Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease

Ferruh Artunc and Teut Risler

Department of Internal Medicine IV, Section of Nephrology and Hypertension, Eberhard-Karls-University of Tuebingen, Germany

Abstract

Background. Renal anaemia is caused by a relative erythropoietin (EPO) deficiency. Due to difficult interpretation of serum EPO concentrations adapted to anaemia and renal function, the diagnostic value of measuring serum EPO concentrations is limited.

Methods. We retrospectively analysed the relationship between haemoglobin and serum EPO concentrations routinely measured in in- and out-patients of our university hospital from 2001–04. Patients under EPO substitution or those with acute renal failure, polycystic kidney disease, renal carcinoma or polycythaemia due to pulmonary disease were excluded. The study population (n = 500) was then stratified according to the presence or absence of chronic kidney disease (CKD) and to the stage if CKD was present. EPO concentrations were expressed in percentiles corrected for the severity of anaemia and based on the EPO response in patients without CKD.

Results. In patients without CKD (n = 167) there was a strong parametric correlation between severity of anaemia and increase in EPO (r = −0.81). Linear regression of the log-transformed EPO values revealed the equation log EPO (mIU/ml) = −0.135 × Hb (g/dl) + 2.821 (r² = 0.65). With increasing stages of CKD the correlation between haemoglobin and EPO concentrations was gradually attenuated and was completely lost in CKD stage four and five. In anaemic patients with Hb < 11 g/dl, relative EPO deficiency defined as EPO concentrations below the 25th percentile was present in 38%, 67%, 93% and 100% of the patients with CKD stages 1–5, respectively.

Conclusions. Expression of EPO concentrations in percentiles improves the diagnostic value of measuring EPO concentrations for diagnosing relative EPO deficiency and renal anaemia.

Keywords: CKD; diagnostic value; erythropoietin; renal anaemia; response

Introduction

Renal anaemia is a common and early complication in chronic kidney disease (CKD) and is found in nearly all patients with severe renal failure [1,2]. Identifying renal disease as a cause of anaemia remains difficult since specific laboratory tests are lacking. Renal anaemia is suspected in a patient with CKD who presents with normochromic anaemia after exclusion of other causes such as haemodialysis, deficiency of iron, vitamin B₁₂ or folate or malignant haematological disease. The latter requires bone marrow aspiration with core biopsy and is still not able to rule out early stages of myelodysplastic syndrome [3]. As a result, renal anaemia remains often under-diagnosed and thus untreated. In the aforementioned study of Obrador et al. [1], 51% of the patients had a haematocrit (Hct) < 28% and only 20% of the patients had been treated with erythropoietin (EPO) before start of dialysis showing gross under-utilization of EPO.

The normal renal response to anaemia is characterized by an exponentially increased EPO production in the peritubular fibroblasts which is rapidly up-regulated at the transcriptional level and results in >100-fold elevated serum EPO concentrations when Hct falls below 20% [4,5]. In patients with CKD this response is disturbed and causes anaemia with low EPO concentrations consistent with a relative deficiency [6,7]. Although serum EPO concentrations can easily be measured with radioimmunoassay (RIA) or enzyme-linked assay (ELISA), routine measurement of serum EPO concentrations is not practised widely. K/DOQI guidelines do not recommend EPO measurements to establish the diagnosis of renal anaemia in patients with creatinine concentrations >2 mg/dl [8]. The low utilization of measuring serum EPO concentrations can partly be explained by the difficult...
interpretation since EPO concentrations have to be assessed in relation to the severity of anaemia and to the renal function. Relative EPO deficiency may be easily overlooked without correction for these factors since reference values given by the manufacturer are mainly derived from healthy non-anaemic subjects.

Given the difficulties in diagnosing renal anaemia and the resulting underdiagnosis and undertreatment of this complication, we evaluated the diagnostic value of measuring serum EPO concentrations and aimed to determine how the relative EPO deficiency in CKD can be quantified and applied in anaemic patients with or without CKD.

