Hemostasis assessment in patients treated with recombinant human erythropoietin

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Uraemia represents one of the most important causes of acquired coagulation defect. The haemostatic defect in hemodialysis patients has been attributed either to platelet or plasma alterations. A contributing role for anaemia has also been demonstrated.

The availability of recombinant human erythropoietin (r-HuEPO) has allowed to obtain the full correction of anaemia in uraemic dialysis patients. The increased incidence of thrombotic side effects observed after correction of anaemia supports an important role for anaemia in inducing some haemostatic defects in dialysis patients and gives new insights on the interaction among haemostasis, thrombosis and haematocrit in these patients.

Pathophysiology of uremic anaemia

It has been definitely established that the kidney is the major site of production of erythropoietin [1]. The existence of this hormone was first postulated by Carnot [2] and many years later human erythropoietin was isolated [3] and characterized [4].

Anaemia in uraemic patients is due to the failure, by the diseased kidneys, to stimulate erythropoiesis enough to fulfill the increased demand. Recently, a number of papers have shown that the infusion of erythropoietin rich plasma in normal and uraemic sheeps can reverse anaemia [5]. The results observed in animal studies have been confirmed in humans treated with the recombinant hormone [6]. Although several other factors may contribute to anaemia in uraemic patients (inhibitors of the heme synthesis in the bone marrow [7, 8], reduced life span of red cells due to uraemic toxins [9] and increased aluminum body burden [10, 11]), the deficient erythropoietin production is the primary cause, even though not the only one in the pathogenesis of uraemic anaemia. High levels of parathyroid hormone [12] and the increased blood-losses due to gastrointestinal bleeding or to occasional clotting in the extracorporeal circuit have also been involved in the pathophysiology of uraemic anaemia [13].

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Coagulation defects in uraemia

a) Primary haemostasis

Disorders of haemostasis have been widely described and investigated in dialysis patients [14-18]. The preminent haemostatic alteration is the defective formation of the primary haemostatic plug at the site of the injured vessels, reflected by a prolonged bleeding time [18, 19]. The bleeding time which is primarily affected by platelet number and function [20], is the test which correlates most closely with clinical bleeding in uraemic patients being a sensitive laboratory index of primary haemostatic function [21].

Platelet number in uraemic patients is frequently reduced, but in the normal range, while a number of alterations of platelet function have been described. They include: abnormal adenosin diphosphate and serotonin content [22], reduced cyclooxygenase activity [23], reduced ability to generate thromboxane [24], altered platelet adhesion due to defective binding of Von Willebrand factor (VWF) to subendothelium and impaired platelet adhesion to vessel subendothelium [25] and defective adhesion to glass beads [26]. The haematocrit has also been reported to influence the bleeding time [20]. In fact, when in uraemic patients blood transfusions had been given in amounts able to rise the haematocrit above 26 % [27] or above 30 % [19] the prolonged bleeding time was shortened or normalized. Of course, it cannot be excluded that the improvement in bleeding time after the transfusions of washed red cells might be related to haemostatic components from the donor tightly bound to the red cell membrane and made available for plug formation. However, this event can be excluded if the rise in

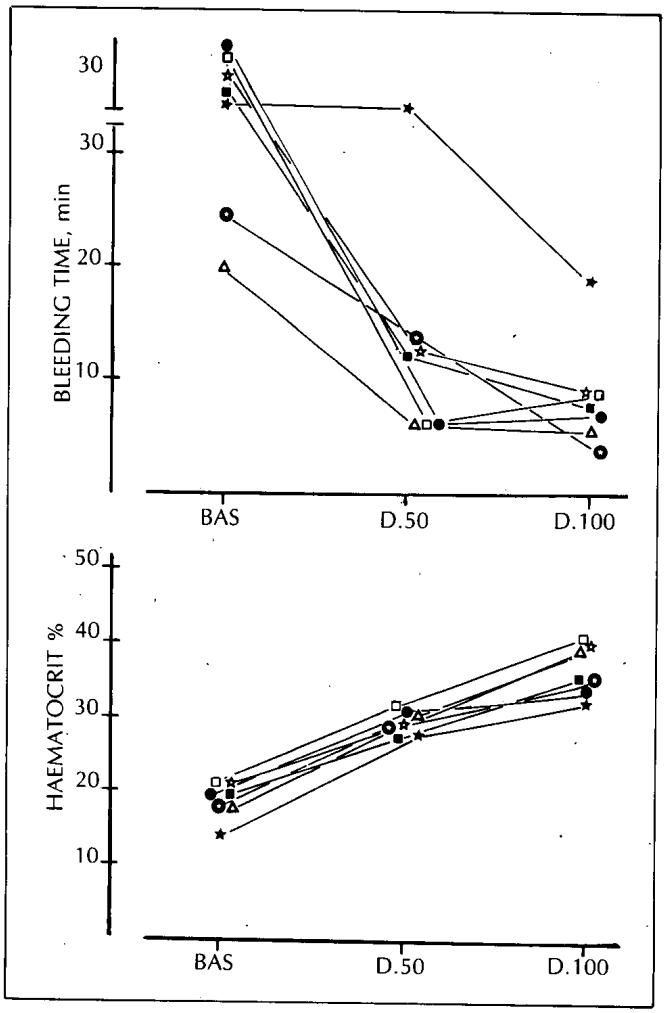

Fig. 1.—Changes in bleeding time during treatment with r-HuEPO in 7 chronic haemodialysis patients with pre-r-HuEPO treatment prolonged bleeding time (upper part).
Changes in haematocrit levels in the same patients during r-HuEPO treatment (lower part).
Bas = before r-HuEPO treatment; D100 = target haematocrit; D50 = mid-point increase of the target haematocrit.

