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Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia

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Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia.

Background. Chronic renal failure leads to hyporegenerative anemia due to erythropoietin deficiency. The creatinine clearance and hemoglobin levels, at which anemia treatment with recombinant erythropoietin should be started, are unclear. Interpretation of serum erythropoietin levels in the context of renal insufficiency remains controversial and was addressed in this study.

Methods. Three hundred and ninety-five patients were randomly chosen out of over 5000 consecutive patients investigated by coronary angiography at a single center between 1997 and 2001. Laboratory values and clinical information were prospectively collected in a central registry. Serum samples were frozen before angiography and now used to measure serum erythropoietin levels and evaluate the relationship between erythropoietin and hemoglobin levels in the context of various degrees of renal insufficiency.

Results. The patients with the lowest renal function (creatinine clearance <20 mL/min) had significantly lower hemoglobin levels than the group with normal renal function. However, erythropoietin levels were identical indicating a lower set point for erythropoietin regulation. Above a creatinine clearance of 40 mL/min a significant inverse correlation between erythropoietin and hemoglobin levels was observed and described with the formula erythropoietin [U/L] = 2.5 × (140 − hemoglobin [g/L]) or alternatively Δerythropoietin (U/L) = −2.5 × Δhemoglobin (g/L). Below 40 mL/min no significant correlation was found.

Conclusion. A cut-off level for an altered set point of erythropoietin regulation was determined at 40 mL/min creatinine clearance. Above this cut-off hemoglobin negatively regulates erythropoietin. Below the cut-off erythropoietin levels remain stable. Pathophysiologic concepts for this finding and clinical implications in patients with moderate renal failure are discussed.

Key words: erythropoietin, chronic renal failure, creatinine clearance, hemoglobin, anemia.

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Hyporegenerative anemia is a common manifestation of chronic renal failure and considered at least partially responsible for the symptoms of chronic fatigue and a reduced general health condition associated with uremia. The importance of anemia treatment was underlined by recent studies indicating a reduction in morbidity and mortality due to a reduced incidence of left ventricular hypertrophy and consecutive heart failure, a condition now addressed as cardiorenal syndrome [1, 2]. In addition data indicating a positive effect of anemia treatment on the progression of chronic kidney failure were presented [3], although no large randomized trial on this topic has been published so far and therefore a recent Cochrane database review did not support this therapy indication [4].

With clinical approval of recombinant human erythropoietin the treatment of anemia of chronic renal failure got much less cumbersome and risky than before, when regular blood transfusion had to be performed. However, erythropoietin treatment is expensive, and the recently described pure red cell aplasia due to antierythropoietin antibodies has raised new questions on its safety [5]. Although the evidence for beneficial effects of anemia treatment in advanced renal failure is overwhelming [6], the question at what stage of renal dysfunction erythropoietin treatment should be started in order to prevent cardiovascular complications is still a matter of debate. One reason for this uncertainty is the lack of a measurable parameter for inadequate erythropoietin synthesis. Patients with renal failure may have normal erythropoietin levels although low for their degree of anemia, a condition addressed as "relative erythropoietin deficiency." This study attempts to establish a quantitative association between erythropoietin levels and hemoglobin at different levels of creatinine clearance. Based on this a threshold level of creatinine clearance is determined, where "relative erythropoietin deficiency" occurs. Possible pathophysiologic explanations and consequences for anemia treatment are discussed.

