Pediatr Nephrol (1996) 10: 578–581


Pediatric Nephrology

*Original article*

# Mechanism underlying early anaemia in children with familial juvenile nephronophthisis

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Received October 26, 1995; received in revised form and accepted March 6, 1996

**Abstract.** Familial juvenile nephronophthisis (NPH) is a hereditary form of chronic tubulointerstitial nephritis with onset in childhood. About one-third of patients develop anaemia before renal insufficiency. We investigated the pathogenetic mechanisms leading to anaemia by comparing 6 patients with NPH and 12 reference patients with other renal diseases. We studied their iron metabolism and measured transferrin receptor-ferritin ratios. There was no evidence for iron deficiency or haemolysis. The serum erythropoietin concentrations of the patients with NPH ($12 \pm 2.3$ U/l) were low compared with the 12 reference patients($25 \pm 18.9$ U/l). In the 2 patients with NPH who were fully investigated, the pharmacokinetics of recombinant human erythropoietin appeared normal. Thus, anaemia in patients with NPH does not result from iron deficiency or correlate with impaired iron status. The mechanism underlying the anaemia of NPH appears to affect the function or regulation of the cells producing erythropoietin.

**Key words:** Familial juvenile nephronophthisis – Renal insufficiency – Anaemia – Erythropoietin – Iron – Transferrin receptor

## Introduction

Familial juvenile nephronophthisis (NPH) is a hereditary form of chronic tubulointerstitial nephritis characterised by polyuria, polydipsia, anaemia, growth retardation and progressive renal failure. The mean age at onset of renal failure is around 10 years. The renal insufficiency leads to end-stage renal disease (ESRD) within about 4 years of the first symptoms [1, 2]. Extrarenal manifestations of NPH may include retinitis pigmentosa, liver fibrosis, cerebellar ataxia and cone-shaped epiphyses of the bones [3]. Renal histology reveals features typical of chronic tubulointerstitial nephritis which are not specific to NPH. However, the thickening and layering of the tubular basement membrane is so extensive that recent research concerning the pathogenesis of NPH has been concentrated on the components of the tubular basement membrane [4]. In children, NPH has been estimated to account for 15% of the cases of ESRD [3].

In contrast to other renal diseases, in which the degree of anaemia is dependent on the stage of renal insufficiency, some patients with NPH present with an earlier anaemia. Gardner [5] reviewed 238 reported patients with NPH. Among the 110 patients studied most intensively, 34% of those not yet having renal failure presented with anaemia. No studies have focused on the pathogenesis of the anaemia, but the limited information available indicates that it is normochromic and normocytic. In this prospective controlled study, we aimed to investigate several of the mechanisms possibly involved in the anaemia, including the role of erythropoietin (EPO) and iron metabolism.

## Patients and methods

*Patients.* We studied 6 patients with NPH (4 males, 2 females) who had been followed at the Children's Hospital, University of Helsinki. Their mean age at the time of the study was $10.4 \pm 2.8$ years (range 6.8–13.8 years). The diagnostic criteria were as follows: polyuria, polydipsia, no haematuria or proteinuria, and progressive renal failure leading to ESRD [2, 3, 5]. We did not find evidence of extrarenal involvement. Histological examination revealed chronic tubulointerstitial nephritis with thickened tubular basement membranes. Infectious and toxic causes were excluded. Of the 6 patients, 3 were related to each other and 3 were sporadic cases.

Our patients were compared with 12 reference patients who had a mean age of $11.1 \pm 3.2$ years. Of these 12 patients, 6 had urethral valve obstruction, 2 had juvenile recessive polycystic kidney disease, 2 had anomalous dysplastic kidneys, 1 had Alport's syndrome and 1 had chronic glomerulonephritis. Renal insufficiency developed within 1–3 years of the onset of first symptoms. The 12 patients were divided into two reference groups, one matched for the serum creatinine value ($n = 6$) and the other for haemoglobin ($n = 6$), and the resulting study

