


**Does uraemia potentiate bacteraemia-associated haemolysis in patients receiving erythropoietin?**

Sir,

In patients with bacterial infection, anaemia is attributed chiefly to reduced red-cell production by the bone marrow and is usually not evident until several days after the acute infection [1,2]. Impaired release of iron from reticuloendothelial cells to the marrow as well as a relative deficiency of erythropoietin also partly contribute to anaemia following acute infections or inflammation [2–5].

However, the relative contribution of acute haemolysis to anaemia following acute infections is unquantified, especially in those conditions where there are abnormalities of red blood cell (RBC) metabolism such as uraemia.

Prompted by the clinical observation of an abrupt fall in haematocrit in erythropoietin-treated haemodialysis patients with bacteraemia not explicable solely by bone-marrow suppression, we theorized that uraemia may potentiate bacteraemia-associated haemolysis.

We conducted a retrospective study comparing the haematocrit and the serum lactate dehydrogenase (LDH) concentration in the first 2 in-hospital days in patients with or without end-stage renal disease (ESRD) and bacteraemia, and those who have ESRD without bacteraemia.

**Methods.** Patients hospitalized during the preceding 12 months (1995) with bacteraemia were identified from the hospital medical records computer and screened for eligibility. Patients with malignancy, gastrointestinal bleeding, human immunodeficiency virus infection, haemoglobinopathy, liver disease, or myelofibrosis were excluded.

The bacteraemic patients were sorted into two groups according to whether they had normal renal function (serum creatinine concentration on admission $\leq 1.5$ mg/dl) (group I, $n=30$), or had ESRD and were receiving maintenance haemodialysis as well as recombinant erythropoietin prior to hospitalization (group II, $n=23$). Group III ($n=26$) included all erythropoietin-treated haemodialysis patients without bacteraemia (no fever or leukocytosis, not receiving antibiotics) hospitalized on the same day as were group II patients.

Haematocrit and LDH values (measured predialysis in groups II and III) were extracted from patients’ records. One-way analysis of variance (ANOVA) was used for group comparisons. Tukey Honestly Significant Difference post hoc tests were used to test for differences between individual means. Student $t$ test was used for paired comparison. Values are mean±SEM.

**Results.** Basic clinical and demographic data are shown in Table 1. The dose of erythropoietin (U per pound of body weight) administered intravenously thrice weekly prior to hospitalization was $32.3±3.3$ U in group II and $26.1±2.2$ U in group II ($P=0.001$).

On day 1, the mean haematocrit was higher in group I ($37±1.1%$) than in group II ($29.1±2.0%$) or group III ($29.6±1.2%$) ($P=0.001$); but by the second hospital day, mean haematocrit had dropped by only $5.4%$ to $35.0±1.6%$ in group I, and by $5.4%$ to $28.0±0.94%$ in group III, compared to a $12%$ drop to $25.6±1.2%$ in group II ($P=0.001$) (Figure 1).

The patients with ESRD and bacteraemia (group II) had higher serum LDH concentration on admission and on day 2 than those without bacteraemia (group III) or those with normal renal function and bacteraemia (group I) ($P=0.04$) (Table 1). There was no evidence of disseminated intravascular coagulation in any study subject. All bacteraemic patients were treated with parenteral antibiotics from day 1.

In group I bacteraemia was due to Gram-positive organisms in 21 (70%) subjects and Gram-negative organisms in nine (30%) subjects, while in group II bacteraemia was due to Gram-negative organisms in 21 (70%) subjects and Gram-positive organisms in 14 (65%) subjects and Gram-negative bacilli in nine (35%) subjects ($P=0.32$). In both groups there was as steep a drop in haematocrit among those with Gram-positive than in those with Gram-negative bacteraemia, but this difference did not achieve statistical significance (Figure 2).

The change in haematocrit in group I subjects with Gram-positive bacteraemia was $6.8±1.9%$ vs $3.2±2.4%$ among those with Gram-negative bacteraemia; also in group II, change in haematocrit among those with Gram-positive bacteraemia was $13.8±4.4%$ compared with $6.9±1.8%$ among those with Gram-negative bacteraemia ($P=0.07$).