Table 1. Patient characteristics[a]

	Group A[b]		Group B[b]		Group C	Group D	Group E	All patients
Creatinine clearance *mL/min*[c]	<20		20–40		40–60	60–90	90–120	0–120
Number	20		89		125	98	63	395
Females *number* (%)	7 (35)		33 (37)		49 (39)	38 (39)	28 (44)	155 (39)
Age *years*	63.3 ± 16.3	$P < 0.03$	72.9 ± 10.3	$P < 0.001$	72.0 ± 9.1	61.9 ± 9.5	54.4 ± 7.9	66.5 ± 12
Body mass index kg/m^2	23.3 ± 6.1	$P < 0.001$	24.4 ± 3.3	$P < 0.001$	24.9 ± 3.1	27.2 ± 3.8	30.6 ± 4.4	26.2 ± 4.2
Creatinine clearance Cockcroft *mL/min*	13.5 ± 4.1	$P < 0.001$	32.1 ± 5.9	$P < 0.001$	48.2 ± 6.0	75.7 ± 8.7	102.0 ± 7.7	58.2 ± 26.8
Glomerular filtration rate MDRD *mL/min*	12.0 ± 5.2	$P < 0.001$	37.3 ± 11.9	$P < 0.001$	53.6 ± 9.8	69.8 ± 8.1	77.9 ± 10.9	55.8 ± 19.9
Ferritin[d] *μg/L* ($N = 220$)	196 ± 100	$P = 0.53$	136 ± 122	$P = 0.2$	123 ± 101	142 ± 144	174 ± 150	146 ± 129
Vitamin B_{12}[d] *ng/L* ($N = 203$)	431 ± 286	$P = 0.87$	327 ± 136	$P = 0.05$	307 ± 142	346 ± 185	418 ± 246	352 ± 192
Folic acid[d] *μg/L* ($N = 203$)	16.4 ± 43.5	$P = 0.35$	6.4 ± 3.0	$P = 0.32$	6.0 ± 2.1	6.8 ± 2.9	5.8 ± 2.8	7.1 ± 12.4
C-reactive protein[d] *mg/L* ($N = 347$)	11.2 ± 14.6	$P = 0.01$	8.3 ± 9.4	$P = 0.02$	7.7 ± 15	5.2 ± 4.3	5.1 ± 4.1	6.9 ± 10.7

[a]Values indicate mean ± standard deviation where appropriate.
[b]Statistical significance was calculated with unpaired Student *t* test and is indicated for comparison between group E (normal creatinine clearance) with groups A or B, respectively.
[c]Creatinine clearance range in mL/min that was used for definition of groups.
[d]*N* = number of patients, for whom the respective laboratory value was available.

METHODS

Patient populations

A total of 5120 consecutive patients were investigated by coronary angiography at a single center between 1997 and 2001. These patients were prospectively entered in a registry containing main clinical data, laboratory values, and results of angiography. For each patient serum samples were frozen immediately before angiography and now used for analysis of erythropoietin levels. Patients on dialysis and/or with previous erythropoietin treatment were excluded. The opening of the registry and its anonymous analysis were approved by the local ethics committee. In addition, each patient gave written informed consent for coronary angiography and respective laboratory investigations.

For 4760 (93%) of the mentioned 5120 patients hemoglobin levels and complete information to calculate creatinine clearance according to the Cockcroft formula were available. Data on this population are shown in Figures 1 and 2A, and we refer to it as the "whole population." These 4760 patients were then stratified according to the calculated creatinine clearance, and a random sample of 395 patients was chosen to measure erythropoietin levels from frozen serum samples. We refer to this population as the "sample population," and data on them are shown in Figures 2 and 3 and in Table 1.

The sample population of 395 patients was then divided into five subgroups based on the calculated creatinine clearance according to Cockcroft (Table 1). The groups E (clearance >90 mL/min; normal renal function) and D (clearance 60 to 89 mL/min; mild renal insufficiency) correspond to the staging of chronic kidney disease according to the National Kidney Foundation [7]. For groups C (moderate), B (moderate to severe), and A (severe renal failure) the cut-off levels for chronic renal failure suggested by the National Kidney Foundation had to be changed from 30 and 15 to 40 and 20 mL/min, respectively, for two reasons: (*1*) group A was considerably smaller than the other groups because coronary angiography was performed very restrictively in this patient group due to the high risk of contrast media-associated renal failure; elevation from 15 to 20 mL/min allowed adequate sampling of this group; and (*2*) analysis of the whole data set revealed that pathophysiologic changes in the erythropoietin response to anemia occur already at a level of 40 rather than 30 mL/min.

