

## **Timing and determinants of erythropoietin deficiency in chronic kidney disease.**

Lucile Mercadal, Marie Metzger, Nicole Casadevall, Jean-Philippe Haymann, Alexandre Karras, Jean-Jacques Boffa, Martin Flamant, François Vrtovsnik, Bénédicte Stengel, Marc Froissart

► **To cite this version:**

Lucile Mercadal, Marie Metzger, Nicole Casadevall, Jean-Philippe Haymann, Alexandre Karras, et al.. Timing and determinants of erythropoietin deficiency in chronic kidney disease.: Erythropoietin and chronic kidney disease. Clinical Journal of the American Society of Nephrology, American Society of Nephrology, 2012, 7 (1), pp.35-42. <10.2215/CJN.04690511>. <inserm-00739807>

**HAL Id: inserm-00739807**

**<http://www.hal.inserm.fr/inserm-00739807>**

Submitted on 9 Oct 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Timing and determinants of erythropoietin deficiency in chronic kidney disease

Lucile Mercadal<sup>1 2</sup>, Marie Metzger<sup>1 2</sup>, Nicole Casadevall<sup>3 4</sup>, Jean Philippe Haymann<sup>5 6</sup>,  
Alexandre Karras<sup>7</sup>, Jean-Jacques Boffa<sup>6 8</sup>, Martin Flamant<sup>9 10</sup>, François Vrtovsnik<sup>10 11</sup>,  
Bénédicte Stengel<sup>1 2</sup>, Marc Froissart<sup>1 12</sup> on behalf of the Nephrotest Study Group

<sup>1</sup> Inserm, CESP Centre for research in Epidemiology and Population Health, U1018, Epidemiology of Diabetes, Obesity, and Kidney Diseases Team, F-94807, Villejuif, France

<sup>2</sup> Université Paris Sud 11, UMRS 1018, F-94807, Villejuif, France

<sup>3</sup> Department of Hematology Hôpital Saint Antoine, Assistance Publique-Hôpitaux de Paris, université Pierre et Marie Curie, Paris

<sup>4</sup> Inserm U1009, Villejuif

<sup>5</sup> Department of Physiology and Nephrology, Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie, Paris

<sup>6</sup> Inserm U702, Paris

<sup>7</sup> Department of nephrology, Hôpital Européen G Pompidou, Assistance Publique-hôpitaux de Paris, université Pierre et Marie Curie, Paris

<sup>8</sup> Department of nephrology, Hôpital Tenon, Assistance Publique-hôpitaux de Paris, université Pierre et Marie Curie, Paris

<sup>9</sup> Department of physiology, Hôpital Bichat, Assistance Publique-hôpitaux de Paris

<sup>10</sup> Inserm U699, Paris

<sup>11</sup> Department of nephrology, hôpital Bichat, Assistance Publique-hôpitaux de Paris, université Pierre et Marie Curie, Paris

<sup>12</sup> Department of Physiology, Hôpital Européen G Pompidou, Assistance Publique-hôpitaux de Paris, Université Paris Descartes, Paris, France.

Running title: Erythropoietin and chronic kidney disease

Subject of manuscript (categories): epidemiology, chronic kidney disease, anemia

Word count for text: 2953

Word count for abstract: 235

Corresponding Author:

L Mercadal

Inserm, CESP, U 1018,

16 avenue Paul Vaillant Couturier, 94 807 Villejuif, France.

[lucile.mercadal@inserm.fr](mailto:lucile.mercadal@inserm.fr)

phone 0033145595108 fax 0033147269454

## **Abstract**

**Background and objectives:** Anemia in patients with chronic kidney disease (CKD) is related to a large extent to impaired erythropoietin (EPO) response, the timing and determinants of which remain unknown.

**Design, settings, participants, measurements:** We measured EPO levels and studied their relations to glomerular filtration rates measured by  $^{51}\text{Cr}$ -EDTA renal clearance (mGFR) in 336 all-stage CKD patients not receiving any erythropoiesis-stimulating agent.

**Results:** In patients with WHO-defined anemia (hemoglobin (Hb)  $<13$  (12) g/dL in men (women)), EPO response to Hb level varied by mGFR level: above 30 mL/min/1.73m<sup>2</sup>, EPO and Hb levels were negatively correlated ( $r = -0.22$ ,  $p=0.04$ ), and when mGFR was less than 30, they were not correlated ( $r = 0.09$ ,  $p=0.3$ ;  $p$  for interaction, 0.01). In patients with WHO-defined anemia, the ratio of observed EPO to the level predicted by the equation for their Hb level decreased from 0.72 [0.57-0.95] for  $\text{mGFR} \geq 60$  mL/min/1.73m<sup>2</sup> to 0.36 [0.16-0.69] for  $\text{mGFR} < 15$ . Obesity, diabetes with nephropathy other than diabetic glomerulopathy, absolute iron deficiency, and high C-reactive protein concentrations were associated with increased EPO levels, independent of Hb and mGFR.

**Conclusions:** Anemia in CKD is marked by an early relative EPO deficiency, but several factors besides Hb may persistently stimulate EPO synthesis. While EPO deficiency is probably the main determinant of anemia in patients with advanced CKD, in those with mGFR greater than 30 mL/min/1.73m<sup>2</sup>, presence of anemia calls for other explicative factors.

## Introduction

Erythropoietin (EPO) was first isolated in 1971<sup>1</sup> and its gene cloned in 1985.<sup>2</sup> Produced in the kidney and the liver, EPO stimulates the differentiation and proliferation of erythroid progenitors. The subsequent synthesis of recombinant human erythropoietin markedly changed the management of end stage renal disease (ESRD)-related anemia. Recent findings that erythropoiesis stimulating agents (ESA) did not improve cardiovascular and renal outcomes in patients with non-end-stage chronic kidney disease (CKD)<sup>3 4 5</sup> have raised questions about the timing and degree of EPO deficiency in renal anemia.<sup>6</sup> Our knowledge of the underlying cause of impaired EPO production is also incomplete.