**Subjects and methods**

**Study population**

We retrospectively analysed EPO measurements from in- and out-patients of the University Hospital of Tuebingen, Germany, from the years 2001 through 2004. EPO values were collected retrospectively from a database. Serum EPO concentrations were determined unselectively from patients as part of their anaemia work-up. In some patients, serum EPO concentrations were determined related to polycythaemia. Being routinely available at our institution, EPO was determined using a manual ELISA method according to the manufacturer’s instructions (Medac, Hamburg, Germany). The assay is calibrated according to the 3rd International Standard for EPO, rDNA derived (41st Meeting of the WHO ECBS, October 1990). The assay has a detection limit of 1.25 mIU/ml and is linear up to 160 mIU/ml. Samples with values >160 mIU/ml were re-measured with a higher dilution. The manufacturer’s reported normal range was 4–24 mIU/ml which was derived from 100 healthy blood donors.

From all patients corresponding haemoglobin (Hb) and serum creatinine concentrations were obtained from the laboratory archives which were measured within ±3 days of the EPO measurement. Hb and serum creatinine were measured with auto-analysers from Bayer (Leverkusen, Germany), the latter according to a kinetic Jaffé method. Regularly conducted external quality assessments revealed correct measurements of calibrators with assigned values traceable to isotope dilution mass spectrometry (IDMS) within 10% deviation in accordance with German regulations. Glomerular filtration rate (GFR) was calculated according to the IDMS-corrected simplified MDRD formula [9] whereby MDRD–GFR = 175 × serum creatinine (mg/dl)^−1.254 × age (years)^−0.203. This value was multiplied by 0.742 to correct for female gender.

All patients were screened for the presence of CKD by reviewing the charts and the available laboratory tests on serum creatinine, MDRD–GFR, urine dipstick and 24 h protein or albumin excretion. If, according to the K/DOQI guidelines, an MDRD–GFR <60 ml/min/1.73 m² or the presence of renal damage as evidenced by haematuria, proteinuria or microalbuminuria was found in repeated samples over at least 3 months, the patient was considered as having a yet unknown CKD. To identify the underlying reason, the charts were screened for the presence of systemic diseases affecting the kidneys such as diabetes mellitus, hypertension, multiple myeloma, systemic lupus erythematoses or vasculitis. When no underlying disease could be found, CKD could not be specified and a yet unknown origin was assumed.

Patients with acute renal failure, polycystic kidney disease, renal carcinoma or polycythaemia due to pulmonary disease were excluded since these entities may result in increased EPO concentration independent of renal function [10]. Acute renal failure was defined as a rise in serum creatinine concentration >0.5 mg/dl preceding the EPO measurement by up to four weeks. Patients with CKD and EPO substitution were excluded as well as patients on chronic haemodialysis or peritoneal dialysis without EPO treatment. Also, patients were not eligible when the presence of CKD could not be ruled out due to missing charts and missing repeated laboratory tests over 3 months. When multiple EPO values of a single patient were available, only the first value was taken and the subsequent values were excluded.

The study population was stratified according to the presence or absence of CKD and to the stage if CKD was present. All subjects were assigned to one of the following groups: no CKD, CKD stage one and two, CKD stage three, CKD stage four and CKD stage five. Since only n = 9 patients with CKD stage one were identified, these were added to the patients with CKD stage two. As a matter of fact, it should be noted that the MDRD formula is not validated for values over 60 ml/min/1.73 m² and probably underestimate the true GFR [11]. Hence, GFR values for patients of CKD stages one and two as well as for patients without CKD are only approximate GFR values.

**Statistical analysis**

EPO concentrations and its decadic logarithm were found to be normally distributed according to the Kolmogorov–Smirnov test, thus the correlation of EPO to the Hb concentrations was calculated parametrically with the Pearson’s test. Multivariate regression of log-transformed EPO values with haemoglobin, gender and age was performed to test for age and gender dependence. Linear regression was applied using the haemoglobin (Hb) values as the independent variable and the log-transformed EPO concentrations as the dependent variable. Linearity between Hb and log EPO values was tested by the runs test which showed equally distributed values above and below the regression line. Predicted log EPO values were calculated according to the equation obtained from the linear regression. The residuals were calculated as the difference between the predicted and the actual log EPO values. Analysis of the distribution of the residuals showed a normal Gaussian distribution confirming the linear relationship between the two variables.