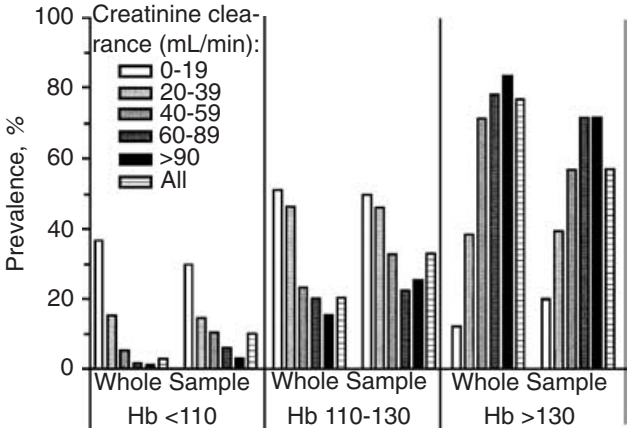

Fig. 1. Prevalence of anemia stratified to creatinine clearance within the whole and the sample population. The prevalence of hemoglobin level <110 g/L, 110 to 130 g/L, and >130 g/L is shown for the whole population of 4760 patients and for the sample population of 395 patients, which were then further analyzed for erythropoietin levels. Bars indicate percentages of patients with the respective hemoglobin level within the indicated stratum of renal function. Stratification of renal function corresponds to that presented in Table 1 and the following figures.

Laboratory values

Erythropoietin was measured in frozen serum samples in two batches by a solid-phase, two-site

