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Serum Erythropoietin Level and Mortality in Kidney Transplant Recipients

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Summary

Background and objectives Posttransplant anemia is frequently reported in kidney transplant recipients and is associated with worsened patient survival. Similar to high erythropoiesis-stimulating agent requirements, resistance to endogenous erythropoietin may be associated with worse clinical outcomes in patients with ESRD. We examined the association between serum erythropoietin levels and mortality among kidney transplant recipients.

Design, setting, participants, & measurements We collected sociodemographic, clinical, medical, and transplant history and laboratory data at baseline in 886 prevalent kidney transplant recipients (mean age 51 ± 13 [SD] years, 60% men, 21% diabetics). A solid-phase chemiluminescent immunometric assay was used to measure serum erythropoietin. Cox proportional hazards regression was used to model the association between baseline serum erythropoietin levels and all-cause mortality risk.

Results During the median 39-month follow-up, 99 subjects died. The median serum erythropoietin level was 10.85 U/L and hemoglobin was 137 ± 16 g/L. Mortality rates were significantly higher in patients with higher erythropoietin levels (crude mortality rates in the highest to lowest erythropoietin tertiles were 51.7, 35.5, and 24.0 per 1000 patient-years, respectively [$P = 0.008$]). In unadjusted and also in adjusted Cox models each SD higher serum erythropoietin level significantly predicted all-cause mortality: HR_{1SD increase} 1.22 and 1.28, respectively. In adjusted Cox models each SD higher serum erythropoietin/blood hemoglobin ratio also significantly predicted all-cause mortality: HR_{1SD increase} 1.32. Serum erythropoietin predicted mortality in all analyzed subgroups.

Conclusions In this sample of prevalent kidney transplant recipients, higher serum erythropoietin levels were associated with increased mortality.

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Introduction

Posttransplant anemia (1–4) is associated with worsened clinical outcomes (5–10).

Previous studies demonstrated an association between erythropoietin (EPO) resistance or reduced hematopoietic response to erythropoiesis-stimulating agents (ESAs) and poor clinical outcomes among chronic kidney disease (CKD) patients (11–17). It has been suggested that the dose of ESA may be a frequently neglected confounder for the association between higher targeted hemoglobin and mortality in randomized trials. It seems likely that the variable ESA requirements may potentially and plausibly generate confounding by indication (18,19). Patients with higher ESA requirements may be at higher risk for adverse outcomes due to the underlying reasons for their ESA resistance (such as inflammation), due to potential “off-target,” nonerythropoietic effects of the higher administered ESA doses or conditions precipitated by the higher doses of ESA (such as iron defi-

ciency) (20), or due to a combination of these. Moreover, a recent secondary analysis of the Trial to Reduce Cardiovascular Events with Aranesp Therapy showed that a poor initial response to ESA therapy was associated with increased cardiovascular and all-cause mortality (18).

In recent observational studies elevated endogenous EPO levels were predictive for mortality both in diabetic patients with CKD (21) and among people aged 85 years and older (22). Moreover, serum EPO was inversely associated with hemoglobin in kidney transplant recipients (23). Finally, resistance to endogenous EPO is reportedly associated with mortality in patients with heart failure (24,25). The association among serum EPO levels, endogenous EPO resistance, and mortality among kidney transplant recipients has not been elucidated.

Based on the above, we hypothesize that similarly to resistance to exogenous ESA (16,17) resistance to endogenous EPO, represented by increased serum EPO

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levels, may represent a state of heightened risk in patients with CKD, in particular, those with posttransplant anemia. We investigated the association of serum EPO concentration and its ratio to hemoglobin (as a marker of endogenous EPO resistance) with mortality in a prospective cohort of prevalent kidney transplant recipients.

Materials and Methods

Patient Population and Data Collection

We invited all prevalent kidney transplant recipients 18 years of age or older ($n = 1214$) who were followed at a single transplant outpatient clinic at the Department of Transplantation and Surgery at Semmelweis University Faculty of Medicine, Budapest, Hungary, on December 31, 2006, to participate in this prospective, observational study. Exclusion criteria were acute rejection within the previous 4 weeks, current hospitalization, transplantation in the previous 3 months, and acute infection or bleeding (26–30). Baseline assessments were conducted between February 2007 and August 2007 (Malnutrition-Inflammation in Transplant—Hungary Study) (26–30).