To quantify the EPO concentrations in relationship to the haemoglobin value, EPO values were expressed as a function of Hb using percentiles. To this end, the equation obtained from linear regression of the patients without CKD served as a reference to calculate a z-value of each measured actual EPO value according to the formula:

\[ z = \frac{\text{actual log EPO} - \text{predicted log EPO}}{s_{y,x}} \]

where \( s_{y,x} \) denotes the standard deviation of the residuals obtained from linear regression (\( s_{y,x} = 0.38 \)). The z-value was converted to a percentile value (in %) according to the
normal distribution. All statistical analysis was done using JMP IN release 5.1.2, SAS Institute (Cary, NC, USA) and GraphPad Prism version 3.00 for Windows, GraphPad Software (San Diego, CA, USA). A P-value < 0.05 was considered significant.

Results

From 2001–04, a total of n = 1091 EPO measurements were done on 821 patients. A total of 591 values had to be excluded due to repeated measurements (n = 268), concurrent EPO use (n = 98), presence of acute renal failure (n = 84), renal carcinoma, polycystic kidney disease, polycythemia due to pulmonary disease (n = 81) or missing chart or laboratory values (n = 58). The remaining 500 patients were stratified according to the presence or absence of CKD and to the stage if CKD was present. The selection of the study population is depicted in Figure 1; the characteristics of these groups are shown in Table 1. Gender distribution and mean age were similar. Median haemoglobin and range was largest in the patients without CKD and both decreased with increasing stages of CKD. As listed, malignant haematological disease was the main cause of anaemia in the group without CKD (45%) and to a lesser extent carcinoma, bleeding, iron deficiency or haemodialysis. In 5% of the cases no specific cause of anaemia could be established. In contrast, in the patients with CKD the cause of anaemia was largely not specified. The proportion of these anaemic patients without a known cause increased with increasing stages of CKD and reached 97% in the patients with CKD stage five. Malignant haematological disease was present in only 16% of all patients with CKD and few patients had anaemia due to bleeding or iron deficiency. The underlying disease leading to CKD was mainly diabetes mellitus, glomerulonephritis or due to toxicity. In a significant proportion of patients (20–34%), no specific cause of CKD could be identified.

In the group of patients without CKD (n = 167) a strong negative correlation between severity of anaemia represented by the haemoglobin values and serum EPO concentrations (r = −0.81) was found (Table 2). With increasing stages of CKD the correlation between haemoglobin and serum EPO concentrations was gradually attenuated (r = −0.61 in CKD stage one and two and r = −0.41 in CKD stage three) and was completely lost in CKD stage four and CKD stage five.

To test for gender and age dependence of the renal erythropoietin response, EPO concentrations in patients without CKD were further analysed. Multivariate regression of log-transformed EPO values with haemoglobin, gender and age as covariates showed no significant impact of age and gender on the EPO values. Hence the data of the patients of all ages and each gender without CKD were pooled for further analysis. The equation derived from regression analysis was log EPO (mIU/ml) = −0.135 × Hb (g/dl) + 2.821 (r² = 0.65, Figure 2A). As shown in Figure 2B, linear regression of the patients with CKD showed gradual reduction in both slope and y-intercept with increasing stages of CKD. While the regression line was not different between patients without CKD or patients with CKD stage one and two, the regression line from patients of CKD stage three was significantly different from patients without CKD. In CKD stage four and five the slope was not significantly different from zero consistent with the abolished correlation.

To quantify the EPO concentrations in relation to the haemoglobin value, a percentile value of the measured actual EPO values was calculated using the predicted EPO values which were derived from the equation obtained in patients without CKD. As a result, EPO values from patients with CKD were related and normalized to the EPO response of the patients without CKD. Figure 3 depicts the distribution of these Hb-adapted EPO percentiles in the various groups. As a matter of principle, patients without CKD showed an even distribution of the EPO percentiles. This was also largely true for the patients with CKD 1+2. With increasing stages of CKD, an uneven distribution of the EPO percentiles was evident. The median EPO percentile was only 15%,
4% and 3% in patients with CKD stage three, CKD stage four and CKD stage five, respectively. Figure 4 depicts the distribution of these patients in the low EPO percentiles (5th, 10th and 25th, respectively). When considering all patients regardless of the Hb value, EPO quantiles were below the 25th percentile in 63%, 89% and 95% of the patients with CKD stages three, four and five, respectively. These percentages were higher in anaemic patients. In anaemic patients with Hb ≤11 g/dl, 38% and 67% of the patients with CKD stage 1 + 2 and three were below the 25th percentile, respectively, and virtually all patients with CKD stage four and five (93% and 100%, respectively). In anaemic patients with Hb < 9 g/dl, the majority of the patients with CKD 3 (68%), virtually all patients with CKD 4 (96%) and all patients with CKD 5 (100%) were below the 25th percentile. In contrast, the percentage of patients without CKD