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**Table 1.** Clinical details of the patients with familial juvenile nephronophthisis (NPH), creatinine-matched reference patients (I), and haemoglobin-matched reference patients (II) (means ± SEM)

| | NPH | Reference I | Reference II | Reference I + II |
|---|---|---|---|---|
| *n* | 6 | 6 | 6 | 12 |
| Age (years) | 10.4± 2.76 | 13.0± 3.10 | 10.0± 3.07 | 11.1± 3.2 |
| Haemoglobin (g/dl) | 8.4± 1.4 | 9.6± 2.3 | 8.2± 1.2 | 9.5± 2.3 |
| Haematocrit (%) | 25 ± 4.6 | 29 ± 7.2 | 26 ± 3.6 | 29 ± 6.0 |
| MCV (fl) | 84.3± 4.9 | 87.2± 4.0 | 86.2± 4.9 | 86.2± 4.6 |
| MCH (pg) | 28 ± 2 | 28 ± 1 | 28 ± 1 | 28 ± 1 |
| Serum iron (µmol/l) | 13.3± 2.4 | 14.4± 3.2 | 10.6± 3.4 | 13.5± 4.9 |
| Serum transferrin (g/l) | 2.8± 0.6 | 2.2± 0.4 | 2.8± 0.4 | 2.5± 0.4 |
| Serum transferrin saturation (%) | 19 ± 5.0 | 22 ± 6.4 | 17 ± 5.4 | 22 ± 7.0 |
| Serum TfR (mg/l) | 3.0± 1.0 | 4.1± 1.5 | 3.6± 1.0 | 3.9± 1.3 |
| Serum ferritin (µg/l) | 37 ± 10 | 76 ± 38 | 31 ± 15 | 47 ± 34 |
| TfR/ferritin (µg/µg) | 85 ± 33 | 75 ± 56 | 131 ± 53 | 119 ± 60 |
| Serum EPO (U/l) | 12 ± 2.3 | 24 ± 11.1* | 26 ± 26.1** | 25 ± 18.9*** |
| Serum creatinine (µmol/l) | 387 ±200 | 392 ±188 | 535 ±147 | 405 ±167 |

MCV, Mean corpuscular volume; MCH, mean corpuscular haemoglobin; TfR; transferrin receptor; EPO, erythropoietin
* $P = 0.01$; ** $P = 0.05$; *** $P = 0.005$

patient – reference patient pairs were used to analyse the cause of the anaemia further.

Blood samples were collected over 4 years and 3 months from study patients and reference patients at different stages of renal insufficiency before initiation of recombinant human EPO (rhEPO) medication and dialysis treatment. We studied the pharmacokinetics of rhEPO in 2 patients. By that time, the 4 other patients had already received kidney tansplants. Patient 1 had been on dialysis for 3 months and had been receiving rhEPO for 14 months. Patient 2 was newly diagnosed with juvenile nephronophthisis. Her serum creatinine was 150 µmol/l.

*Methods.* Venous blood samples were drawn for measurement of haemoglobin, haematocrit, red blood cell indices and serum concentrations of iron, transferrin, transferrin receptor (TfR), ferritin and EPO. The sera for measurement of concentrations of TfR and EPO were stored at –20° C prior to assay. Haemoglobin, haematocrit and red blood cell counts were measured with an automatic counter (Coulter counter T540). Serum iron concentrations were measured colorimetrically without deproteinisation (Kit 1010 Iron, Orion Diagnostica, Espoo, Finland). Serum transferrin concentrations were measured with an immunoturbidimetric method (Antihuman transferrin, Orion Diagnostica). We used a two-step sandwich-type time-resolved immunofluorometric assay for measurement of the serum concentration of TfR [6]. The serum ferritin concentrations were determined using a chemiluminometric immunoassay with an automatic analyser (Ciba Corning ACS Ferritin, Switzerland). The TfR-ferritin ratio (µg/µg) was calculated. Serum EPO concentrations were measured with a commercial EPO-Trac radioimmunoassay kit (Incstar, Stillwater, Minn., USA).

Pharmacokinetic study of rhEPO was carried out after giving 100 IU/kg of rhEPO intravenously (Recormon, Boehringer Mannheim) within 1 min. This dose replaced one of the three weekly doses of rhEPO in patient 1. Blood samples were drawn through an intravenous cathether from the contralateral hand just before the rhEPO dose and at 10 min, and 1, 2, 4, 6, 8 and 16 h. The pharmacokinetic parameters were calculated using apparent values of serum EPO, because the radioimmunoassay does not distinguish between endogenous and exogenous EPO. The pharmacokinetic data were analysed using the computer programme MK MODEL, version 5, BIOSOFT (Ferguson, Mo., USA). For statistical analyses we used the Mann-Whitney U test. A $P$ value $\leq 0.05$ was considered significant. Values are expressed as means ± SEM.