Decrease in the endogenous EPO response to anemia, is thought to be one of the major mechanisms of CKD anemia. In subjects without CKD, EPO and Hb levels are negatively correlated, a feedback regulation that tends to be reversed in CKD patients.<sup>7 8 9</sup> In ESRD patients, the feedback regulation process is blunted, even though it is somewhat preserved after hemorrhage<sup>10 11</sup> and possibly at high altitudes.<sup>12</sup> EPO levels remain in the normal range in CKD patients compared to non-anemic healthy controls.<sup>7 13 14</sup> But for the degree of their anemia, CKD patients had EPO level lower than expected. The decrease in the endogenous EPO response to anemia has not been quantified in CKD patients, however. In addition, all studies conducted before 1980 used bioassays that provided higher EPO levels than those measured with present double-antibody sandwich immunoassays. Moreover, although inflammation and iron deprivation are well-documented risk factors for ESA resistance in dialysis patients, the determinants of EPO production have not been systematically studied in early-stage CKD.

We therefore used reference methods to investigate the timing of EPO deficiency and its determinants according to renal function in 336 CKD patients. In those with anemia, we also quantified the endogenous EPO response to Hb decreases according to GFR level.

## **Methods**

### **Study population**

The Nephrotest study is a prospective hospital-based cohort, enrolling adult patients with all CKD diagnoses, stages 1 through 5, not pregnant and not on dialysis or living with a kidney transplant.<sup>15</sup> By the end of 2009, a total of 1294 patients had been referred to 2 physiology departments for work-ups to assess CKD progression and complications, including measured GFR (mGFR), according to standard methods. All patients provided written informed consent before inclusion in the cohort. In this study, we analyzed a subgroup of 336 patients who were receiving neither ESA nor intravenous iron and whose endogenous EPO was assayed. They did not differ from those without EPO measures for baseline age, gender, diabetes, median mGFR, or Hb level (data not shown).

### **Information**

Demographic, clinical, and laboratory data were collected. Twenty-one percent of all patients had biopsy-proven nephropathy, but less than 10% of those with diabetes. Clinical criteria based on the presence of a history of albuminuria > 300 mg/g creatininuria and of another microangiopathy damage (retinopathy or/and neuropathy) were used to classify diabetic patients without renal biopsy as having diabetic glomerulopathy. Other diabetic patients were classified as having other nephropathy types, most of them having probably a vascular nephropathy. We used Lipschitz's index<sup>16</sup> to define iron status based on transferrin saturation (TSAT) and ferritin as follows: normal, TSAT  $\geq$  20%; TSAT < 20% and ferritin < 40 ng/mL, absolute iron deficiency; TSAT < 20% and ferritin  $\geq$  40 ng/mL, functional iron deficiency. We primarily used WHO sex-specific thresholds to define anemia: Hb concentration < 12 g/dL for women and <13 g/dL for men. GFR was measured by renal clearance of <sup>51</sup>Cr-EDTA

as described earlier<sup>17</sup> and categorized according to Kidney Disease Improving Global Outcomes guidelines.<sup>18</sup>

### **Erythropoietin measurement and responsiveness to Hb level**

Endogenous EPO levels were determined in serum (100  $\mu$ L) with the Quantitine IVD Epo double-antibody sandwich ELISA method from R&D Systems (Minneapolis, MN).

To assess the response of endogenous EPO to Hb levels, we used a method previously developed and validated in non-CKD patients with anemia.<sup>19 20 21</sup> We predicted the erythropoietin value for a given Hb level for all patients with WHO-defined anemia according to the following equation: predicted  $\log_{10}$ EPO (p- $\log_{10}$ EPO) = 4.46 - (0.274 x Hb in g/dL), so that predicted EPO (p-EPO) =  $10^{(\text{p-}\log_{10}\text{EPO})}$  in IU/L.<sup>19</sup> We then calculated the ratio of observed/predicted (O/P) EPO in these patients by dividing the observed endogenous EPO levels by those predicted by equation. Unlike others,<sup>19</sup> however, we divided crude rather than log-transformed EPO values to express results as a percentage of EPO deficiency.

### **Statistical analyses**

Because there were not normally distributed, EPO values were log-transformed in the analyses. The relations between EPO, Hb, and mGFR levels were studied in three steps. We first analyzed EPO response to decreased Hb according to mGFR level, independent of anemia status. For this purpose, we assessed the correlation between Hb and  $\log_{10}$ EPO and tested the interactions with renal function level for different mGFR thresholds: 15, 30, and 45 mL/min/1.73m<sup>2</sup>. We then studied the correlations between endogenous EPO and Hb levels according to WHO-defined anemia status and mGFR in 2 classes, < or  $\geq$  30 mL/min/1.73m<sup>2</sup>. Finally, we studied Pearson correlations between EPO and mGFR in anemic and non anemic

patients. In a sensitivity analysis, we repeated this step after classifying patients according to anemia defined with only one cut-off value of 12 g/dL irrespective of sex.

Other factors associated with EPO levels were first studied with bivariate analyses that used Kruskal-Wallis correlations for qualitative and Pearson correlations for quantitative variables. Interactions with mGFR, anemia status, or sex in the relations between the studied factors and EPO level were systematically tested. We then conducted a multivariate regression analysis of determinants of EPO levels, using three nested models that we compared according to maximum likelihood criteria. Model 1 included age in years, center, and a combined variable derived from previous step findings: no anemia, anemia and mGFR  $\geq 30$  mL/min/1.73m<sup>2</sup>, anemia and mGFR  $< 30$ . Models 2 and 3 sequentially added other risk factors identified from the bivariate analysis, namely BMI and diabetes with glomerular or other types of nephropathy in model 2, and iron index and CRP in model 3. We studied  $\log_{10}$ EPO and expressed the results in percentage of EPO change in the exposed compared with the reference category. All analyses were performed with SAS 9.1 (SAS Institute, Cary, NC).