Demographic data and details of medical history were collected at baseline, when information on age, gender, menopause status, etiology of CKD, transplantation-related data including immunosuppressant medication, ESA (epoetin-beta) use (yes/no), and comorbidities (the modified Charlson Comorbidity Index [CCI]) (31) were obtained. Estimated GFR (eGFR) was calculated using the four-variable Modification of Diet in Renal Disease study formula (32).

The study was approved by the Ethics Committee of the Semmelweis University (49/2006). Before enrollment, patients received detailed written and verbal information regarding the aims and protocol of the study and gave written consent to participate.

Laboratory Data

All laboratory data were measured at the baseline visit in a fasting state and included among others blood hemoglobin, serum C-reactive protein (CRP) and creatinine, BUN, and serum albumin levels. Serum samples were also collected at the time of the baseline assessment and stored at -70°C for future use. Serum EPO concentration was measured by IMMULITE 2000 EPO, a solid-phase, chemiluminescent immunometric assay using human recombinant EPO in a nonhuman serum matrix (EURO/DPC Ltd., United Kingdom; normal range: 3.7 to 31.5 mIU/ml), and high sensitivity IL-6 level was measured using immunoassay kits based on solid-phase sandwich ELISA (R&D Systems, Minneapolis, Minnesota).

Transplantation-Related Data and Donor Characteristics

Transplant-related data extracted from the medical records included current medications (including current immunosuppressive treatment), transplant vintage, *i.e.*, time elapsed since the date of transplantation, length of time on dialysis, type of allograft, history of treated acute rejection(s) after transplantation, HLA mismatch, panel reactive antibodies titer, cold ischemia time, donor age and gender, and history of delayed graft function. Total time with ESRD was defined as the total time on any type of renal

replacement therapy (including any type of dialysis or kidney transplant).

Immunosuppressive Therapy

Standard maintenance immunosuppressive therapy consisted of prednisolone, with either cyclosporine A microemulsion formulation (Neoral) or tacrolimus, combined with mycophenolate-mofetil or azathioprine or sirolimus.

Follow-up

Patients were followed for 39 months: median (interquartile range [IQR]): 38.8 (37.2 to 40.3) months. The primary outcome variable was all-cause mortality, which included all deaths with a functioning graft and deaths occurring after a return to dialysis. Deaths and reinitiations of maintenance dialysis were ascertained from the hospital database. Deaths were validated by cross-referencing with data from the Hungarian Central Office of Administrative and Electronic Public Service, the government agency maintaining official vital status records.

Statistical Analyses

Statistical analyses were carried out using STATA 11.1 (StataCorp, College Station, Texas) and SAS version 9.1 (SAS Institute Inc., Cary, North Carolina) software. Data were summarized using proportions, means (\pm SD), or medians (IQR) as appropriate. Categorical variables were compared with chi-squared test and continuous variables were compared using *t* test or the Mann-Whitney *U* test, Kruskal-Wallis *H* test, or ANOVA as appropriate. In all statistics, two-sided tests were used, and the results were considered statistically significant if P was <0.05 .

Our principal analyses were restricted to patients who were not receiving ESA therapy. Additionally we also analyzed the association between serum EPO and mortality in the total sample ($n = 993$) as a sensitivity analysis. The association between baseline serum EPO level and all-cause mortality was assessed using Cox proportional regression analysis and Kaplan-Meier plots with log-rank test. Proportional hazards assumptions were tested using scaled Schoenfeld residuals. As a sensitivity analysis we also assessed this association using left-truncated analysis accounting for the fact that ours was a prevalent cohort. The variables entered in the multivariable-adjusted models were selected based on theoretical considerations; we included predictors in the models that were known to be associated both with EPO levels and with mortality based on external evidence and clinical experience and that were available in our database. The hierarchical regression modeling was carried out in five steps: (1) unadjusted model; (2) adjusted for eGFR; (3) adjusted for eGFR, age, and gender; and (4) final model: age, gender, eGFR, serum albumin, log-transformed CRP (ln-CRP), CCI, systolic BP, total time with ESRD, history of delayed graft function, smoking status, and history of acute rejection. In the fifth step we adjusted for all of the above variables and also for blood hemoglobin.