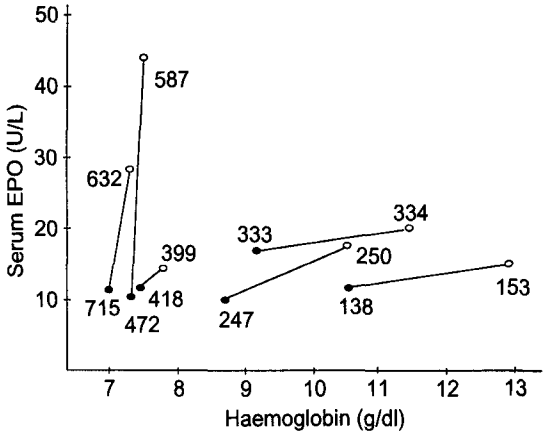


**Fig. 1.** Relationship between serum eryhtropoietin (*EPO*) concentration and haemoglobin in the study patient-reference patient pairs matched for similar serum creatinine values (µmol/l, figures). *Closed circles*, patients with familial juvenile nephronophthisis (NPH); open circles, reference patients

## Results

Serum EPO concentrations were low in the patients with NPH. The difference was significant after comparing these patients with either the creatinine – ($P = 0.01$) or the haematocrit – ($P = 0.05$) matched reference patients, or with the combined group ($P = 0.005$) (Table 1). The anaemia of the patients with NPH did not result from iron deficiency. There were no differences in iron-related parameters between these patients and the reference patients (Table 1). However, when we examined the study patient-reference patient pairs, we found differences in the degree of anaemia and renal failure between the individuals. Figure 1 shows the relationship between serum EPO concentration and blood haemoglobin in study patient-reference patient pairs matched individually for serum creatinine values. We found higher EPO levels in the reference patients with advanced

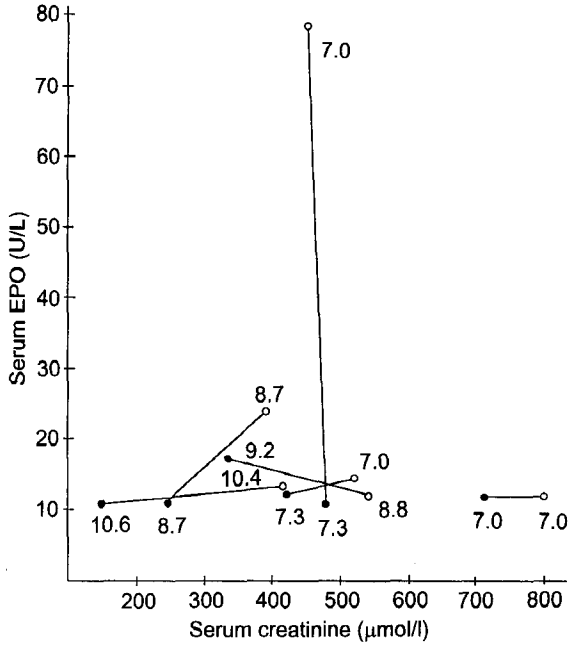


**Fig. 2.** Relationship between serum EPO concentration and serum creatinine in the study patient-reference patient pairs matched for similar haemoglobin values (g/dl, figures). *Closed circles*, patients with NPH; *open circles*, reference patients

renal insufficiency compared with the study patients in these pairs, both having low haemoglobin values. When the renal insufficiency was not yet advanced (serum creatinine $<350$ μmol/l), the study patients had lower haemoglobin values than the reference patients (Fig. 1).

The serum creatinine values were similar in the patients with NPH and the reference patients matched for haemoglobin (Table 1). However, when we considered the study patient-reference patient pairs matched individually for haemoglobin, the relationship between serum EPO concentration and creatinine values showed that renal insufficiency was more advanced in the reference patients in 5 of the 6 pairs (Fig. 2). The clearances, volumes at steady state and elimination half-times of patients 1 and 2 are shown in Table 2. Figure 3 shows the serum EPO concentration-time curves of these 2 patients with NPH.

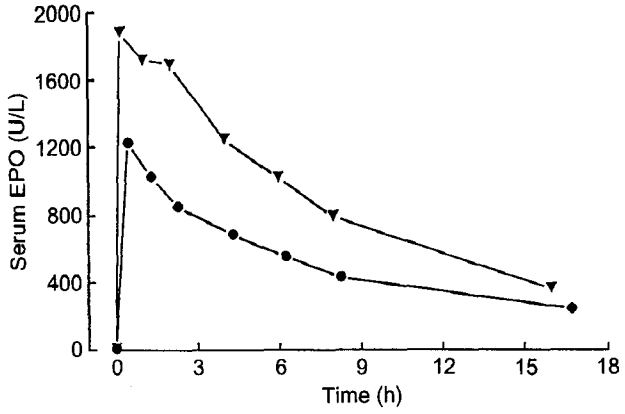


**Fig. 3.** Serum EPO concentration-time curves for 2 patients with NPH. The upper curve (*triangles*) represents patient 1, the lower curve (*circles*) represents patient 2