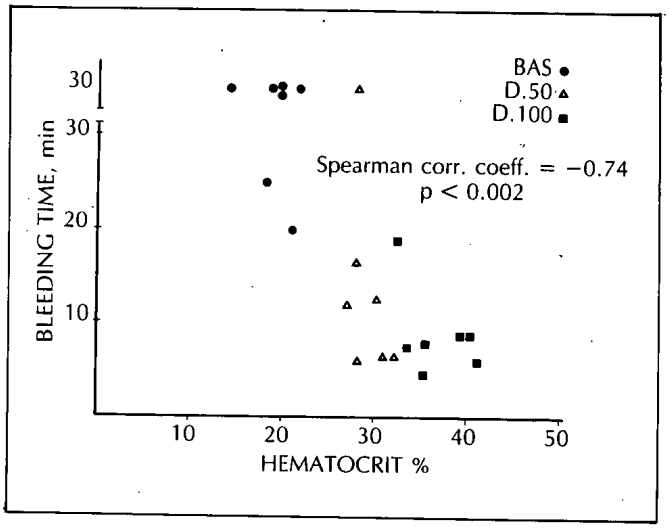

Fig. 2.—Inverse relationship between haematocrit and bleeding time in 7 chronic haemodialysis patients treated with recombinant human erythropoietin.
Bas = before r-HuEPO treatment; D100 = target haematocrit; D50 = mid-point increase of the target haematocrit.

autologous red cell number is obtained with r-HuEPO.

In a recent paper[28] we described a significant shortening of the abnormally prolonged bleeding time in 6 out of 7 anaemic dialysis patients when a partial correction of anaemia was obtained with a prolonged r-HuEPO treatment. Further improvement of bleeding time was observed at a higher haematocrit, becoming almost normal in all but one patient (Fig. 1). A strong inverse relationship between the increase in haematocrit and the reduction in bleeding time was shown, definitely establishing a preminent role for red cells in the pathophysiology of uraemic haemostatic defects (Fig. 2). It can be speculated that the rise in the total mass of circulating red cells produces an increasing rate of radial transport of centrally flowing platelets toward the vessel endothelium and consequently induces a rise in platelet adhesion to the vessel wall[29]. Recently, nitric oxide, an endothelium derived relaxing factor, which can influence platelet adhesion, has been claimed to be involved[30]. Haemoglobin binds nitric oxide, hence the rise in haemoglobin concentration observed under r-HuEPO therapy may bind greater amounts of nitric oxide so influencing platelet adhesion and aggregation. A major objection to this theory is that the effect of the endothelium derived relaxant factor should be completely inhibited for haemoglobin concentrations as low as 4 g/dl[31]. A further theory supports the role of red cells in binding prostacyclin which is known to inhibit platelet adhesion and aggregation[32]. Hence, the rise in the red cell number could favour aggregation and adhesion by removing prostacyclin. Recombinant human erythropoietin can also stimulate thrombopoiesis in a dose dependent fashion, with changes in both number and function of platelets[33, 34]. A significant improvement in platelet aggregation activity was reported by Van Geet[33] in a group of 9 chronic haemodialysis patients under r-HuEPO treatment. This phenomenon reached its peak between 4 and 12 weeks of r-HuEPO treatment reaching almost the normal range, but tended to decline at a longer observation period (Table I). The transitoriness of this direct effect of r-HuEPO on platelets was confirmed by the lack in correlation, in the long term, between platelet activity and bleeding time[33].

b) Plasma factors

Different opinions still exist on the role of plasma coagulation factors on the haemostatic defect in dialysis patients. Coagulation plasma factors have been extensively investigated in dialysis patients. Different

