

DEFECTIVE EPO PRODUCTION CONTRIBUTES
TO ANEMIA PERSISTENT AFTER ARF
IN NEPHROPATHIA EPIDEMICA (NE)

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SUMMARY : NE is an infectious, newly recognized form of acute renal failure (ARF) caused by Hantavirus. In NE cases, anemia is a rare finding of unclear etiology, since NE-induced ARF is most often mild and of short duration, and since hemorrhages are only minor or absent altogether.

We describe here 3 male patients with serologically proven (IFA IgG & ELISA IgM positive) NE, who all developed normocytic normochromic anemia early in their clinical course, without any evidence of internal hemorrhage. Analysis of hematological and clotting parameters on admission pointed to a condition suggestive for *microangiopathic hemolytic anemia* with or without (subclinical) DIC. Later in the clinical course, measurement of newly established indicators of erythropoiesis disclosed in 2 patients with persistent anemia inappropriately low serum levels of *erythropoietin* (EPO) and of *transferrin receptor* (TfR). In contrast, EPO and TfR were within normal range in the patient who showed only a mild and transient degree of anemia.

In conclusion : rapid-onset anemia in the early phase of NE is

consistent with microangiopathic hemolysis. A delayed correction however of this anemia in the convalescent phase of NE seems linked to defective EPO production, probably as a consequence of (peri)tubular injury in the kidney, a frequent finding in acute Hantavirus-nephritis.

INTRODUCTION

Surprisingly little has been published concerning the pathophysiology of anemia in acute renal failure (ARF) (1,2). Erythropoiesis is basically regulated by [1] the red cell mass (trigger), determining in the kidney production of [2] erythropoietin (stimulator), which in turn stimulates in the bone marrow the [3] erythropoietic activity (effector). These 3 successive steps are assessed by : 1/ hematocrit, 2/ circulating erythropoietin (EPO) levels, 3/ serum levels of transferrine receptor (TfR), which was recently showed to be a reliable quantitative assay of total erythropoiesis in animal as well as in man (3).

To our knowledge, these 3 parameters were not assessed together so far in cases of anemia linked to ARF.

We were struck by an outspoken and/or prolonged degree of anemia in some cases of ARF due to Nephropathia epidemica (NE). This is a mild European variant of an infectious form of acute interstitial nephritis, caused by a rodent-borne Hemorrhagic fever virus, called *Hantavirus*. Whereas in the Far East, more severe forms of Hantavirus infections may occur with internal bleedings, hemorrhages are only minor (epistaxis, petechiae) or absent altogether in European NE. Thus, anemia in NE is a rare finding of unclear etiology, since NE-induced ARF is most often mild and of short duration. The aim of this paper is to try to elucidate

mechanisms of protracted anemia in 3 recently confirmed NE-cases.

SUBJECTS AND METHODS : 1. Subjects

Patients A, B and C are white males aged 24, 26 and 32 years respectively, who each presented with a history of sudden fever, general malaise, outspoken lumbar pain and gastro-intestinal disturbances. A transient ARF developed in each, with spontaneous remission within 2 to 3 weeks. Patient C underwent a percutaneous kidney biopsy, which showed mild interstitial infiltrates and edema without glomerular alterations, i.e. a picture typical for NE. The diagnosis was confirmed in each case by the demonstration in IFA of rising titers of IgG antibodies, and in μ -capture ELISA of IgM antibodies against *Puumala* virus, the etiologic agent of NE. Patient A was thoroughly examined on a hematological ward.

2. Methods

* Erythropoietin (EPO) Assay : Circulating EPO levels were measured by a commercially available RIA (Incstar Corp., Stillwater, Minnesota, USA) using recombinant human Epo (rHu Epo)

* Transferrin Receptor (TfR) Assay : Human placental receptor-transferrin complex was purified and injected repeatedly into rabbits. Rabbit anti-TfR antibodies were removed by passing through a column of human diferric transferrin coupled to Affigel 15 (Bio-Med, Richmond, CA). These specific anti-TfR Abs were incorporated in an ELISA assay. (3).