Table 1. Characteristics of the patient groups; Medians with interquartile range; to convert serum creatinine in mg/dl to μmol/l multiplied by 88.4. High Hb value due to myeloproliferative syndrome

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Median age, y</th>
<th>Gender, male (%)</th>
<th>Median serum creatinine, mg/dl</th>
<th>Median MDRD-GFR, ml/min/1.73m²</th>
<th>Median Hb, g/dl</th>
<th>Hb range, g/dl</th>
<th>Cause of anaemia, (%)</th>
<th>No anaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CKD</td>
<td>167</td>
<td>58 (42; 68)</td>
<td>49</td>
<td>0.9 (0.8; 1.0)</td>
<td>80 (68; 95)</td>
<td>10.6 (8.8; 14.8)</td>
<td>5.5–20.4</td>
<td>Leukaemia, lymphoma</td>
<td>15</td>
</tr>
<tr>
<td>CKD 1 + 2</td>
<td>45</td>
<td>60 (40; 70)</td>
<td>69</td>
<td>1.0 (0.90; 1.1)</td>
<td>74 (65; 86)</td>
<td>10.1 (9.2; 13.0)</td>
<td>7.0–19.3</td>
<td>Myelodysplastic syndrome</td>
<td>11</td>
</tr>
<tr>
<td>CKD 3</td>
<td>150</td>
<td>69 (55; 76)</td>
<td>69</td>
<td>1.6 (1.4; 1.85)</td>
<td>40 (34; 48)</td>
<td>10.2 (9.0; 11.3)</td>
<td>6.1–19.2</td>
<td>Myeloproliferative syndromes</td>
<td>19</td>
</tr>
<tr>
<td>CKD 4</td>
<td>99</td>
<td>68 (59; 75)</td>
<td>68</td>
<td>2.7 (2.3; 3.15)</td>
<td>22 (18; 26)</td>
<td>9.7 (8.9; 11.1)</td>
<td>4.6–17.2</td>
<td>Cancer, leukaemia, lymphoma</td>
<td>11</td>
</tr>
<tr>
<td>CKD 5</td>
<td>39</td>
<td>67 (52; 77)</td>
<td>67</td>
<td>4.90 (3.95; 5.7)</td>
<td>12 (10; 14)</td>
<td>9.7 (9.0; 10.7)</td>
<td>6.7–13.2</td>
<td>Cancer, myelodysplastic syndrome</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2. Relationship between log EPO and haemoglobin values. CI confidence interval.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>r</th>
<th>slope with 95% CI</th>
<th>y-intercept with 95% CI</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CKD</td>
<td>167</td>
<td>−0.81</td>
<td>−0.135 (−0.150; −0.120)</td>
<td>2.821 (2.635; 3.007)</td>
<td>0.65</td>
</tr>
<tr>
<td>CKD 1 + 2</td>
<td>45</td>
<td>−0.61</td>
<td>−0.101 (−0.141; −0.061)</td>
<td>2.334 (1.861; 2.807)</td>
<td>0.37</td>
</tr>
<tr>
<td>CKD 3</td>
<td>150</td>
<td>−0.42</td>
<td>−0.093 (−0.126; −0.060)</td>
<td>2.013 (1.662; 2.363)</td>
<td>0.17</td>
</tr>
<tr>
<td>CKD 4</td>
<td>99</td>
<td>−0.16b</td>
<td>−0.029 (−0.066; 0.008)</td>
<td>1.058 (0.674; 1.442)</td>
<td>0.02b</td>
</tr>
<tr>
<td>CKD 5</td>
<td>39</td>
<td>−0.03b</td>
<td>−0.007 (−0.092; 0.076)</td>
<td>0.847 (0.011; 1.683)</td>
<td>0.001b</td>
</tr>
</tbody>
</table>

*significant difference to patients with no CKD.
*no significant correlation.
*slope not significantly different from 0.
below the 25th percentile decreased with increasing severity of anaemia and was 20% and 16% when analysing patients with a HB <11 g/dl and <9 g/dl, respectively (Figure 4).