## Discussion

In the patients with NPH the serum EPO concentrations were lower than in the reference patients with other slowly progressive renal diseases. This finding may afford a clue to the pathogenesis of NPH. EPO is a glycoprotein hormone which controls the differentiation of erythroid progenitor cells in the bone marrow and the production of erythrocytes. After birth, the production of EPO occurs mainly in the kidneys [7]. Recent studies show that EPO is produced by peritubular fibroblasts [8]. It is widely accepted that an oxygen sensor in the kidney has an important role in the production of EPO via a feedback mechanism [9]. We speculate that, in patients with NPH, the cells responsible for the regulation of EPO production may be impaired. The low serum EPO concentrations in these patients also suggest that the pathogenetic mechanisms involved affect the function of the cells producing EPO. As the main reason for anaemia in renal insufficiency is commonly accepted to be a relative deficiency of EPO [10], our findings indicate that the anaemia is more severe in patients with NPH than in other chronic renal diseases.

The concentration of serum TfR is a new parameter reflecting iron status and the level of iron available for erythropoiesis [11]. Thus, an increase in serum TfR concentration reflects impaired iron status or increased erythropoiesis [12]. In situations with iron overload or erythoid

**Table 2.** Pharmacokinetics of intravenous recombinant human EPO (rhEPO)

| | Patient 1 | Patient 2 | Healthy adults[a] | Infant with obstructive nephropathy[b] | |
|---|---|---|---|---|---|
| Age | 8.66 years | 8.62 years | | 31 days | 103 days |
| rhEPO dose (IU/kg) | 100 | 100 | 150 | 100 | 250 |
| Earlier rhEPO treatment | yes | no | no | no | yes |
| CL (ml/h per kg) | 13.2 | 7.7 | 4.98 ±0.81 | 12.6 | 14.8 |
| $V_{ss}$ (ml/kg) | 154 | 79 | 49.2 ±8.12 | 120 | 79.8 |
| $t½$ (h) | 8.0 | 6.4 | 6.10±0.88 | 7.4 | 4.8 |

CL, Total clearance; $V_{ss}$, steady-state volume of distribution; $t½$, elimination half-time

[a] From reference [15]

[b] From reference [17]

hypoplasia, as in chronic renal failure, values are decreased [13]. The normal level depends on the method used, but values are a few milligrams per litre [10]. In this study the serum TfR concentrations of the patients with NPH were low and similarly low to those of the reference patients. This finding supports the conclusion that the anaemia is not due to iron deficiency or haemolysis.

The TfR-ferritin ratio may be a more sensitive indicator of iron status than TfR alone [14]; R Anttila, JD Cook, MA Siimes, unpublished observation), although data on the role of this ratio are limited. The TfR-ferritin ratios of the patients with NPH were low. This observation also argues against iron deficiency and haemolysis.

Pharmacokinetic studies of intravenous doses of EPO indicate that the elimination half-times are dose dependent in normal healthy men. The reported values are $4.42 \pm 1.18$ h after a tiny dose of 10 IU/kg, $6.10 \pm 0.88$ h after a therapeutic dose of 150 IU/kg and $11.02 \pm 0.03$ h after a large dose of 1,000 IU/kg [15]. Patients with renal insufficiency have similar elimination half-times: from 4 to 13 h. It has also been reported that the half-times decrease during EPO treatment [16]. EPO appears to be eliminated primarily by non-renal routes. Thus, the half-times of patients with renal insufficiency are not expected to differ from those of healthy individuals. The elimination half-times of our 2 patients with NPH fit well within the ranges of the reported values when taking into consideration the dosage used (Table 2). This indicates that in patients with NPH the catabolism of EPO is not stimulated, but the decreasing synthesis of EPO may be the reason for low serum concentrations of EPO.

The greater total clearance and steady-state volume of distribution reported in an infant [17] than in healthy men may result from the larger extracellular distribution volume in infants [17]. The total clearance and steady-state volume of distribution in our 2 patients were greater than in healthy adults and closer to those seen in the infant (Table 2).

*Acknowledgement.* This work was supported by grants from the Kidney Foundation and the Finnish Society of Nephrology. The authors thank Dr. Juhani Rapola who kindly analysed the renal biopsies.

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