As sensitivity analysis we also tested a separate model in which we included additional markers of inflammation and iron deficiency (because of the relatively limited number of events, we could only include a limited number of

independent variables in these models). In this model we adjusted for age, gender, eGFR, serum albumin, Charlson Comorbidity index, serum IL-6, serum tumor necrosis factor- α , serum ferritin, serum total iron-binding capacity, and use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs).

We also performed prespecified analyses in relevant subgroups of patients. In these multivariable models we restricted the independent covariates to age, eGFR, CCI, systolic BP, and total time with ESRD to comply with the general rule of having at least 10 outcome events/covariate included in the model.

Nonlinear associations were assessed using fractional polynomials and restricted cubic splines. Variance inflation factors were used to assess collinearity between independent variables. Patients were censored at the time of either death or at the end of the follow-up period. Less than 1% of our data were missing for any variables and at any time point analyzed; thus we used a complete-case analysis approach.

Results

Demographics and Baseline Characteristics

Of the 1214 potential patients 205 (17%) refused to participate in the study, and 16 (1%) patients were excluded based on exclusion criteria. The final study sample therefore included 993 patients. The proportion of men among participants was lower than among those who refused to participate (57% versus 67% men; $P = 0.008$), but there was no difference in age between the two groups (51 ± 13 versus 52 ± 13 years, $P = 0.66$).

Of the 993 patients 102 (10%) were not included in the primary analyses because they were receiving ESA therapy, and five patients did not have data on serum EPO levels, resulting in a cohort of 886 ESA-naïve patients for our primary analyses.

Baseline patient characteristics overall and in patients grouped according to their EPO levels are shown in Table 1. The mean age was 51 ± 13 years, mean eGFR was 53 ± 20 ml/min per 1.73 m^2 (2), 60% were men, and 21% were diabetic subjects, and mean hemoglobin was 137 ± 16 g/L (Table 1). At the time of enrollment 81% of the patients were taking prednisolone, and almost half of the patients were on cyclosporine A therapy (49%). The percentage of patients taking mycophenolate-mofetil was 79%. Forty-two percent of the patients were taking tacrolimus, 4% were on azathioprine, and 8% were on sirolimus. The median serum EPO level was 10.85 U/L (IQR: 7.6 to 15 U/L). The distribution of serum EPO level is presented in Supplementary Figure 1. Patients with a higher serum EPO level were older, were less likely to be active smokers, and had lower serum albumin and higher serum CRP levels (Table 1). We found a weak but statistically significant positive correlation between serum EPO (Supplementary Figure 2A), ln-transformed serum EPO (Supplementary Figure 2B), and blood hemoglobin ($P < 0.001$ for all).

Mortality

During a median follow-up of 39 months 99 patients died, and none were lost to follow-up. The crude mortality rate was 36.8/1000 patient-years (95% confidence interval

[CI]: 30.2 to 44.8 patient-years). The unadjusted mortality rate was significantly higher among patients with the highest EPO levels (crude mortality rates in the highest tertile: 51.7/1000 patient-years [95% CI: 38.6 to 69.3 patient-years]; middle tertile: 35.5/1000 patient-years [95% CI: 25 to 50 patient-years]; lowest tertile: 24.0/1000 patient-years [95% CI: 15.8 to 36.4 patient-years]; $P = 0.008$), also shown in the Kaplan-Meier plot (Figure 1A). A similar result was found with serum EPO/blood hemoglobin ratio (Figure 1B).

Table 2 and Supplementary Table 1 show the association of all-cause mortality with serum EPO in 886 kidney transplant recipients. In unadjusted Cox proportional regression analyses a 1 SD higher serum EPO level significantly predicted all-cause mortality (hazard ratio [HR]_{1 SD increase} = 1.22; 95% CI: 1.12 to 1.33). This association remained significant after adjusting for relevant covariates in our fully adjusted model (HR_{1 SD increase} = 1.28; 95% CI: 1.02 to 1.62). Moreover, adjustment for blood hemoglobin did not attenuate this association (HR_{1 SD increase} = 1.29; 95% CI: 1.02 to 1.62). The association of serum EPO level (Figure 2A) and serum EPO/blood hemoglobin ratio (Figure 2B) with mortality was uniformly increasing when modeled as a continuous variable and using fractional polynomials and cubic splines. We found qualitatively similar results when using left-truncated analysis (Supplementary Table 2) and also when we analyzed the total population (including patients who received treatment with ESA) in sensitivity analyses (Supplementary Table 3). We also examined the association between serum EPO and mortality within different hemoglobin tertiles. The association was statistically significant in all except the third tertile. We reanalyzed our data after adjusting for a different set of markers of inflammation and iron deficiency. We found that results were qualitatively similar to the original model (HR_{1 SD increase} = 1.10; 95% CI: 1.00 to 1.21).