As an alternative to calculations, a nomogram for the estimation of the quantile values from measured serum EPO concentrations was created (Figure 5A). It requires the current HB value and is based on the EPO response in patients without CKD. The curves represent the 5th, 10th, 25th percentile and the median of the serum EPO concentrations. Figure 5B and C show the actual percentile values of patients with CKD stages 1+2 and three as well as stages four and five, respectively.

Discussion

In this study we provide detailed quantitative data on the renal erythropoietin response in relation to the severity of anaemia and renal function in a large patient cohort with various causes of anaemia seen in a university hospital from 2001–04. As already known, main determinants of the renal EPO response were the severity of anaemia and the presence and stage of CKD. Thus, for the correct interpretation of measured EPO concentrations, current HB concentration as well as the kidney function have to be taken into account. In this study, we expressed EPO concentrations in percentiles which related EPO concentrations to both the severity of anaemia and to the normal response seen in anaemic patients without CKD. In addition to the calculation by the given formula, the percentile of the EPO concentration can be graphically determined using the nomogram given in Figure 5. The use of percentiles is in analogy to the interpretation of growth in paediatric medicine based on percentile curves [12].

The approach using quantiles has distinct advantages. First, it facilitates the assessment of the adequacy of the renal EPO response especially in patients with CKD since the percentile value relates the measured EPO concentration to the severity of anaemia and to the normal EPO response at the same time. Thus, these values are directly comparable to each other and cut-off values for the detection of relative EPO deficiency or overproduction can be derived. Inherent to quantiles, this approach also accounts for patients without CKD who may show low EPO concentration and as a result low percentile values. From our data, we propose a cut-off of less than the 25th percentile for defining relative EPO deficiency in patients with CKD which is based on the distribution of the percentiles in CKD patients. In our study population, the vast majority of anaemic patients (HB <11 g/dl) with CKD stage three had EPO percentiles less than this threshold (67%) and virtually all patients with CKD stage four and five (93% and 100%, respectively). In contrast, only 20% of the anaemic patients without CKD had EPO concentrations below the 25th percentile.

Paralleling the cumulation of CKD patients in low EPO percentiles, the proportion of patients with unspecified anaemia increased with increasing stages
of CKD and reached 100% in CKD stage five (Table 1). This strongly suggests that anaemia as a complication of renal disease was increasingly present but not identified.

Since a gold-standard for the diagnosis of renal anaemia is lacking, the true diagnostic value of measuring EPO cannot be analysed using receiver-operating curves (ROC). Due to this it is not possible to calculate sensitivity and specificity. Although a diminished or absent EPO response in a renal patient indicates renal anaemia, additional investigations have to be performed to rule out other causes of anaemia. When renal anaemia is suspected by the presence of CKD, normochromic anaemia and relative EPO deficiency, EPO substitution is warranted in these patients to target levels of 11–13 g/dl according to the

Fig. 3. Percentile distribution of the EPO concentrations according to the group.

Fig. 4. Percentage of patients with EPO values <5, 10 and 25th percentile in all patients and in anaemic patients with Hb<11 g/dl or Hb<9 g/dl.
K/DOQI guidelines provided sufficient iron stores (ferritin > 100 ng/dl).

In a similar study, Fehr et al. [13] investigated EPO concentrations with various degrees of renal insufficiency and anaemia. In patients with advanced renal failure (calculated creatinine clearance < 40 ml/min) they also could not find a significant correlation between EPO and Hb concentrations whereas for patients with a clearance > 40 ml/min a negative correlation of −0.35 was obtained. From linear regression analysis they derived a formula to calculate EPO from Hb levels (EPO in U/l = 2.5 × (140 − Hb in g/l)), which mathematically does not account for the exponential rise of the EPO levels with decreasing Hb levels. Furthermore, this equation is only valid for Hb values > 140 g/l otherwise negative EPO values are calculated.