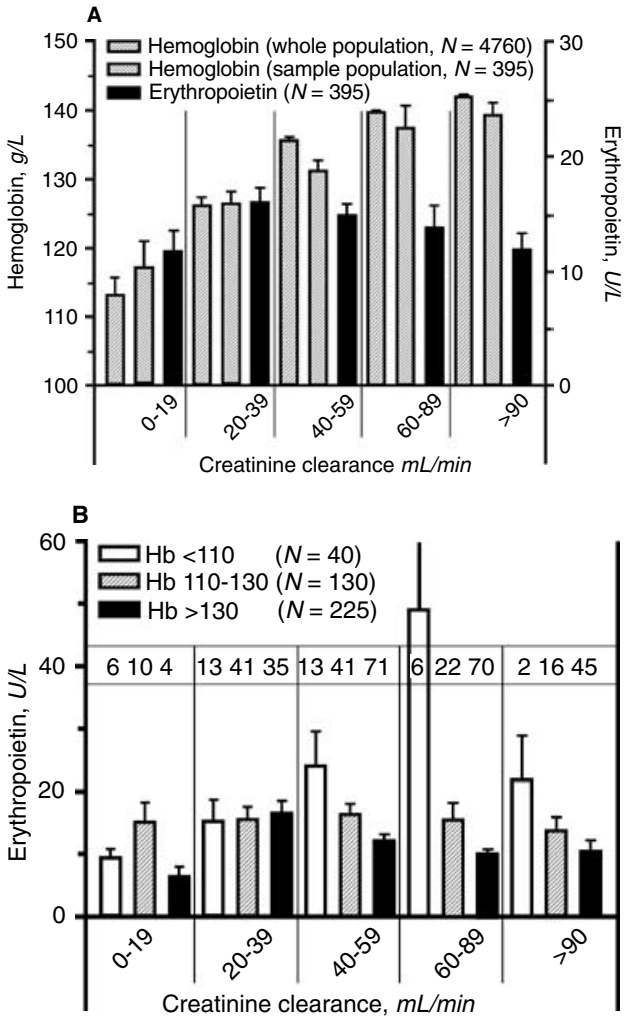

Fig. 2. Distribution of erythropoietin levels stratified to creatinine clearance and hemoglobin. The patient cohort was divided into five different groups according to the calculated creatinine clearance (mL/min): 90 to 120, normal renal function ($N = 1490$ in whole/63 in sample population); 60 to 89, mild renal insufficiency ($N = 2319/98$); 40 to 59, moderate renal insufficiency ($N = 751/125$); 20 to 39, moderate to severe renal insufficiency ($N = 151/89$); and <20, severe renal failure ($N = 49/20$). (*A*) Levels of hemoglobin (g/L) and erythropoietin (U/L) in the five different patient groups. (*B*) Serum erythropoietin levels (U/L) stratified to the degree of anemia within the five patient groups. Over each bar the number of patients analyzed in the respective subgroup is indicated. All bars indicate means ± SEM.

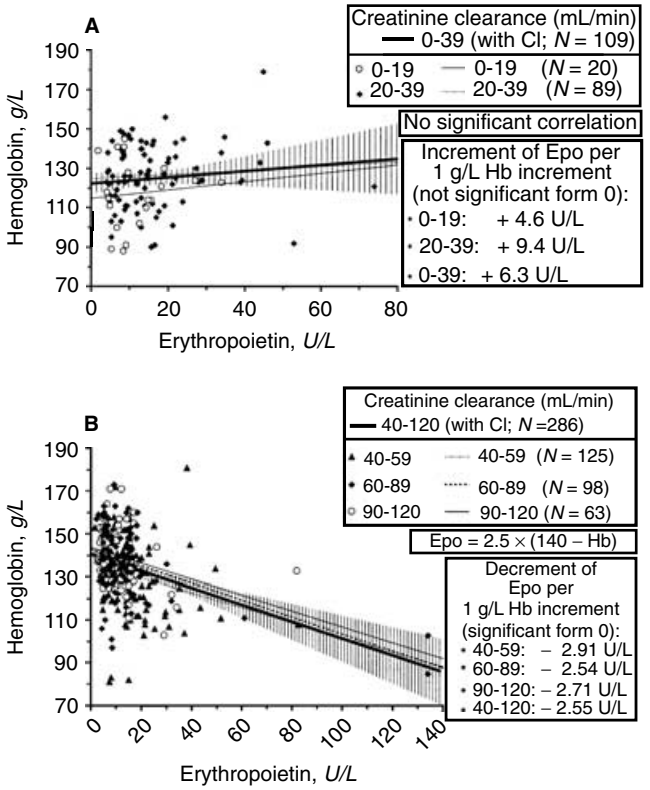

Fig. 3. Distribution of individual erythropoietin and hemoglobin levels with linear regression analysis. The same patient groups were analyzed as described in legend to Figure 1. Bold regression lines are valid for the whole population shown in the respective panel, fine and dotted regression lines apply to the different subgroups as indicated. Vertical bars indicate 95% CIs for the bold regression line. (*A*) Patients with moderate to severe renal insufficiency (creatinine clearance < 40 mL/min) showed no significant correlation between hemoglobin and erythropoietin levels ($r = 0.11$, $P = 0.25$). However, a tendency for a positive slope of the linear regression line can be observed in both subgroups. (*B*) Patients with normal renal function or mild to moderate renal insufficiency (creatinine clearance > 40 mL/min) showed a significant negative correlation between hemoglobin and erythropoietin ($r = -0.35$, $P < 0.0001$). For all three patient subgroups the slope of the linear regression line is statistically significant different from zero ($P < 0.02$), but not different among each others.

chemiluminescent enzyme immunometric assay on an Immulite analyzer (DPC, Llanberis, UK). This assay has a sensitivity of 0.24 mIU/mL and no high dose hook effect up to 50,000 mIU/mL. Reference values in a healthy population are indicated as 3 to 30 U/L. There is no cross-reactivity to α_2-macroglobulin, γ-globulin, transferrin, and human serum albumin. Total coefficient of variation is around 6.8%.