We also analyzed the ratio of serum EPO/blood hemoglobin, a potential marker of endogenous EPO resistance. In unadjusted Cox proportional regression analyses a 1 SD higher serum EPO/blood hemoglobin ratio was a significant predictor of all-cause mortality (HR_{1 SD increase} = 1.22; 95% CI: 1.12 to 1.32). This association remained significant after multivariable adjustments (HR_{1 SD increase} = 1.32; 95% CI: 1.05 to 1.67).

Figure 3 shows adjusted HRs (95% CI) of mortality associated with a 1 SD higher serum EPO level (Figure 3A) and serum EPO/blood hemoglobin ratio (Figure 3B) in various prespecified patient subgroups. The association with mortality was similar in all examined subgroups. All tests of interactions were not statistically significant, indicating no effect modification by the examined characteristics (age, gender, presence of diabetes, eGFR, hemoglobin, serum IL-6, albumin, and time since transplant).

Discussion

In this prospective cohort study of 886 prevalent kidney transplant recipients we report independent associations between serum EPO level and mortality. To the best of our knowledge this study is the first to demonstrate this association in kidney transplant recipients.

We found the hazard for mortality to be linear for both increasing serum EPO levels and the EPO/hemoglobin ratio,

Table 1. Patients' baseline characteristics

	All Patients	EPO Tertile 1 (U/L)	EPO Tertile 2 (U/L)	EPO Tertile 3 (U/L)	P-for Trend	P-ANOVA
Number of patients	886	296	297	293	N/A	N/A
serum EPO (U/L) ^a	10.85 (7.6 to 15.0)	6.6 (5.4 to 7.6)	10.9 (9.6 to 12.1)	17.5 (15.1 to 22.9)	<0.001	<0.001
Age (years) ^b	51 ± 13	49 ± 13	52 ± 13	53 ± 12	<0.001	<0.001
Gender, female (%)	40	39	35	46	0.06	0.01
Time since last transplant (month) ^a	72 (39 to 113)	73 (44 to 115)	63 (33 to 109)	75 (39 to 115)	0.57	0.28
Previous time on dialysis (month) ^a	20 (9 to 39)	22 (9 to 42)	19 (9 to 37)	20 (11 to 42)	0.74	0.22
Total ESRD time (month) ^a	107 (68 to 154)	115 (75 to 156)	100 (61 to 147)	104 (68 to 155)	0.46	0.18
eGFR (MDRD) (ml/min per 1.73 m ²) ^b	53 ± 20	53 ± 20	52 ± 20	54 ± 21	0.82	0.49
Charlson Comorbidity index ^a	2 (2 to 3)	2 (2 to 3)	2 (2 to 3)	2 (2 to 4)	0.02	0.17
Presence of hypertension (%)	93	92	95	93	0.53	0.001
Presence of diabetes (%)	21	20	21	24	0.24	0.51
Presence of coronary heart disease (%)	9	7	9	10	0.18	0.40
Smoking (%)	19	25	17	16	0.004	0.009
Hb (g/L) ^b	137 ± 16	137 ± 17	137 ± 16	136 ± 15	0.47	0.81
Serum albumin (g/L) ^b	41 ± 4	41 ± 4	41 ± 4	40 ± 4	<0.001	<0.001
CRP (mg/L) ^a	3.2 (1.5 to 7)	2.6 (1.4 to 6)	3.1 (1.6 to 6)	3.9 (1.7 to 8.2)	0.001	0.11
In-CRP (mg/L) ^b	1.17 ± 1.13	1.06 ± 1.15	1.11 ± 1.08	1.34 ± 1.13	0.001	0.005
Use of ACE inhibitor or ARB (%)	28	32	30	22	0.008	0.02
Primary cause of ESRD (%)					0.33	0.43
chronic GN	23	25	26	19		
chronic TIN	13	15	13	11		
PKD	17	12	17	24		
diabetic nephropathy	7	4	5	4		
hypertensive nephropathy	7	8	6	6		
others or unknown	33	34	33	36		
Panel reactive antibody titer (%) ^c	0 (0 to 80)	0 (0 to 85)	0 (0 to 55)	0 (0 to 70)	0.03	0.001
Cold ischemic time (minute) ^b	1250 ± 345	1275 ± 325	1237 ± 344	1237 ± 366	0.30	0.30
History of delayed graft function (%)	26	24	30	24	0.86	0.16
History of acute rejection (%)	32	34	33	31	0.42	0.69
HLA mismatches (%)					0.66	0.92
0	1	1	2	0		
1	5	5	4	5		
2	22	22	24	21		
3	45	46	43	46		
4	22	18	23	23		
5	4	6	3	4		
6	1	1	1	1		