## **Results**

### **Patient characteristics**

Mean age was close to 60 years, and patients were predominantly men and of European origin (Table 1). Vascular nephropathy was the most common type of CKD. Two thirds of the patients had CKD stage 3B or 4. WHO-defined anemia (Hb <13 (12) g/dL in men (women)) was present in 55% of men and 63% of women. EPO levels ranged from 1.9 to 47.8 IU/L.

### **Relations between erythropoietin, hemoglobin, and measured GFR levels**

Endogenous EPO response to Hb level varied according to mGFR level. The strongest interaction between Hb and mGFR in the relation with EPO was observed for an mGFR threshold of 30 mL/min/1.73 m<sup>2</sup> (p for interaction=0.01); interaction was not statistically significant for mGFR of 45 and 15 mL/min/1.73m<sup>2</sup> (p=0.72 and 0.18). EPO and Hb levels were negatively correlated (r = -0.2, p=0.04) in patients with mGFR value ≥ 30 mL/min/1.73m<sup>2</sup>, but were not when mGFR level was < 30 (r = 0.07, p=0.3). Analysis by anemia status, however, showed that this pattern was limited to patients with anemia (Figure 1).

Moreover, there was no correlation between EPO and mGFR levels in patients with or without anemia (r = 0.08 and 0.01, respectively, both ns). In anemic patients, predicted EPO value was always consistently greater than observed EPO at all mGFR (Figure 2). The observed to predicted (O/P) EPO ratio fell markedly with mGFR decline from 0.72 [0.57-0.95] for mGFR ≥ 60 mL/min/1.73m<sup>2</sup> to 0.36 [0.16-0.69] for mGFR < 15, the relative EPO deficits ranging so from 28 to 64% from CKD stages 1-2 to 5.

### **Other factors associated with erythropoietin level**

EPO was significantly higher in patients aged 60 to 69 years, in those overweight or obese, or with absolute iron deficiency, diabetes with nephropathy other than diabetic glomerulopathy,



or C-reactive protein (CRP) > 8 mg/L, compared with their counterparts without these conditions (Table 2). EPO levels were not related to gender, geographic origin, treatment with renin angiotensin system inhibitors, or urine protein to creatinine ratio, vitamin D or folate levels. There was a significant interaction ( $p = 0.04$ ) between serum albumin and mGFR: higher EPO levels with lower albumin values were observed only in patients with  $mGFR \geq 30$  mL/min/1.73 m<sup>2</sup>. No other significant interaction was seen with mGFR, gender, or anemia (all  $p \geq 0.2$ ). Mean Hb level was significantly higher in patients overweight or obese than in those with normal BMI,  $12.6 \pm 1.5$  g/dL vs  $12.2 \pm 1.3$  g/dL ( $p=0.03$ ), but did not significantly differ with CRP level  $\leq$  or  $> 8$  mg/L,  $12.5 \pm 1.5$  g/dL vs  $12.1 \pm 1.5$  g/dL ( $p=0.1$ ).

### **Multivariate analysis of factors associated with change in erythropoietin levels**

In view of the observed interaction between mGFR and anemia status, we created a combined variable for multivariate analyses (Table 3). After adjusting for covariates, EPO levels were 17.3% higher in patients with WHO-defined anemia and  $mGFR \geq 30$  mL/min/m<sup>2</sup> than in the reference group without anemia, whereas they were similar in those with anemia and  $mGFR < 30$  mL/min/1.73m<sup>2</sup> (Table 3). The fully-adjusted EPO increase was associated with overweight, obesity, diabetes with nephropathy types other than diabetic glomerulopathy, absolute iron deficiency, and CRP > 8 mg/L and ranged from 22 to 55%. Including these variables significantly improved the log likelihood of the fully-adjusted model compared to that with anemia and mGFR alone to explain EPO changes. Serum albumin and EPO levels were no longer associated after adjusting for the covariates. Similar results were obtained throughout the analysis when using a unique cut-off value of 12 g/dL for both genders. EPO level for patients with anemia and  $mGFR \geq 30$  mL/min/1.73m<sup>2</sup> was 13% higher than in those without anemia ( $p=0.04$ ), and relations with other factors were unchanged.

## Discussion

This study confirms the existence of an interaction between hemoglobin, GFR and erythropoietin in non-end-stage CKD, but its contribution and novelty lie in four key points. First, in contrast with earlier studies, the analysis was conducted by anemia status, which enabled to show that there was no relation between EPO concentration and mGFR levels in patients without anemia, whereas EPO deficiency increases with mGFR decline in anemic patients. Second, the most original findings indicate that several factors other than Hb and GFR levels may stimulate EPO production in CKD. Third, this study also quantified the relative EPO deficiency throughout the range of mGFR pointing out its importance as early as stages 1-2 that worsens as mGFR declines to reach 64% at stage 5. Finally, the quality of patient phenotype, both regarding GFR and EPO measurements resulted in a most accurate assessment of the EPO deficiency in non end-stage CKD.

In both normal and disease conditions, serum EPO must be interpreted according to Hb level.<sup>8</sup> Under non-anemic conditions, there is no need for higher EPO since Hb concentration and oxygen supply to tissues is still sufficient. In contrast, in patients with anemia, serum EPO is expected to be inversely correlated with Hb, as a result of the feedback process described in the introduction.<sup>7,8</sup> Impairment of this physiological feedback process in CKD was first described in dialysis patients.<sup>7</sup> Fehr et al. first suggested that it began below a GFR of 40 mL/min/1.73m<sup>2</sup>, but did not stratified the analysis by anemia status.<sup>9</sup> Here, we studied the interaction between Hb and GFR according to anemia status and found a slightly lower GFR threshold in patients with anemia, at 30 mL/min/1.73m<sup>2</sup>, probably explained by their use of Cockcroft-Gault estimated GFR while we used measured GFR.