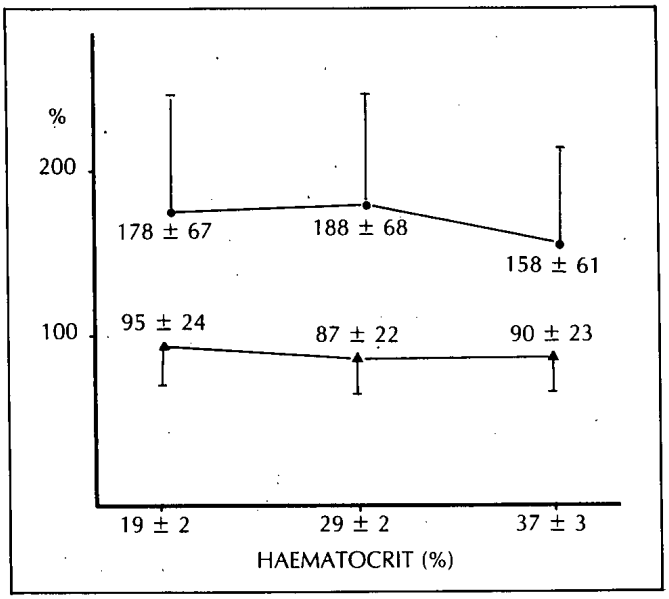

Fig. 3.—Factor VIII activity (●) and factor X levels (▲) at different haematocrit levels in a group of chronic haemodialysis patients treated with recombinant human erythropoietin. Data are presented as % difference from FVIII activity and Factor X values of a pool of sera from normal subjects.

findings have been described: Factor VIII activity (FVIIIAct) has been reported to be abnormally high [15, 35] or normal [36] in uraemic patients. Von Willebrand factor antigen (vWFAg) was described generally increased [15]. Ristocetin cofactor activity (vWFRCo) was found both elevated [15, 36] as well as decreased in dialysis patients [37].

In order to evaluate the possible influence of r-HuEPO treatment on coagulation plasma factors we measured several haemostatic parameters before, during and after correction of anemia with r-HuEPO [28].

Fibrinogen, antithrombin III and Factor VII were in the normal range before r-HuEPO administration and did not change after the correction of anaemia. Factor X was slightly below the normal range and remained substantially unchanged during r-HuEPO treatment (Fig. 3). FVIIIAct, vWAg and vWFRCo pretreatment levels were above the normal values and were not influenced by the r-HuEPO treatment with the exception of FVIIIAct which showed a slight unexplainable decrease (Fig. 3).

Pretreatment multimeric structure of vWFAg did not show apparent alterations remaining unchanged after r-HuEPO therapy. The perusal of the available data on coagulation plasma factors in uraemic patients suggests that they do not play an evident role in the pathogenesis of the haemostatic defect. This finding is confirmed also in r-HuEPO treated patients in whom plasma coagulation factors are normal or high before treatment and unchanged afterwards.

Clinical implications

It has been suggested that raising the haematocrit might increase thrombotic complications, particularly to dialysis vascular accesses. As a matter of fact we observed seven episodes of thrombosis of the vascular access in 4/16 r-HuEPO treated patients during a 16 ± 4 months follow-up [38]. In a well matched control group, two episodes of thrombosis of the vascular access were encountered in only 2/50 patients.

A transient ischaemic cerebral attack was observed in one r-HuEPO treated patient after complete correction of anaemia. Myocardial infarct was diagnosed in two patients, one in the treated group and one in the untreated [38]. In another multicentre study on a larger haemodialysis population the incidence of myocardial infarct and of cerebral vascular accidents episodes was not changed by r-HuEPO treatment [39], while thrombosis of the vascular access was reported in 14 of 150 r-HuEPO treated haemodialysis patients.

Tabla I. Platelet aggregation before and during treatment with r-HuEPO in haemodialysis patients

Weeks of treatment	0	4	8	12	28	Control group
Platelet count PPRP (×10/μl)	249	299	331	325	325	
Aggregation induced by:						
ADP (2.10 M):						
max amplitude	38.4	47.4 *	46.5 *	48.5 *	39,0	51
max velocity	41.7	48.3	45.2	53.8 *	43.0	59
Collagen (2 μg/ml):						
max amplitude	38.5	41.5	44.8	42.8	37.0	59
max velocity	38.5	41.6	42.8	39.8	33.5	56.6
Ristocetin (1.45 mg/ml)						
max amplitude	40.0	50.6	45.0 **	56.7 **	42.0	64.5
max velocity	36.8	49.8	60.2 *	67.3 **	51.0	79.5

Results are expressed as means of 8 patients.
* $p < 0.05$. ** $p < 0.01$. vs pretreatment.
(Modified from: C. van Geet, *Thromb Haemost* 61:117-121, 1989.)

However, in 6 of 14 patients thrombosis had occurred before a clinically significant elevation of haemoglobin.

These data indicate that the increase in haematocrit and in platelet adhesion to subendothelium together with the shortened bleeding time can increase the risk of thrombotic complications in r-HuEPO treated patients. As other Authors pointed out[40] since an uncontrolled rise of haemoglobin in dialysis patients may be harmful, the risk of thrombotic complications might be reduced or avoided and haemostasis still be improved by aiming to lower increments in haematocrit. This problem might also be approached through the administration of drugs reducing the risk of platelet aggregation in order to prevent thrombotic accidents. These events might occur particularly in the early phases of correction of anaemia when the increase in platelet activity[33] and number[34] is potentially maximally dangerous.

Only a longer observation period may better define whether this aproach is useful in preventing thrombosis without increasing the haemorrhagic risk in uraemic patients with a fully corrected anaemia.

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