EPO, serum erythropoietin; N/A, not available; eGFR, estimated GFR; MDRD, Modification of Diet in Renal Disease; Hb, hemoglobin; In-CRP, log-transformed C-reactive protein; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; GN, glomerulonephritis; TIN, tubulo-interstitial nephritis; PKD, polycystic kidney disease.

^a Values are median with interquartile range in parentheses.

^b Values are mean ± SD.

^c Values are median with minimum to maximum in parentheses.

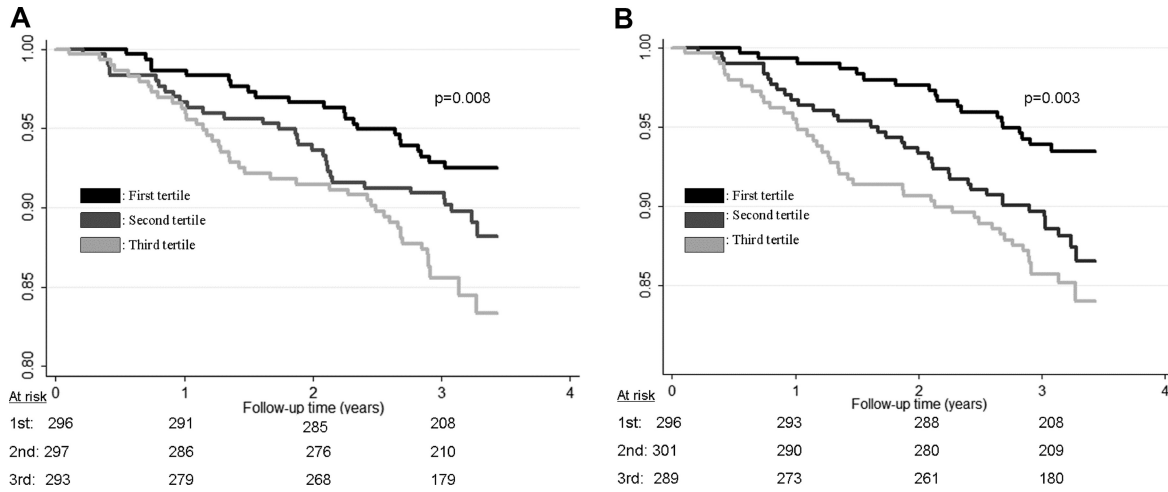


Figure 1. | Kaplan-Meier analysis of overall survival for 886 kidney transplant recipients grouped according to serum erythropoietin level (A) and serum erythropoietin/blood hemoglobin ratio (B).

Table 2. Association of serum EPO with mortality using Cox regression analyses in the study sample of 886 kidney transplant recipients

	Unadjusted		+eGFR		+eGFR, Age, and Gender		Final Model		Final Model + Hb	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
EPO (per 1 SD)	1.22	1.12 to 1.33	1.18	1.09 to 1.28	1.14	1.06 to 1.24	1.28	1.02 to 1.62	1.29	1.02 to 1.62
ln-EPO (1 ln point increase)	1.81	1.28 to 2.56	1.91	1.37 to 2.67	1.79	1.27 to 2.53	1.56	1.06 to 2.31	1.58	1.06 to 2.34
EPO/Hb (1 SD increase)	1.22	1.12 to 1.32	1.17	1.07 to 1.27	1.14	1.06 to 1.23	1.32	1.05 to 1.67	—	—

The total number of deaths was 99. Independent variables in the final model were age, gender, eGFR, serum albumin, serum log-transformed C-reactive protein, Charlson Comorbidity index, average of systolic BP, total time in ESRD, history of delayed graft function, smoking status, and history of acute rejection. serum EPO, serum erythropoietin; eGFR, estimated GFR; Hb, hemoglobin; HR, hazard ratio; CI, confidence interval; ln-EPO, log-transformed EPO; —, not available.