Hemoglobin levels, serum creatinine, and in 88% of patients also C-reactive protein levels were determined immediately before coronary angiography as part of routine clinical workup. The upper normal value for C-reactive protein was 8 mg/L. Creatinine was measured by the Jaffé method on a Hitachi 917 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) (age-dependent reference ranges). Creatinine clearance was then calculated according to the Cockcroft formula (140 − age [year]) × body weight [kg]/creatinine [μmol/L] with correction factors ×1.23 for men and ×1.04 for females [8], and glomerular filtration rate (GFR) was estimated with the MDRD formula $186 \times (\text{creatinine [mg/dL]})^{-1.154} \times (\text{age [years]})^{-0.203}$ with a correction factor of ×0.742 for females [9]. In more than 50% of patients, serum ferritin, folic acid, and vitamin B_{12} were also measured depending upon the amount of available material. Ferritin was measured by MEIA (Abbott, Wiesbaden, Germany)

(reference range 10 to 400 μg/L), folic acid by ion capture technology on an Axsym analyzer (Abbott) (reference range 3.5 to 16 μg/L) and vitamin B_{12} by chemoluminescence on an Immulite 2000 analyzer (DPC) (reference range 200 to 1000 ng/L).

Statistics

Comparisons between different groups were calculated by unpaired Student *t* test. Pearson's correlation coefficient *r* was calculated where indicated. All these calculations were performed with the statistical software SPSS (version 11.0). Linear regression lines and there 95% CIs were calculated and plotted with GraphPad Prism (version 3.03).

RESULTS

Patient characteristics

The sample population of 395 patients was divided in five different groups based on calculated creatinine clearance (see **Methods** section). Patients in groups A (severe renal failure) and B (moderate to severe renal insufficiency) were older and had a significantly lower body mass index when compared to group E (normal renal function). Before analyzing the correlation between erythropoietin and hemoglobin, we determined additional parameters in order to exclude other forms of anemia caused by deficiency of iron, vitamin B_{12}, and folic acid. No significant differences for these parameters were found between groups A and E. Notably, group A had even slightly higher levels of vitamin B_{12}, folic acid, and ferritin indicating that these patients received adequate supplies of these nutrients. The only significant difference was observed for vitamin B_{12} levels between groups B and E. However, since the mean of group B was still >50% above the lower reference range, a significant vitamin B_{12} deficiency is unlikely. C-reactive protein showed significantly higher levels in groups A and B compared to group E. However, the mean levels (group A = 11.2 mg/L and group B = 8.3 mg/L) were only slightly above the upper normal value (8 mg/L).

Prevalence of anemia stratified by renal function

Anemia is a feature of renal insufficiency. We therefore analyzed the prevalence of anemia in the whole population of 4760 patients as well as in the sample population, which was later used to investigate the erythropoietin response to anemia. Anemia was defined as a hemoglobin level <110 mg/L, since this is a level widely used as cut-off to start erythropoietin therapy (see European Best Practice Guidelines, [10]). Figure 1 shows two major points: (*1*) the prevalence of anemia with hemoglobin <110 mg/L is around 30% to 40% in patients with severe renal failure and around 15% in patients with clearance of 20 to 39 mL/min, but rare in the other groups; and (*2*) the hemoglobin levels over various degrees of renal failure is virtually identical between the whole and the sample population, which supports an adequate sampling of the patients further used for erythropoietin analysis.

Erythropoietin and hemoglobin levels stratified by renal function

Analysis of erythropoietin and hemoglobin levels in groups A to E is depicted in Figure 2A. Groups E (creatinine clearance >90 mL/min) and D (creatinine clearance 60 to 90 mL/min) had identical levels of hemoglobin and erythropoietin. As expected groups A to C showed increasing anemia due to progressive renal insufficiency. However, erythropoietin levels only rose mildly in groups C and B compared to E (significant only for B, $P = 0.04$), but were equal between groups A and E.

We then looked for the response of erythropoietin to different degrees of anemia within each patient group (Fig. 2B). As expected, an inverse relationship of erythropoietin and hemoglobin levels was found in groups C, D, and E. However, the erythropoietin response to anemia was blunted in groups A and B, indicating a relative erythropoietin deficiency already in patients with moderate renal insufficiency (creatinine clearance <40 mL/min).