According to the K/DOQI guidelines, measurement of EPO concentrations can be omitted in patients with creatinine > 2.0 mg/dl [8]. Indeed, when analysing patients with CKD stage four and five, no correlation of the EPO concentrations to Hb values was observed. In CKD stage three, there was a weak correlation and still 67% of the anaemic patients were below the 25th percentile. In contrast, the remaining 33% exhibited a normal erythropoietin response excluding renal cause of anaemia. Thus, measurement of EPO levels aids in the diagnosis of relative EPO deficiency in this particular subgroup. Based on these findings, we propose measuring EPO levels in the initial work-up of an anaemic renal patient with CKD stages one, two and three in addition to investigations to rule out other causes of anaemia. In patients with CKD stage four and five, routine EPO measurements can be omitted since this subgroup no longer showed a correlation of EPO levels with the severity of anaemia, indicating lost erythropoietin response.

The data demonstrate clearly that there is a relative EPO deficiency in renal anaemia rather than an absolute lack; even in patients with severe renal failure (CKD 5) EPO concentrations were neither lower than the values from renal patients without anaemia nor different from the normal range. When analysing the regression lines of patients with CKD, we found a continuous decline in both slope and y-intercept with increasing stages of CKD. This indicates both reduced responsiveness and reduced capacity of EPO secretion. Nevertheless, EPO remains detectable even in the most advanced stages of CKD maintaining minimal erythropoiesis. This might be attributable to residual renal function or alternatively hepatic production [14,15]. Renal retention of EPO due to its low molecular weight has been assumed in earlier studies [16]. However, more recent work shows that renal clearance of EPO is thought to play only a minor role. In a study in sheep [17], the elimination of 125I-labelled recombinant human EPO was not affected by bilateral
nephrectomy and only <1% of the tracer was found in the urine.

EPO percentiles presented in this study were calculated in the context of long-standing chronic anaemia. Patients with acute anaemia due to haemorrhage were not studied, hence the EPO concentrations may be different in these situations. In healthy volunteers, serum EPO concentrations are increased after acute blood loss due to phlebotomy [18]. This response is still detectable even in the most advanced stages of CKD, as haemodialysis patients were also shown to increase EPO secretion after phlebotomy, albeit the response was markedly blunted [19]. Enhanced erythropoesis was also demonstrated in an old study looking at dialysis patients in high altitude even though EPO levels were not directly measurable at that time [20].

The EPO response is not dependent on age and gender. The latter is in agreement with a previous study [21]. Despite the gender difference of the normal range for Hb (12–16 g/dl for females vs 14–18 g/dl for males) this difference did not translate into a shift of the regression line between the genders.

The cohort studied here reflects anaemic patients with or without CKD whose serum EPO concentrations were measured as part of their anaemia work-up. Hence, the CKD population of this study might not be representative for the general CKD population and will contain a disproportionate amount of anaemic patients at each CKD stage in comparison with the whole CKD population in each stage. This is also suggested by the median age of the study population which does not vary with the stage of CKD as epidemiological studies of CKD demonstrate an increase in median age with stage of CKD. Hence, the percentile distribution of the EPO values of the patients with CKD might not be generalizable. However, this selection bias does not affect the quantitation of the normal EPO response as the latter was derived from anaemic patients without CKD. It also does not affect the proposed cut-off at the 25th percentile for the detection of relative EPO deficiency which can only be applied to anaemic patients. A non-anaemic patient with CKD is expected to have normal serum EPO concentrations and hence an EPO percentile >25%.

The concept of detecting a relative EPO deficiency is limited by the fact that a significant proportion of anaemic patients with normal renal function (20%) also showed inadequately low EPO concentrations. This proportion decreased to 16% in more severely anaemic patients with an Hb <9 g/dl and might be related to the underlying disorder which may have caused depression of EPO secretion. This had been previously shown for patients with various tumours [22] or rheumatoid arthritis [23]. However, the term renal anaemia in these patients is not applicable since CKD is not present. Theoretically, these patients could be treated with recombinant erythropoiesis-stimulating agents, which are able to correct the anaemia and reduce the transfusion frequency. However, it remains unclear whether tumour response and overall survival are also affected [24,25].

In summary, we propose a new quantitation approach by expressing measured EPO concentrations in percentiles corrected for the severity of anaemia and based on the normal erythropoietin response in patients without chronic kidney disease. From our data, a cut-off of >25th percentile was adopted for defining relative EPO deficiency in patients with CKD indicative of renal anaemia.

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