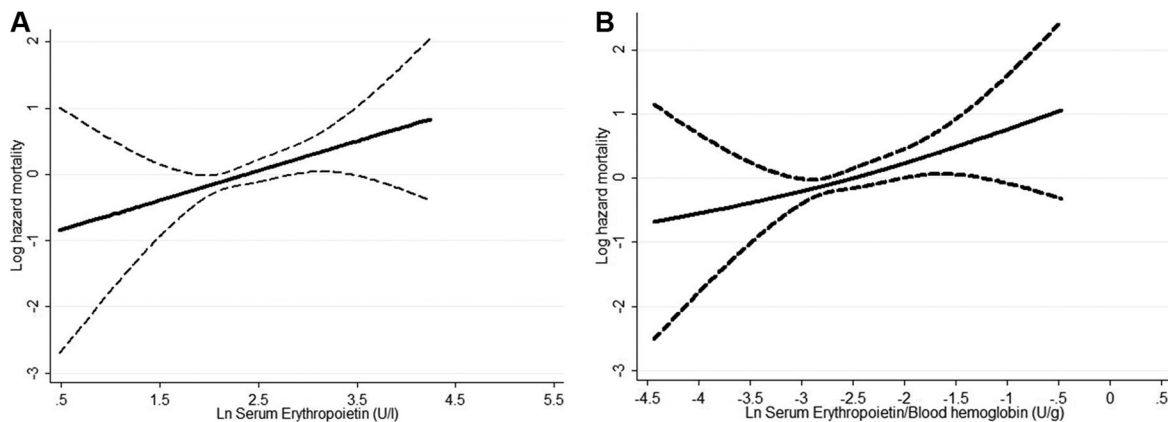


Figure 2. | Association of serum erythropoietin (A) and serum erythropoietin/blood hemoglobin ratio (B) with mortality in Cox models. Models were adjusted for age, gender, estimated GFR, serum albumin, serum ln-CRP, Charlson Comorbidity index, average systolic BP, total time in ESRD, history of delayed graft function, smoking status, and history of acute rejection.

a marker of endogenous EPO resistance. In our study population, each 1 SD increase of serum EPO level was associated with a 28% higher risk of mortality. Moreover, we did not

find significant interactions when we explored relevant subgroups, including age groups, gender, diabetes status, CKD stage, or inflammation and nutrition parameters.

