregation response [6, 8], impaired $TxB_2$ production [8, 12] and increased release of vascular prostacyclin [13] have been described, but anaemia seems to be the main cause of bleeding [3, 7]. In these subjects correction of anaemia with transfusion or recently with rHu-EPO [14], shortens the bleeding time [3, 4] which is strictly related to the haemorrhagic tendency [5]. Only one of our patients with end-stage renal disease (no. 7) had a history of bleeding, but all had prolonged bleeding times. This same subject had no response to collagen and a very low production of $TxB_2$.

Treatment with rHu-EPO clearly increased the haemotocrit level, which correlated with a shortening in bleeding time in all our patients. This finding confirms the key role of the red cell mass in the pathogenesis of uraemic bleeding. However, anaemia is not the only factor associated with abnormal bleeding time. In fact, compared with non-uraemic subjects with the same degree of anaemia, uraemic patients show a significantly longer bleeding time [3]. In addition, a partial improvement in platelet function was obtained in the patients following dialysis [8, 15].

Platelet aggregation studies showed a significant subnormal response to collagen in all our patients and to ADP

**Table 3.** Changes of platelet aggregation and serum thromboxane $B_2$ ($TxB_2$) during treatment with rHu-EPO

| Patients no. | ADP 2 μM (%) | | | Collagen 2 μg | | | $TxB_2$ (ng $10^8$ platelets) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | +6 weeks | +12 weeks | Before | +6 weeks | +12 weeks | Before | +6 weeks | +12 weeks |
| 1 | 63 | 74 | 60 | 46 | 57 | 48 | 12 | 8 | 20 |
| 2 | 64 | 57 | 54 | 49 | 63 | 83 | 17 | 22 | 17 |
| 3 | 27 | 73 | 59 | 25 | 62 | 46 | 36 | 62 | 7 |
| 4 | 30 | 58 | 42 | 43 | 60 | 100 | 25 | 70 | 28 |
| 5 | 22 | 49 | 43 | 42 | 72 | 66 | 19 | 23 | 30 |
| 6 | 58 | 66 | – | 53 | 83 | – | 23 | 46 | – |
| 7 | 24 | 40 | 49 | 7 | 10 | 30 | 2 | 1 | 37 |
| 8 | 71 | 64 | 49 | 67 | 57 | 43 | 23 | 15 | 54 |
| 9 | 62 | 42 | 57 | 26 | 27 | 29 | 17 | 32 | 31 |
| Mean(SD) | 46(19) | 58(11) | 51(6.5) | 39(16)** | 54(21)* | 55(24)* | 19(8.8)** | 31(22)* | 27(13)* |
| Controls | 56(9) | | | 59.7(14) | | | 44(10) | | |

* $P < 0.05$ vs before rHu-EPO values; ** $P < 0.05$ vs control values

in 44%. After correction of anaemia with rHu-EPO we obtained an improvement in platelet aggregation, as reported by van Geet et al. [16]. This finding cannot be explained by the haematocrit increase since no effect of red cell transfusion on platelet aggregation has been reported in uraemic patients [17]. In our patients $TxB_2$ generation during blood clotting was significantly reduced, as already reported [8, 12, 18]. Moreover $TxB_2$ production improved significantly after rHu-EPO treatment. So the improvement of bleeding time observed in our patients might be due to the increase in both haematocrit level and platelet function. During anaemia correction, we also observed a significant increase in platelet number after both 6 and 12 weeks of therapy and a related reduction in platelet volume. A relative iron deficiency could be also responsible for rising platelet count. However the iron store balance was effectively stabilized by administering oral iron during treatment with rHu-EPO. Therefore, a direct effect of rHu-EPO on thrombopoiesis may be advanced. The mechanism that regulates thrombopoiesis remains to be understood. Thrombopoietin is the putative major regulator but other regulator factors should be considered in steady-state thrombopoiesis [19]. For instance, the continuous infusion of granulocytic-macrophage-colony-stimulating factor induced a platelet count increase and the concomitant MPV fall in monkeys [20]. On the other hand, the effect of erythropoietin on thrombopoiesis is doubtful. Nevertheless erythropoietin affects the differentiation of murine megakaryocytes in vitro [21] and a temporary increase in thrombocytes was observed after rHu-EPO infusion in humans [16, 22].

Platelet count and MPV are inversely related and constitute the main control of thrombopoiesis [20]. In normal subjects it was demonstrated that the product of platelet count and volume (platelet mass) remains constant over a platelet range of 150–500 platelets $10^9$/l [20]. In fact, after rHu-EPO treatment, we observed an increase of platelet count ranging from 180–600 platelets $10^9$/l and a concomitant reduction in platelet volume which contributed to the maintenance of a normal platelet mass. The secondary increase in the PDW was due to the inverse relationship existing between platelet volume and PDW [11]. The smaller platelets seen after rHu-EPO therapy could reflect either release by the bone marrow of smaller platelets, or fragmentation of platelets during blood circulation. However, platelet size seems to be determined during platelet production and this may well occur in response to a thrombopoietic stimulus [20].