Correlation of erythropoietin and hemoglobin

A quantitative correlation of erythropoietin and hemoglobin was then attempted (Fig. 3). As expected from Figure 1B, no correlation of erythropoietin and hemoglobin was found in patients with a creatinine clearance < 40 mL/min (Fig. 3A) (combined groups A and B $r = 0.11$, $P = 0.25$). The slope of the linear regression line was not significantly different from zero, but tended to be positive. In contrast a highly significant inverse correlation of erythropoietin and hemoglobin was found for patients with creatinine clearance >40 mL/min (Fig. 3B) (combined groups C, D, and E $r = -0.35$, $P < 0.0001$). The slope of the linear regression line was clearly negative and did not differ between the three subgroups, as indicated by the regression lines of the individual groups, which all lied within the 95% CIs. The association of erythropoietin with hemoglobin above a creatinine clearance of 40 mL/min can approximately be described by the formula erythropoietin [U/L] = 2.5 × (140 − hemoglobin [g/L]) or alternatively Δerythropoietin (U/L) = −2.5 × Δhemoglobin (g/L).

DISCUSSION

Hyporegenerative anemia is a common symptom of chronic renal failure and has an important impact on morbidity and mortality of kidney patients. Recently two large studies with more than 12,000 patients each have

investigated the incidence of anemia in patients with various degrees of renal insufficiency and observed a higher incidence already in patients with moderate renal insufficiency (creatinine clearance <60 mL/min) [11, 12]. These results were confirmed in our whole population of almost 5000 patients. With recombinant human erythropoietin an easy although expensive treatment for renal anemia is available, but the time point when to initiate erythropoietin treatment is still a matter of debate, because no parameter to measure erythropoietin deficiency is available.

Erythropoietin levels are difficult to interpret in the context of renal failure. We are aware of only three studies that investigated erythropoietin levels in relation to hemoglobin in patients with various degrees of renal insufficiency. Radtke et al [13] measured hemoglobin and erythropoietin levels in 135 patients with creatinine clearances between 2 and 90 mL/min and found elevated erythropoietin levels in all five groups compared to control subjects with normal renal function (creatinine clearance >90 mL/min). Below a creatinine clearance of 40 mL/min erythropoietin levels were inadequate for the degree of anemia. However, correlation of erythropoietin and hemoglobin within the different groups was not analyzed in this study. Chandra, Clemons, and McVicar [14] performed a very similar investigation in 48 children with various degrees of renal insufficiency. They showed a significant correlation of hemoglobin and creatinine clearance below a clearance of 40 mL/min, but again no analysis of erythropoietin levels at various degrees of anemia in those groups was shown. Our group recently presented a small investigation in 17 patients with either normal renal function (creatinine <100 μmol/L) or mild renal insufficiency (creatinine 100 to 130 μmol/L) and found a significantly altered regulation of erythropoietin in patients with mild renal insufficiency [15]. However, these results should be interpreted cautiously due to the small patient sample and the fact that renal function was analyzed only by serum creatinine.

This study for the first time presents a three-dimensional quantitative analysis of erythropoietin, hemoglobin, and renal function assessed by calculated creatinine clearance in a homogenous population of almost 400 patients. The three main findings of this study are (*1*) anemia is already observed below a creatinine clearance of 60 mL/min; this finding is in line with recent large epidemiologic studies and therefore supports the adequate sampling of our patient population; (*2*) inadequate erythropoietin synthesis in response to chronic anemia was found below a creatinine clearance of 40 mL/min; (*3*) the inverse correlation between erythropoietin levels and hemoglobin above a creatinine clearance of 40 mL/min can be described by the formula erythropoietin [U/L] = 2.5 × (140 − hemoglobin [g/L]) or alternatively Δerythropoietin (U/L) = −2.5 × Δhemoglobin (g/L); no correlation was found below this clearance level.