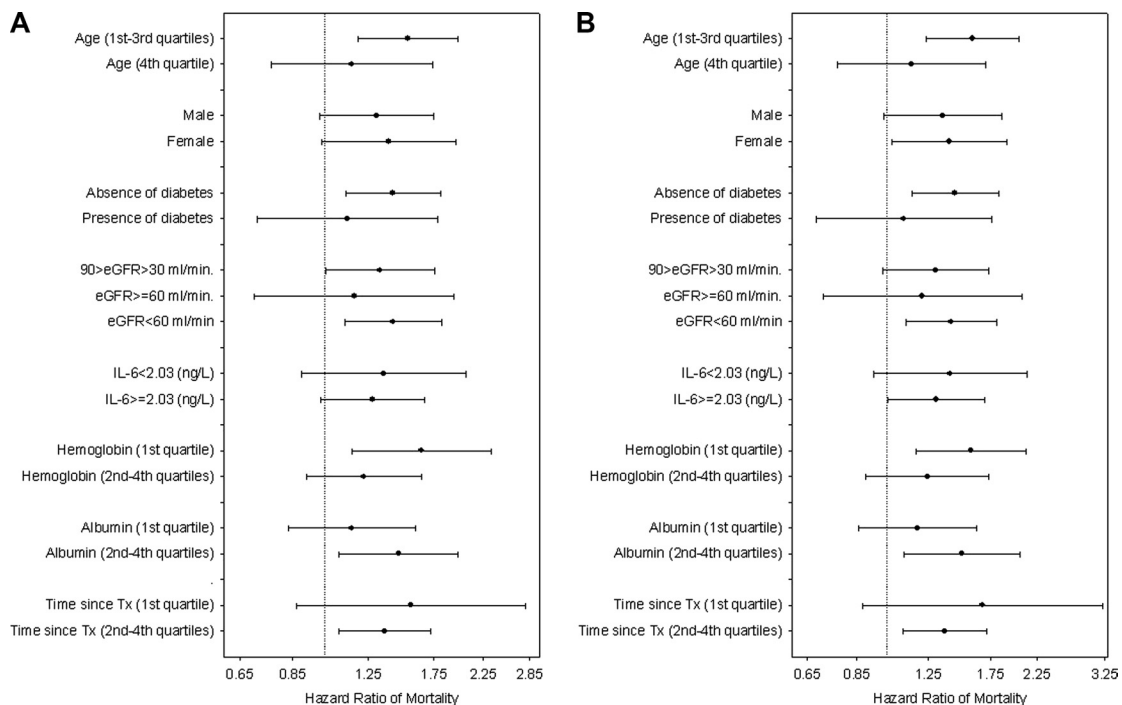


Figure 3. | Hazard ratios (95% confidence intervals) of mortality associated with serum erythropoietin levels (per 1 SD increase) (A) and serum erythropoietin/blood hemoglobin ratio (per 1 SD increase) (B), using multivariable adjusted Cox regression analyses in 886 kidney transplant recipients, in various subgroups of patients. Models were adjusted for age, estimated GFR, Charlson Comorbidity index, average of systolic BP, and total time in ESRD.

The association between serum EPO and mortality remained significant after adjusting for hemoglobin level. This suggests that the association is largely independent of its impact on anemia. Previous studies in nontransplant CKD patients have demonstrated an association between EPO resistance or reduced hematopoietic response to ESAs and poor clinical outcomes, including mortality (11–17). Resistance to endogenous EPO and mortality has also been reported in patients with chronic heart failure and patients with CKD (21,24,25,33).

The EPO/hemoglobin ratio, a potential marker of endogenous resistance to EPO, was also associated with mortality. These results are consistent with the hypothesis that resistance to EPO may be linked, directly or indirectly, to pathologic processes leading to death. A potential interaction between EPO resistance and high exogenous ESA dose may also be postulated, as was also suggested by recent studies (34). However, there is a difference between immunologically detectable and biologically active EPO (35). In ESRD patients the level of immunologically detectable hormone was significantly higher than that of the bioactive hormone (35). It is also not known whether there are different mechanisms responsible for the actions of endogenous and exogenous (recombinant) EPO (36). Investigating the different mechanisms of endogenous and exogenous (recombinant) EPO, Conlon *et al.* pointed out that ACE inhibitors interact with endogenous EPO secretion but not with the effect of exogenous EPO (36).

Several potential mechanisms may explain the observed association between higher serum EPO levels and mortality. Higher serum EPO may be a surrogate marker of

underlying disease or pathophysiologic processes. In our analysis, we accounted for important confounders, including measures of inflammation, nutrition, and comorbidity, but EPO maintained an independent association with mortality. Although this may diminish the argument for serum EPO as simply a surrogate marker, we cannot exclude the impact of residual confounding. Moreover, resistance to endogenous EPO is likely to be affected by factors similar to those causing resistance to extrinsic ESAs in patients with CKD, that is, protein-energy wasting, inflammation (37) and co-morbidity (13), and perhaps additional, yet unmeasured factors. These factors are also associated with mortality in kidney transplant recipients (29). Alternatively (and perhaps less likely in this context), a high EPO level itself may be harmful. A number of unexpected nonhematopoietic functions of EPO have also been identified and may contribute to a poor outcome because of its established prothrombotic, platelet-activating, or tumor-promoting effects (38–40).

Several factors need to be considered when interpreting our results. A study suggested that endogenous serum EPO has circadian fluctuations (41), but this was not detected in healthy men (42). Furthermore, this may not be a source of bias in our cohort because blood was taken in the morning hours uniformly. Smoking may modulate serum EPO (22). Interestingly, smoking was associated with lower serum EPO in our cohort. The explanation of this finding is not known, although multiple factors will influence the EPO level in this patient population. For example, drugs might have an effect on serum EPO level. ACE inhibitor ARB use was associated with lower serum EPO

level in our study. Furthermore, immunosuppressive drugs may also have an effect on endogenous EPO level in kidney transplant patients (43,44).