We further showed that despite very early relative EPO deficiency in the course of CKD, physiological response to anemia was somewhat preserved in patients with GFR above 30 mL/min/1.73m<sup>2</sup>. Conversely, below 30 mL/min/1.73m<sup>2</sup>, patients with anemia had severe

EPO deficiency, with lower EPO levels than patients without anemia. At this stage, declining EPO concentration is likely to explain a major part of CKD anemia.

Beyond Hb and mGFR levels, a number of other factors were related to EPO concentration. First, we found absolute iron deficiency was associated with an increased EPO level. This association has previously been described in non-CKD patients and in dialysis patients.<sup>22 23 24 25</sup> It was also expected based on recent findings about the involvement of EPO in iron metabolism and particularly about hepcidin.<sup>26</sup> EPO reduces circulating hepcidin in humans<sup>27</sup> and directly affects the intestinal handling of iron in rat models.<sup>28</sup> Inversely, absolute iron deficiency is associated with increased EPO levels and early blockage of erythroid differentiation.<sup>29</sup> Transferrin receptor 2 (TfR2), recently described as a sensor of body iron levels, may mediate EPO change with absolute iron deficiency.<sup>30</sup>

Interestingly, diabetes with nephropathy other than diabetic glomerulopathy was associated with enhanced EPO production, while diabetic glomerulopathy was not. To the best of our knowledge, this finding is new and requires confirmation by others. In contrast, other authors<sup>31 32</sup> suggest that EPO response may be blunted in diabetic patients with microangiopathy, thus explaining their higher rate of anemia compared to non diabetic patients. Although we previously described such a higher rate of anemia, our findings here do not support this hypothesis, as EPO levels did not differ between patients with diabetic glomerulopathy and those without diabetes.

Another novel finding is that higher EPO levels were associated with increasing BMI. Several studies have shown no excess risk of anemia in patients with obesity despite increased inflammation and functional iron deficiency related to the hepcidin increase.<sup>15 33 34</sup> We have previously described a 40% lower risk of anemia in obese patients in the NephroTest cohort.<sup>15</sup> This apparently protective effect of obesity on anemia may result from the positive relation we observed between BMI and EPO levels. Further research is needed to investigate whether

sleep apnea syndrome and its consequence, the chronic hypoxia common in obese patients, is a plausible trigger for the EPO increase.

Finally, we found a positive association between inflammation and EPO level, independent of Hb and mGFR levels. Wagner et al recently found the same association in diabetic patients with CKD.<sup>35</sup> Experimentally, it is well established that EPO production is down-regulated by some cytokines that also influence EPO action.<sup>36 37 38</sup> In clinical studies, however, patients with chronic inflammation have preserved EPO feedback to changes in their Hb level.<sup>39 40 41</sup> Their EPO level is somewhat less stimulated than it is in the presence of absolute iron deficiency, which has the strongest impact on EPO production. Of note both Wagner and our results suggest a stimulatory role of inflammation on EPO independent of Hb level. It is likely that the variations of the different pro- and anti-inflammatory cytokines are far from explaining the variations in EPO levels. Other pro-ischemic factors may participate in EPO stimulation.

The major strengths of our study include its large sample size of well-phenotyped patients with a wide range of renal function and types of kidney disease compared with previous studies, and its use of measured GFR. Misclassification of patients according to renal function is therefore unlikely. This study also had the power to analyze a number of determinants and to test potential interactions with renal function in their relation with EPO, which has facilitated understanding of the underlying mechanisms. The higher number of men than women in this cohort reflects the well-established higher risk for CKD in men, but the lack of interaction with gender in the studied associations allows generalizability of the results to both genders. This study however has limitations, mainly related to its cross-sectional design that prevents causal inferences. For example, whether EPO deficiency precedes decreased Hb and causes anemia in the early stage of CKD or whether Hb decline is caused by other mechanisms and is insufficiently corrected by EPO cannot be answered. Because the

EPO level was measured only once for each patient, the robustness of the correlations may be affected by the inter-assay variability of the EPO level within any individual. This study clearly showed, however, distinct patterns of relations between EPO and Hb levels according to whether mGFR was  $<$  or  $\geq 30$  mL/min/1.73 m<sup>2</sup>. This interaction suggests that EPO plays a role in different phases of CKD anemia. Similarly, the association between EPO levels and the other factors we studied should be interpreted with caution. Nonetheless, the absence of interaction between these factors and mGFR, in contrast to Hb, suggests that these factors have a persistent impact on EPO level throughout the range of mGFR.

Altogether, our findings may shed some light on the surprising and disappointing results from recent ESA trials, particularly the Trial to Reduce Cardiovascular Events with Aranesp Therapy<sup>5</sup> conducted in diabetic CKD patients. The higher EPO production found in our study associated with diabetes with nephropathy types other than diabetic glomerulopathy may be a missing link. These patients may predominantly have a vascular nephropathy and have enhanced EPO levels, despite their similar anemia rates.<sup>15</sup> This finding suggests resistance to EPO, which has recently been linked to mortality.<sup>42</sup> ESA should thus be administered cautiously to patients with enhanced EPO concentration, as they may benefit less and experience more adverse effects. Endogenous EPO response to anemia may bring new perspectives in the assessment of the potential benefit of an ESA prescription.

In conclusion, CKD anemia is associated with an early relative EPO deficiency, that rises from 28 to 64% as renal function declines. In patients with mGFR greater than 30 mL/min/1.73m<sup>2</sup>, presence of anemia calls for explicative factors other than EPO deficiency, while it is probably the main determinant of anemia in patients with advanced CKD. But several factors besides Hb may stimulate EPO synthesis, bringing a more complex view of EPO response to Hb decrease in CKD population.

Nephrotest study group, collaborators:

Gauci C; Houiller P; Letavernier E; Urena P; Maruani G; Vallet M; Rougier JPH; Rondeau E; Ronco P; Plaisier E; Fessi H; Descamps C; de La Faille R; Dautheville S; Daugas E; d Auzac C; Costa MA; Bouet J.