Erythropoietin secretion is inversely regulated by hemoglobin levels [16], and the measured parameter is tissue oxygenation (pO_2) in the outer medulla of the kidney. A low tissue pO_2 leads to up-regulation of the hypoxia-inducible factor-α (HIF-α), a transcription factor that regulates erythropoietin synthesis [17]. In principle, "relative erythropoietin deficiency" in the context of renal failure may be caused by two different mechanisms: either the production capacity of the kidney is decreased due to the tissue damage by the underlying disease, or the set point for erythropoietin production is lowered in relation to tissue oxygenation. Several studies have shown that the hypoxia-erythropoietin-hemoglobin feedback system for acute hypoxia is functional in patients with chronic renal failure. One of the first and most compelling studies was published 30 years ago. Erythropoietin was measured in six hemodialysis patients at an altitude of 400 m above sea level, before they were transported within 5 hours to the alpine research facility of Jungfraujoch in Switzerland at 3450 m above sea level, where erythropoietin measurements were repeated. An adequate response to the acute drop in inspiratory pO_2 and therefore tissue oxygenation could be demonstrated [18]. Another study performed in children with creatinine clearance below 20 mL/min found an adequate erythropoietin increase after acute hypoxic stress by either pulmonary edema, congestive heart failure, or sepsis-induced hypotension [14]. These findings, together with our results, which show that anemia occurs during early stage of renal failure (creatinine clearance <60 mL/min), where there is still an inverse relationship between hemoglobin and erythropoietin (although on a lower level), strongly support the theory of a lowered set point for erythropoietin synthesis in patients with renal failure.

Oxygen consumption and therefore tissue oxygenation in the kidney is mainly determined by the fractional sodium reabsorption, a highly energy-consuming transport process [19]. Erythropoietin levels were shown to vary directly with fractional sodium reabsorption in the context of diabetic nephropathy [20], and blockade of proximal tubular reabsorption by acetazolamide results in a drop of erythropoietin levels in normal individuals [21]. Decreasing hemoglobin and decreasing fractional sodium reabsorption might therefore represent antagonistic stimuli for erythropoietin production in the failing kidney leading to "normal" erythropoietin levels at the expense of anemia.

Our results indicate that a lowered set point of erythropoietin synthesis is observed below a creatinine clearance of 40 mL/min. If anemia occurs in this context and no other reason (as deficiency of iron, folic acid, or vitamin B_{12}) can be found, erythropoietin therapy should be

evaluated in order to prevent cardiovascular complications [1, 22]. For patients with creatinine clearance above 40 mL/min, we have established a quantitative relationship between hemoglobin and erythropoietin for a normal set point regulation by the formula erythropoietin [U/L] = 2.5 × (140 − hemoglobin [g/L]) or alternatively Δerythropoietin (U/L) = −2.5 × Δhemoglobin (g/L). In a given patient this formula could be used to determine an expected erythropoietin level in the context of a given degree of anemia and therefore allows the determination of a state of relative erythropoietin deficiency when compared with a measured eryhropoietin level.

Since our study was performed in a homogenous patient population referred because of supposed or proven coronary artery disease, the main diagnoses for chronic renal failure may be hypertensive, vascular, and diabetic nephropathy. Therefore, it remains to be established whether these data can be extrapolated to patients with glomerulonephritis, interstitial nephritis, congenital nephropathies or a failing renal allograft.

Taken together, our study shows that an altered set point of erythropoietin regulation occurs below a creatinine clearance of 40 mL/min. Below this cut-off measurement of erythropoietin is not really useful, since the response to anemia is blunted. Above this cut-off we suggest a formula to calculate an expected erythropoietin value, which then can be compared to the effectively measured level in a given patient in order to determine a state of relative erythropoietin deficiency. Further prospective studies have to reveal whether measurement of erythropoietin and application of such a formula may be predictive for the response to exogenous erythropoietin and the prognosis of renal insufficiency and cardiovascular events.

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