RESULTS

1. Assessment of renal & hematological evolution

Normocytic normochromic anemia developed in each patient without any evidence of internal hemorrhage. Renal, hematological and clotting parameters of the 3 patients are summarized on Table I (only the most abnormal values during the clinical course of each patient are given). Stem cell culture and flow cytometry both of

peripheral blood and of bone marrow were normal in patient A. Bone marrow examination disclosed in each patient an enhanced megakaryocytosis and a normal (Pat A) or slightly diminished (Pat B) erythropoietic activity. The most severe anemia (Hematocrit 24%, hemoglobin 8.5 g/dl) was noted 25 days after onset of symptoms (and 10 days after discharge from hospital) in Pat C, at a moment when renal function was almost completely restored (S.Creat down from 12 to 2 mg %). Ambulant gastroscopy, performed to exclude internal hemorrhage, was found within normal limits.

2. Assessment of erythropoiesis factors

EPO-levels and Transferrin receptor (TfR) levels are given on Table II, together with the Hct levels registered the same day.

TABLE I :

	S.Creat.	Hct	Platelets	FSP	APTT	Schisto	Indir.Bili
	mg%	%	$\times 10^9/l$	ng/ml	sec	-cytes %	-rubin mg %
Pat A	3.8	27.1	25	8,000	52.6	3	1.66
Pat B	9.7	30.6	12	2,000	48.6	neg	0.6
Pat C	12	24	192	n.d.	n.d.	n.d.	0.6
Normals	<1.2	40-54	>140	≤ 500	24-39	neg	<0.8

renal, hematological & clotting parameters in Pat A,B,C with NE.

n.d. = not done

TABLE II

PATIENT	Hct	EPO	TfR
sample N°	(%)	(mU/ml)	(ng/ml)
A1	44.4	16.8	4100
A2	<u>27.5</u>	<u>22.4</u>	<u>2800</u>
A3	<u>27.1</u>	<u>20.7</u>	<u>6000</u>
A4	<u>36.4</u>	17.6	5600
A5	42.0	17.0	6400
B1	<u>35.4</u>	34.8	3600
B2	40.3	34.9	3200
C	<u>24</u>	<u>10.2</u>	<u>4900</u>

sample numbers denote successive blood samplings during the clinical course (1 : admission to 4 : discharge). Abnormally low values are underlined.

Lowest "physiological" values :

EPO at Hct 37% : 8 and at Hct 27% : 50 mU/ml

TfR at Hct 37 % : 3500 at Hct 27 % : > 10.000 ng/ml

also lead to *micro-angiopathic hemolysis*, the signs of which were found in our patients on admission : thrombocytopenia together with elevated indirect bilirubin and LDH (Table I). A further step in this pathophysiology may be (subclinical) *diffuse intravascular coagulation* (DIC), as also found initially in some of our patients (Table I).

However, all these clotting alterations disappeared together with the other inflammatory signs during the clinical course of our patients, whereas low hematocrits persisted, thus excluding micro-angiopathic hemolysis as the only explanation of anemia.

Values for EPO and TfR, as found subsequently during the

DISCUSSION

With a possible exception for Pat B, the degrees of normocytic normochromic anemia found in these patients with serologically proven NE were out of proportion with the degree of ARF and persisted after restoration to normal or near normal levels of kidney function. The typical thrombocytopenia, as often found in the initial phase of NE, was probably missed in Pat C, who was hospitalized only 7 days after the onset of symptoms. After admission however, he developed thrombocytosis up to $553 \times 10^9/\text{lit}$, as often seen as a rebound phenomenon after initial thrombocytopenia.

The thrombocytopenia is probably the expression of platelet activation secondary to *endothelial lesions*, the most constant histopathological finding in Hantavirus disease (4,5). This microvascular damage may be a direct effect of virus replication in the endothelial cell (5) but immunomediated endothelial injury triggered by viral infection has also been proposed (6).

Widespread endothelial lesions with platelet activation may clinical course were *inappropriately low* to the degree of anemia in Pats A & C, but only borderline low in Pat B, who showed the least severe and the most transient degree of anemia. This pattern of erythropoiesis, consisting of [1] low Hct, [2] low EPO and [3] low Tfr is consistent with the so-called state of "*defective EPO production*" as clearly demonstrated so far in chronic renal failure (3). Low EPO levels, accounting for the slow increase in hemoglobin following recovery, have been demonstrated in ARF of varied (septic and non-septic) etiology and are not specific for NE. Peritubular injury has been proposed as underlying mechanism (2).

CONCLUSION : A transient state of "defective EPO production" as demonstrated in 3 NE patients with ARF can be interpreted as a late consequence of (peri)-tubular injury in the kidney, a frequent finding in acute Hantavirus-nephritis.

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