#### Acknowledgements

The NephroTest CKD cohort study is supported by grants from: Inserm GIS-IReSP AO 8113LS TGIR (BS); French Ministry of Health AOM 09114 (MF); Inserm AO 8022LS (BS); Agence de la Biomédecine R0 8156LL (BS), AURA (MF) and Roche 2009-152-447G (MF). The Nephrotest initiative was also sponsored by unrestricted grants from F.Hoffman-La Roche Ltd. (LM).

#### Authorship contributions

BS, LM, NC, and MF defined the research theme and designed methods; MF, JPH, and NC carried out the laboratory reference measurements; LM and MM performed the statistical analyses; BS, MF, LM, MM, and NC discussed analyses, interpretation and presentation; CG, MM, BS, and MF managed the data collection; LM, BS, and MF have been involved in drafting the manuscript; MF and BS acquired funding; NC and JPH revised the manuscript critically.

#### Statement of competing financial interest

MF has received consulting or lecture fees or research funds from Affymax, Genzyme, Hoffmann-La-Roche, Novartis, Sandoz, Shire, Takeda, and Vifor International. MF has been employed by Amgen since January 1, 2011, but was a full-time academic assistant professor during the time of study conception, data collection, data analysis and preparation of the manuscript.

NC has received consulting or lecture fees or research funds from Amgen, Roche, Sandoz, and Shire.

LM has received consulting or lecture fees or research funds from Hospal, Gambro, Hoffmann-La-Roche, Vifor.

## References

---

- <sup>1</sup> Goldwasser E, Kung CKH Kung: Purification of erythropoietin. *Proc Nat Acad Sci* 68(4): 697-698, 1971
- <sup>2</sup> Shoemaker CB, Mitsock LD: Murine erythropoietin gene: cloning, expression, and Human gene homology. *Molecular Cell Biol* 6: 849-858, 1986
- <sup>3</sup> Drüeke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, Burger HU, Scherhag A: CREATE Investigators. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *N Engl J Med* 355(20): 2071-84, 2006
- <sup>4</sup> Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D : CHOIR Investigators: Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med*;355(20): 2085-98, 2006
- <sup>5</sup> Pfeffer MA, Burdmann EA, Chen CY, et al: TREAT Investigators. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med* 361(21):2019-32, 2009
- <sup>6</sup> Fishbane S, Miyawaki N, Szczech LA: Hypothesis: an erythropoietin honeymoon phase exists. *Kidney int* ; 78: 646-649, 2010
- <sup>7</sup> Erslev AJ: Erythropoietin. *N Engl J Med* 324(19):1339-44, 1991
- <sup>8</sup> Beguin Y, Clemons GK, Pootrakul P, Fillet G: Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. *Blood* 81: 1067-1076, 1993
- <sup>9</sup> Fehr T, Ammann P, Garzoni D, Korte W, Fierz W, Rickli H, Wüthrich RP: Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. *Kidney Int* 66(3): 1206-11, 2004
- <sup>10</sup> Walle AJ, Wong GY, Clemons GK, Garcia JF, Niedermayer W: Erythropoietin-hematocrit feedback circuit in the anemia of end-stage renal disease. *Kidney Int* 31(5):1205-9, 1987
- <sup>11</sup> Ross RP, McCrea JB, Besarab A: Erythropoietin response to blood loss in hemodialysis patients is blunted but preserved. *ASAIO J*; 40(3): M880-5, 1994
- <sup>12</sup> Brookhart MA, Schneeweiss S, Avorn J, Bradbury BD, Rothman KJ, Fischer M, Mehta J, Winkelmayr WC: The effect of altitude on dosing and response to erythropoietin in ESRD. *J Am Soc Nephrol* 19: 1389-1395, 2008
- <sup>13</sup> McGonigle RJ, Wallin JD, Shadduck RK, Fisher JW: Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney Int*; 25(2): 437-44, 1984
- <sup>14</sup> Radtke HW, Claussner A, Erbes PM, Scheuermann EH, Schoeppe W, Koch KM: Serum erythropoietin concentration in chronic renal failure : relationship to degree of anemia and excretory renal function. *Blood*; 54 (4): 877-884, 1979
- <sup>15</sup> Moranne O, Froissart M, Rossert J and al. Nephrotest study group: Timing of onset of CKD related metabolic complications. *J Am Soc Nephrol* 20(1): 164-71. 2009
- <sup>16</sup> Lipschitz DA, Cook JD, Finch CA: A clinical evaluation of serum ferritin as an index of iron stores. *Nutrition*; 8(6): 443-8, 1992
- <sup>17</sup> Froissart M, Rossert J, Jacquot C, Paillard M and Houillier P: Predictive performance of the modification of diet in renal disease and cockcroft-gault equations for estimating renal function. *J Am Soc Nephrol*; 16: 763-773, 2005