We found that while haematocrit dropped in both bacteraemic and non-bacteraemic patients, a greater absolute and fractional decline in haematocrit was observed in haemodialysis patients with bacteraemia during their first 2 in-hospital days. Descriptions of significant haemolysis-induced by bacterial infections have been limited mainly to patients with sickle-cell disease, glucose-6-phosphate dehydrogenase deficiency, or infection with *Bartonella bacilliformis*, *Clostridium welchii* or *Haemophilus influenzae* type b [2]. No study subject was infected with any of these organisms.

**Proposed mechanisms for haemolysis in bacterial infections include direct invasion of RBCs by bacteria or its toxins, induction of antibodies or cold agglutinins directed against RBC cell-wall antigens, and alteration of RBC cell-wall antigens, rendering them immunogenic** [2].

There is evidence suggesting increased susceptibility of uraemic RBCs to lysis by toxic agents or oxidative stress [6–12]. Patients with ESRD have shortened RBC life-span, but the precise mechanism for shortened RBC survival in ESRD is unknown [6,7,13,14]. Normalization of RBC survival when RBCs from uraemic patients are transfused into persons with normal renal function strongly incriminates uraemic toxins [13,14]. However, dialytic therapy (i.e. partial removal of purported uraemic toxins) does not uniformly...
Table 1. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th></th>
<th>Bacteremia and normal renal function (n = 30)</th>
<th>Bacteremia and ESRD (n = 23)</th>
<th>No bacteremia and ESRD (n = 26)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60 ± 3.1</td>
<td>56.7 ± 4.4</td>
<td>52.8 ± 3.5</td>
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</tr>
<tr>
<td>Gender (M/F)</td>
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<td>10/13</td>
<td>15/11</td>
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<td></td>
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<td>2</td>
</tr>
<tr>
<td>Body temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>100.1 ± 0.42</td>
<td>99.6 ± 0.35</td>
<td>97.9 ± 0.14</td>
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<tr>
<td>Haematocrit</td>
<td></td>
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<tr>
<td>day 1</td>
<td>37 ± 1.1</td>
<td>29 ± 1.2</td>
<td>29.6 ± 1.2</td>
<td>0.001</td>
</tr>
<tr>
<td>day 2</td>
<td>35 ± 1.1</td>
<td>25.6 ± 1.2</td>
<td>28 ± 0.94</td>
<td></td>
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<tr>
<td>Serum LDH (U/l)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>313 ± 24</td>
<td>360 ± 43</td>
<td>253 ± 15</td>
<td>0.04</td>
</tr>
<tr>
<td>day 2</td>
<td>313 ± 37</td>
<td>356 ± 37</td>
<td>256 ± 21</td>
<td></td>
</tr>
</tbody>
</table>

*Reasons for hospitalization included thrombosed vascular access in 17 (65%), angina in 8 (31%), and acute myocardial infarction in 1 (4%).

normalize RBC survival [6], suggesting that abnormalities intrinsic to uraemic RBCs may also predispose them to shortened survival or haemolysis [7,9–11]—uraemic red cells are hypermetabolic even when incubated with sera from persons with normal renal function [9].

As evidenced by decreased levels of reduced glutathione, metabolic antioxidant capacity is reduced in uraemia, thereby limiting the capability of uraemic RBCs to withstand any oxidative stress as in bacterial sepsis [8]. Increased sensitivity to oxidative stress persists in patients with ESRD treated with recombinant erythropoietin [10]. Also, uraemic red cells have reduced membrane deformability, attributed partly to increased RBC calcium [11]. Reduced RBC deformability may accompany bacterial sepsis and enhances the haemolytic elimination of RBCs from the circulation [11,15]. Furthermore, sepsis causes dysfunction of erythrocyte calcium pump, resulting in additional intracellular accumulation of calcium [16].

That haemolysis is principally responsible for the decline in haematocrit in the ESRD patients with infection is supported by concomitant elevation in lactate dehydrogenase concentration. There were no other factors in the patients' histories that might have contributed to the observed changes in haematocrit.

There are several limitations to our study. We have not excluded splenic or tissue sequestration of blood, and did not measure serum haptoglobin or look for schistocytes in peripheral blood smears. Changes in haematocrit may not be the best measure of haemolysis. We cannot exclude the possibility that changes in haematocrit were partly due to alterations in total body water that may accompany fever or bed-rest.