As a result of the increased thrombopoiesis induced by rHu-EPO, younger and probably more active platelets [20, 23] can contribute to the improvement in the in vitro platelet response observed in our patients after rHu-EPO therapy.

In conclusion, during treatment with rHu-EPO we obtained an improvement in the haemostatic balance which may be explained not only by the correction of anaemia but also by the increase in platelet count and function.

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## References

1. Eschbach JW (1989) The anaemia of chronic renal failure, pathophysiology and the effects of recombinant human erythropoietin. Kidney Int 35: 134–148
2. Eschbach JW, Egrie JG, Dowing MR, Browne JK, Adamson JW (1987) Correction of anaemia of end-stage renal disease with recombinant human erythropoietin: results of combined stage I and II clinical trial. N Engl J Med 316: 73–78
3. Livio M, Gotti E, Marchesi D, Mecca G, Remuzzi G, De Gaetano G (1982) Uremic bleeding: role of anaemia and beneficial effect of red-cell transfusions. Lancet II: 1013–1015
4. Moia M, Vizzotto L, Cattaneo M, Mannucci PM, Casati S, Ponticelli C (1987) Improvement in the haemostatic defect of uremia after treatment with recombinant human erythropoietin in anaemic patients on hemodialysis. Lancet I: 73–78
5. Steiner RW, Coggins C, Carvalho ACA (1979) Bleeding time in uremia: a useful test to assess clinical bleeding. Am J Hematol 17: 107–117
6. Woolley AC (1987) Platelet dysfunction in uremia. Kidney 19: 15–19
7. Remuzzi G (1988) Bleeding in renal failure. Lancet I: 1205–1208
8. Di Minno G, Martinez J, McKean ML, De La Rosa J, Burke JF, Murphy S (1985) Platelet dysfunction in uremia. Multifaceted defect partially corrected by dialysis. Am J Med 79: 552–559
9. Fabris F, Randi ML, Sbrojavacca R, Casonato A, Girolami A (1981) The possible value of platelet aggregation studies in patients with increased platelet number. Blut 43: 279–285
10. Luzzatto G, Fabris F, Mazzucato M, Girolami A (1985) PF4 versus BTG as evidence for platelet activation in myeloproliferative disorders. Scand J Haematol 35: 299–304
11. Luzzato G, De Franchis G, Fabris F, Gerunda GE, Girolami A (1985) Increased proportion of giant platelets and platelet distribution width are better indicators of altered platelet homeostasis than mean platelet volume in liver cirrhosis. Folia Haematol (Leipz) 5: 719–726
12. Remuzzi G, Benigni A, Dodesini P, Schieppati A, Livio M, Day JS, Smith L, Pinca E, Patrignani P, Patrono C (1983) Reduced platelet thromboxane formation in uremia. Evidence for a functional cyclooxygenase defect. J Clin Invest 71: 762–768
13. Remuzzi G, Cavenaghi AE, Mecca G, Donati MB, De Gaetano G (1977) Prostacyclin-like activity and bleeding in renal failure. Lancet II: 1195–1197
14. Winearls CG, Oliver DO, Pippard MJ, Reid C, Downing MR, Cotes PM (1986) Effect of human erythropoietin derived from recombinant DNA on the anemia of the patients maintained by chronic haemodialysis. Lancet I: 1176–1178
15. Rabiner SF (1972) The effect of dialysis on platelet function of patients with renal failure. Ann N Y Acad Sci 201: 234–242
16. van Geet C, Hauglustaine D, Verresen L, Vanrusselt M, Vermylen J (1989) Hemostatic effects of recombinant human erythropoietin in chronic haemodialysis patients. Thromb Haemost 61: 117–121
17. Fernandez F, Goudable C, Sie P, Ton-That H, Durand D, Sue JM, Boneu B (1985) Low haematocrit and prolonged bleeding time in uremic patients: effect of red cell transfusions. Br J Haematol 59: 139–148
18. Bloom A, Greaves M, Preston FE, Brown B (1986) Evidence against a platelet cyclooxygenase defect in uremic subjects on chronic haemodialysis. Br J Haematol 62: 143–149
19. Hill J, Levin J (1989) Regulators of thrombopoiesis: their biochemistry and physiology. Blood Cells 15: 141–166
20. Thompson CB, Jakubowski JA (1988) The pathophysiology and clinical relevance of platelet heterogeneity. Blood 72: 1–8
21. Ishibashi T, Koziol JA, Burstein SA (1987) Human recombinant erythropoietin promotes differentiation of murine megakaryocytes in vitro. J Clin Invest 79: 286–289
22. Bommer J, Muller-Buhl E, Ritz E, Eifert J (1987) Recombinant human erythropoietin in anaemic patients in haemodialysis. Lancet I: 392
23. Thompson CB, Jakubowski JA, Quin PG, Deykin D, Valeri CR (1984) Platelet size and age determine platelet function independently. Blood 63: 1372–1375