- 
- <sup>18</sup> Levey AS, Eckardt KU, Tsukamoto Y and al: Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 67(6): 2089-100, 2005
- <sup>19</sup> Belonje AMS, Voors AA, van der Meer P, van Gilst WH, Jaarsma T, vanVeldhuisen DJ: Endogenous erythropoietin and outcome in heart failure. *Circulation* 121: 245-251, 2010.
- <sup>20</sup> van der Meer P, Lok DJ, Januzzi JL and al: Adequacy of endogenous erythropoietin levels and mortality in anaemic heart failure patients. *Eur Heart J* 29(12): 1510-5, 2008
- <sup>21</sup> Opasich C, Cazzola M, Scelsi L and al: Blunted erythropoietin production and defective iron supply for erythropoiesis as major causes of anaemia in patients with chronic heart failure. *Eur Heart J*; 26(21): 2232-7, 2005
- <sup>22</sup> Bray GL, Taylor B, O'Donnell R: Comparison of the erythropoietin response in children with aplastic anemia, transient erythroblastopenia, and iron deficiency. *J Pediatr* 120(4 Pt 1):528-32, 1992
- <sup>23</sup> Joosten E, Van Hove L, Lesaffre E, Goossens W, Dereymaeker L, Van Goethem G, Pelemans W: Serum erythropoietin levels in elderly inpatients with anemia of chronic disorders and iron deficiency anemia. *J Am Geriatr Soc* 41(12):1301-4, 1993
- <sup>24</sup> Theurl I, Mattle V, Seifert M, Mariani M, Marth C, Weiss G: Dysregulated monocyte iron homeostasis and erythropoietin formation in patients with anemia of chronic disease. *Blood* 107: 4142-4148, 2006
- <sup>25</sup> Teruel JL, Marcen R, navaro JF, Villafruela JJ, Fernandez Lucas M, Rivera M, Ortuño J. Influence of body iron stores on the serum erythropoietin concentration in hemodialyzed patients. *Am J nephrol* 1994, 14(2): 95-8
- <sup>26</sup> Nemeth E: Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. *Adv Hematol* 2010: 750643, 2010
- <sup>27</sup> Ashby DR, Gale DP, Busbridge M and al: Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. *Haematologica* 95(3):505-8, 2010
- <sup>28</sup> Srai SK, Chung B, Marks J and al: Erythropoietin regulates intestinal iron absorption in a rat model of chronic renal failure. *Kidney Int* 78(7): 660-7, 2010
- <sup>29</sup> Bullock GC, Delehanty LL, Talbot AL and al: Iron control of erythroid development by a novel aconitase-associated regulatory pathway. *Blood* 116(1):97-108, 2010
- <sup>30</sup> Forejtikova H, Vieillevoye M, Zermati Y and al: Transferrin receptor 2 is a component of the erythropoietin receptor complex and is required for efficient erythropoiesis. *Blood* 116: 5357-5367, 2010
- <sup>31</sup> Biaggioni I, Robertson D, Krantz S, Jones M, Haile V: The anemia of primary autonomic failure and its reversal with recombinant erythropoietin. *Ann Intern Med* 121 181-186, 1994
- <sup>32</sup> Bosman DR, Winkler AS, Marsden JT, Macdougall IC, Watkins PJ: Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. *Diabetes Care* 24: 494-499, 2001
- <sup>33</sup> Ausk KJ, Ioannou GN. Is obesity associated with anemia of chronic disease? A population-based study. *Obesity*. 2008; 16: 2356-2361



- 
- <sup>34</sup> Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Guzman G, Holterman AX, Braunschweig C. Elevated systemic hepcidin and iron depletion in obese premenopausal females. *Obesity*. 2010; 18(7):1449-56
- <sup>35</sup> Wagner M, Alam A, Zimmermann J, Rauh K, Koljaja-Batzner A, Raff U, Wanner C, Schramm L. Endogenous erythropoietin and the association with inflammation and mortality in diabetic chronic kidney disease. *Clin J Am Soc Nephrol* 6(7):1573-9, 2011
- <sup>36</sup> Faquin WC, Schneider TJ, Goldberg MA: Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood* 79(8):1987-94, 1992
- <sup>37</sup> Fandrey J, Jelkmann WE: Interleukin-1 and tumor necrosis factor-alpha inhibit erythropoietin production in vitro. *Ann N Y Acad Sci* 628:250-5, 1991
- <sup>38</sup> Congote LF, Sadvakassova G, Dobocan MC, Difalco MR, Li Q: Erythropoietin-dependent endothelial proteins: potential use against erythropoietin resistance. *Cytokine* 51(2):113-8, 2010
- <sup>39</sup> Cazzola M, Ponchio L, de Benedetti F and al. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. *Blood* 87(11):4824-30, 1996
- <sup>40</sup> Corazza F, Beguin Y, Bergmann P and al: Anemia in children with cancer is associated with decreased erythropoietic activity and not with inadequate erythropoietin production. *Blood* 92: 1793-1798, 1998
- <sup>41</sup> Spivak JL, Barnes DC, Fuchs E, Quinn TC: Serum immunoreactive erythropoietin in HIV-infected patients. *JAMA* 261 (21): 3104-7, 1989
- <sup>42</sup> Solomon SD, Uno H, Lewis EF, Eckardt KU and al: Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) Investigators. Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med* 363(12):1146-55, 2010

**Table 1 - Patient characteristics**

	Mean±SD Median [IQR] % (n)
<b>N</b>	336
<b>Age</b>	58.8 ± 14.6
<b>Men</b>	73 (246)
<b>African origin</b>	7.4 (25)
<b>Diabetes</b>	
With diabetic glomerulopathy	13.6 (46)
With other nephropathy types	12.8 (43)
<b>Kidney disease</b>	
Diabetic nephropathy	13.6 (46)
Glomerular nephropathy	16.9 (57)
Vascular nephropathy	27.8 (94)
Polycystic kidney disease	2.3 (8)
Interstitial nephritis	9.5 (32)
Unknown	29.4 (99)
<b>Body mass index (kg/m<sup>2</sup>)</b>	26.1 ± 4.2
<b>mGFR mL/min/1.73m<sup>2</sup></b>	33.6 [22.9-46.1]
≥ 60	10.4 (35)
45- 60	15.4 (52)
30- 45	34.8 (117)
15 -30	30.6 (103)
<15	8.6 (29)
<b>PCR (mg/mmol)</b>	45.3 [17.7-142.5]
<b>Serum albumin (g/L)</b>	40.2 ± 5.2
<b>Hemoglobin (g/dL)</b>	12.4 ± 1.4
<b>Men</b>	12.7 ± 1.4
<b>Women</b>	11.6 ± 1.3
<b>EPO (IU/L )</b>	9.1 [6.7-13.01]
<b>Oral iron therapy</b>	12.8 (43)
<b>ACEI/ARBs</b>	78.8 (265)

BMI=body mass index; mGFR=glomerular filtration rate measured by <sup>51</sup>Cr-EDTA renal clearance; PCR=ratio of urinary protein to creatinine; EPO= measured erythropoietin; ACEI/ARBs= Angiotensin Converting Enzyme Inhibitors/Angiotensin Receptor Blockers