We acknowledge that the haemoccult stool test is imperfect for detecting gastrointestinal bleeding. We have no explanation for the 5.4% drop in haematocrit in ESRD patients without bacteraemia or for the difference in dose of erythropoietin in groups II and III prior to hospitalization. We limited our study to the first 2 in-hospital days because subsequently it may be difficult to exclude the effect of dialysis and multiple blood drawing on the haematocrit.

Our findings confirm that an abrupt absolute and proportional decline in haematocrit occurs in erythropoietin-treated
haemodialysis patients with bacteraemia, and is not duplicated in bacteraemic patients with normal renal function. The uraemic milieu may potentiate the red blood cell lytic effect of bacterial toxins.

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Changes in adrenomedullin plasma concentrations during haemodialysis in patients with chronic renal failure

Sir,

Adrenomedullin (AM) is a novel hypotensive peptide first isolated from human phaeochromocytoma eliciting a long-lasting vasorelaxant action [1]. AM immunoreactivity and mRNA have been demonstrated in a number of human tissues, including kidney, and circulates in human plasma [2,3].

AM appears to be actively synthesized and secreted by vascular endothelium into both the bloodstream and space between endothelial cells and vascular smooth-muscle cells [4]. Thus it is likely that vascular tissues are one of the main sources of circulating AM [4].

Plasma AM level has been shown to be increased in various cardiovascular disorders, in particular those in which the vascular endothelium is severely injured [5–7]. Vascular endothelial injury occurs during haemodialysis, as suggested by Turney et al. [8]. These authors demonstrated progressive increase of factor-VIII related antigen and antithrombin II during haemodialysis and ascribed this to vascular endothelial damage caused by reinfusion of platelets and other factors activated by the extracorporeal circulation of blood.

In the present observational study we analysed the effect of the single haemodialysis session on plasma concentrations in patients with end-stage renal disease (ESRD) and relationships between plasma AM levels and blood pressure were examined.

Fourteen patients with ESRD (8 men and 6 women; mean age 54.4 ± 12.8 years) and 21 control subjects (13 men and 8 women; mean age 41.8 ± 12 years) were studied. The aetiology of renal failure was as follows: chronic glomerulonephritis, four patients; polycystic renal disease, two; diabetic nephropathy, three; chronic interstitial nephritis, three; unknown aetiology, two patients. The patients were dialysed for 4 h three times a week (for 14 ± 5 months) using cellulose membranes (cuprophane) and a bicarbonate-based dialysate.

Haemodialysis sessions were all done on an early week dialysis. Interdialytic fluid retention was assessed by comparing the last post-dialysis weight. Blood pressure was measured by medical staff before and after the haemodialysis session (mean of the two measures).

After an overnight fast the patients and controls were kept in the supine position for 60 min to stabilize the physical condition, and blood samples, anticoagulated with EDTA and aprotinin, were taken before and after the haemodialysis session. AM was measured in plasma stored at −80°C and extracted with Sep-Pak C-18 cartridges with a commercial radioimmunoassay kit (Phoenal Pharmaceuticals, Mountain View, CA, USA) as previously reported [9].

Table 1 shows the haemodynamic and laboratory data in patients with ESRD, pre- and post-haemodialysis session, and in healthy subjects.

In our patients with ESRD pre- and post-dialysis body weights were 70 ± 13 kg and 67 ± 12 kg (P < 0.0001), respectively. None of patients became hypotensive following dialysis session. There was a difference in AM plasma levels in patients with ESRD before haemodialysis (20 ± 8 pg/ml) and controls (13.2 ± 6 pg/ml) (P < 0.001). In these patients, after 4 h haemodialysis, plasma levels of AM were significantly increased (27.3 ± 10.6 pg/ml) compared with the predialysis value (20.5 ± 8.1 pg/ml) (P < 0.0001). Dialysate levels of AM in patients with ESRD during the observation period were lower than the detection limit of the assay (2 pg per assay tube). There was no correlation between AM and blood pressure in healthy subjects and patients with ESRD in either period of testing, and neither with body weight changes during haemodialysis.

In the current study we found that ESRD is associated with higher plasma AM concentrations, as reported by others [10]. In addition, the most important data in our study was to have shown a time course of AM concentration during

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