**Table 2: Crude analysis of factors associated with erythropoietin levels**

		N	EPO (UI/ml) median [IQR]	p
<b>Gender</b>	men	245	9.3 [6.7-13.5]	0.2
	women	91	8.9 [6.7-11.9]	
<b>Age (years)</b>	< 50	91	8.5 [5.7-11.5]	0.01
	[50-59[	76	8.9 [6.9-11.6]	
	[60-69[	73	10.5 [7.2-16.1] **	
	[70-max[	96	9.2 [7.0-12.7]	
<b>African origin</b>	no	296	8.9 [6.7-12.6]	0.3
	yes	25	9.9 [6.1-13.6]	
<b>Body mass index</b> (kg/m <sup>2</sup> )	< 25	136	8.6 [5.9-11.4]	0.005
	[25-30[	138	9.3 [6.7-13.1] *	
	[30-max[	62	10.6 [7.6-15.7] ***	
<b>Diabetes</b>	No	247	8.8 [6.1-12.2]	0.002
	With diabetic glomerulopathy	46	8.6 [6.8-12.1]	
	With other nephropathy types	43	11.4 [8.7-16.2] ***	
<b>Iron Index</b>	Normal	253	8.9 [6.3-12.7]	0.02
	Absolute iron deficiency	16	14.9 [9.0-19.8] **	
	Functional iron deficiency	67	8.9 [7.0-11.8]	
<b>CRP</b> ≤ 8 g/L	> 8 g/L	289	8.9 [6.3-12.7]	0.006
	> 8 g/L	40	10.8 [7.7-14.6]	
<b>Serum albumin</b> (g/L)	in patient with mGFR ≥ 30 mL/min/1.73 m <sup>2</sup>			0.003
	≥ 40	124	8.5 [5.9-12.6]	
	< 40	80	10.9 [7.6-14.0]	
	in patients with mGFR < 30 mL/min/1.73m <sup>2</sup>			
	< 40	71	8.3 [6.3-12.2]	
<b>PCR</b> (mg/mmoL)	< 30	140	8.9 [5.9-12.7]	0.2
	[30-300[	165	9.8 [7.05-13.5]	
	[300-max[	31	7.6 [6.1-10.5]	
<b>PTH</b> (pg/mL)	< 60	145	8.9 [6.7-11.9]	0.3
	≥ 60	191	9.2 [6.7-14.1]	
<b>1-25OH<sub>2</sub>Vitamin D</b> (pg/mL)	< 20	214	9.6 [7.0-13.5]	0.09
	≥ 20	121	8.7 [5.6-11.8]	
<b>Folates</b> (ng/mL)	< 8	141	9.4 [6.7-15.4]	0.5
	≥ 8	175	8.9 [6.2-12.4]	
<b>ACEI/ ARBs</b>	no	71	9.4 [7.1-12.0]	0.6
	yes	265	8.9 [6.5-13.2]	

\* p < 0.05; \*\* p < 0.01; \*\*\*p < 0.001;

mGFR=glomerular filtration rate measured by <sup>51</sup>Cr-EDTA renal clearance; Iron index: TSAT ≥ 20% normal iron profile; TSAT<20% and ferritin < 40 ng/mL absolute iron deficiency; TSAT < 20% and ferritin ≥ 40 ng/mL functional iron deficiency; CRP=C reactive protein; BMI=body mass index; PCR=ratio of urinary protein to creatinine; PTH=parathyroid hormone; ACEI/ARBs= angiotensin-converting enzyme inhibitors/angiotensin receptor blockers

**Table 3: Multivariate analysis of factors associated with erythropoietin levels in 336 patients**

		Model 1			Model 2			Model 3		
		% change	p	p-LRT	% change	p	p-LRT	% change	p	p-LRT
<b>Anemia*mGFR status</b>										
	No anemia	Ref	-	0.10	Ref	-	0.03	Ref	-	0.05
	Anemia and mGFR ≥ 30	15.7	0.04		20.1	0.01		17.3	0.03	
	Anemia and mGFR < 30	0.3	0.9		2.6	0.7		0.0	0.9	
<b>Body mass index</b>	(kg/m <sup>2</sup> )									
	< 25				Ref	-	0.008	Ref	-	0.005
	[25-30[				15.3	0.03		15.8	0.02	
	≥30				29.8	0.002		30.5	0.001	
<b>Diabetes</b>	No				Ref	-	0.03	Ref	-	0.01
	With diabetic glomerulopathy				-6.4	0.4		-5.5	0.5	
	With other nephropathy types				23.8	0.02		26.0	0.01	
<b>Iron Index</b>	Normal							Ref	-	0.006
	Absolute iron deficiency							55.2	0.001	
	Functional iron deficiency							-0.1	0.9	
<b>CRP</b>	≤ 8 mg/L							Ref	-	0.02
	> 8 mg/L							21.8	0.03	
Log likelihood		1.05			9.6			19.0		
					P<0.01versus Model 1			P<0.001 versus Model 1		

Model 1, adjusted for age in years, centre (ns), combined variable anemia\*mGFR status; model 2 adjusted for age in years, centre (ns), combined variable anemia\*mGFR status, body mass index, diabetes; Model 3 adjusted for age in years, centre (ns), combined variable anemia\*mGFR status, body mass index, diabetes, iron index, C Reactiv Protein  
p is the p-value of each category compared with the reference category; p-LRT is the p-value of the variable in the model  
mGFR=glomerular filtration rate measured by <sup>51</sup>Cr-EDTA renal clearance; BMI=body mass index; CRP=C reactive protein; Iron index: TSAT ≥ 20% normal iron profile; TSAT<20% and ferritin < 40 ng/mL absolute iron deficiency; TSAT < 20% and ferritin ≥ 40 ng/mL functional iron deficiency;

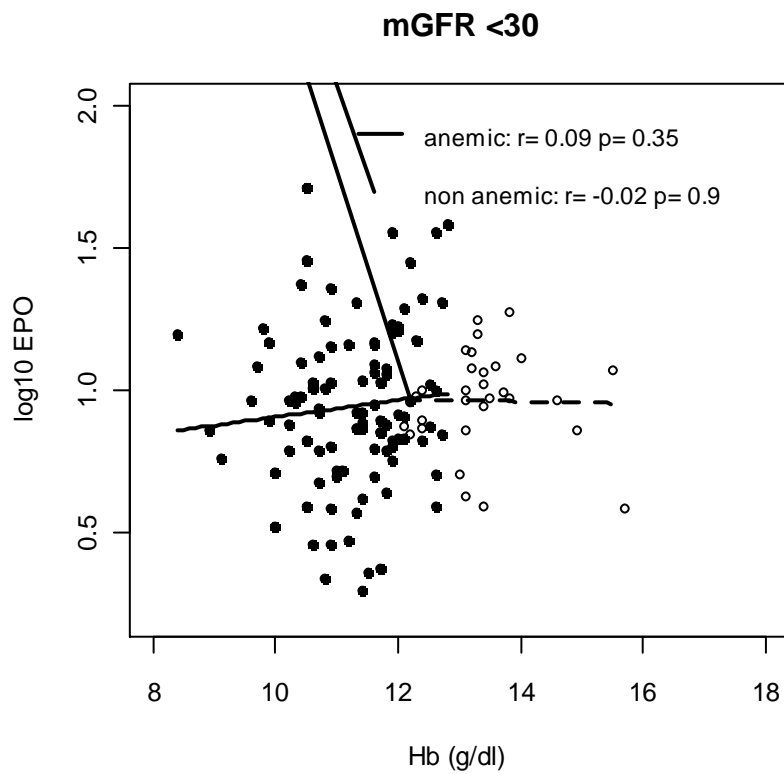
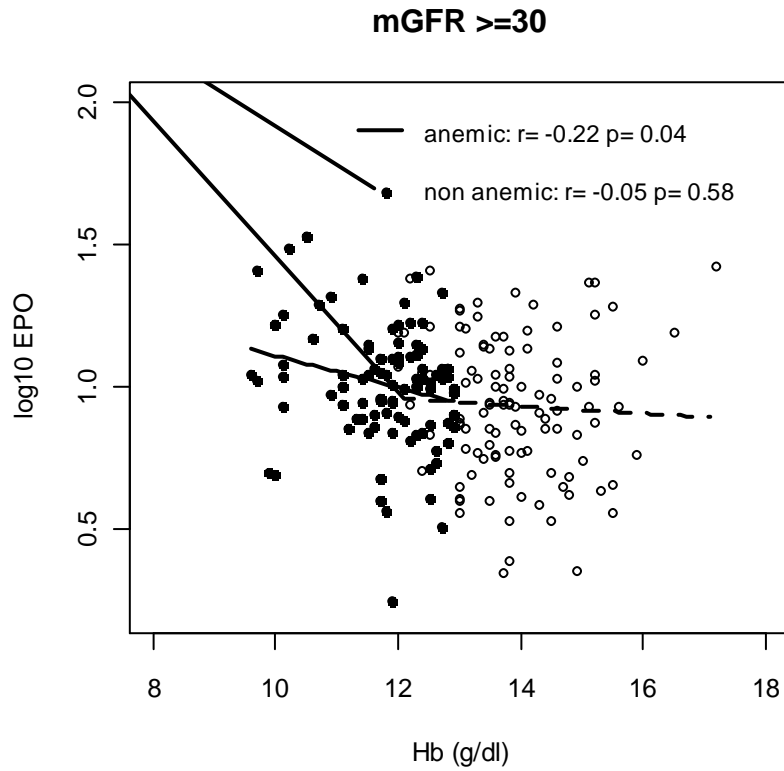
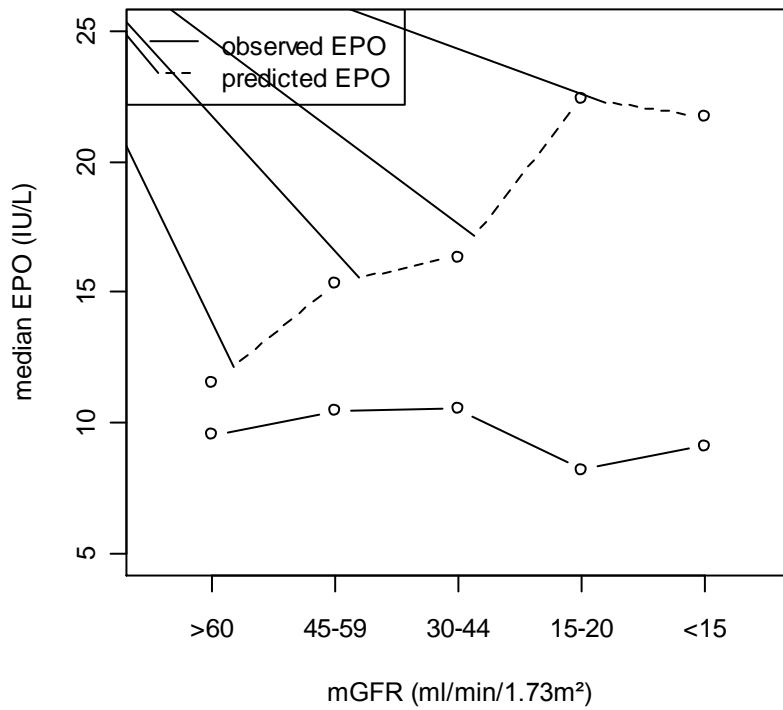


Figure 1 – Relations between EPO (log<sub>10</sub>) and hemoglobin level by WHO-defined anemia status and mGFR level. Open circles: non anemic patients, solid circles: anemic patients



**Figure 2.** Observed and predicted erythropoietin by mGFR levels in patients with WHO-defined anemia.