In CKD and dialysis populations safety concerns have been raised regarding the use of ESA therapy for correcting renal anemia to near-normal levels (45–47), but few data are available in kidney transplant populations. Results from a recent retrospective cohort analysis of more than 1700 kidney transplant recipients also found increased mortality among ESA users who achieved hemoglobin levels above 125 g/L (7). Whether the risk is related to a higher hemoglobin level or to higher ESA use is not clear. We postulate that the resistance to endogenous EPO is also associated with higher risk of mortality in kidney transplant patients.

Our study is notable for its large sample size, prospective design, and comprehensive patient follow-up. As with any observational study we also recognize certain limitations. A study such as ours cannot prove causal association between endogenous EPO level and mortality. This was a single-center study; hence our results may not be generalizable to other populations. Furthermore, we did not have information about some of the variables that are reportedly associated with serum EPO level, such as residual function of the native kidneys, the presence of liver disease, or the exact altitude at which the patients live (48). The last limitation is an unlikely cause of bias in our study, because the altitude difference within Hungary amounts to a few hundred meters only. To address the prevalent nature of the study, we performed a left-censored analysis that showed associations consistent with the principal analysis. Nevertheless, the study is subject to an incidence-prevalence bias. An additional weakness of our study is that we do not have data about cause of death, the presence of proteinuria, or details of cardiovascular comorbidities, and we measured serum EPO only at baseline. We believe it is likely that serum EPO levels are mainly associated with cardiovascular disease and cardiovascular death in this patient population; however, only further studies will be able to answer this question.

Conclusions

In summary, our prospective cohort study demonstrates that higher serum EPO and resistance to circulating EPO are significant predictors of mortality in prevalent kidney transplant recipients. Future studies will be needed to determine mediators of endogenous EPO resistance and how this may influence ESA dosing to improve patient outcomes in kidney transplantation.

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Disclosures

None.

References

1. Vanrenterghem Y: Anemia after kidney transplantation. *Transplantation* 87: 1265–1267, 2009
2. Lorenz M, Kletzmayer J, Perschl A, Furrer A, Horl WH, Sunder-Plassmann G: Anemia and iron deficiencies among long-term renal transplant recipients. *J Am Soc Nephrol* 13: 794–797, 2002
3. Molnar MZ, Novak M, Ambrus C, Kovacs A, Pap J, Rempert A, Szeifert L, Mucsi I: Anemia in kidney transplanted patients. *Clin Transplant* 19: 825–833, 2005
4. Vanrenterghem Y, Ponticelli C, Morales JM, Abramowicz D, Baboolal K, Eklund B, Kliem V, Legendre C, Morais Sarmento AL, Vincenti F: Prevalence and management of anemia in renal transplant recipients: A European survey. *Am J Transplant* 3: 835–845, 2003
5. Molnar MZ, Czira M, Ambrus C, Szeifert L, Szentkiralyi A, Beko G, Rosivall L, Rempert A, Novak M, Mucsi I: Anemia is associated with mortality in kidney-transplanted patients—A prospective cohort study. *Am J Transplant* 7: 818–824, 2007
6. Chhabra D, Grafals M, Skaro AI, Parker M, Gallon L: Impact of anemia after renal transplantation on patient and graft survival and on rate of acute rejection. *Clin J Am Soc Nephrol* 3: 1168–1174, 2008
7. Heinze G, Kainz A, Horl WH, Oberbauer R: Mortality in renal transplant recipients given erythropoietins to increase haemoglobin concentration: Cohort study. *BMJ* 339: b4018, 2009
8. Djamali A, Becker YT, Simmons WD, Johnson CA, Pre-masathian N, Becker BN: Increasing hematocrit reduces early posttransplant cardiovascular risk in diabetic transplant recipients. *Transplantation* 76: 816–820, 2003
9. Rigatto C, Foley R, Jeffery J, Negrijn C, Tribula C, Parfrey P: Electrocardiographic left ventricular hypertrophy in renal transplant recipients: Prognostic value and impact of blood pressure and anemia. *J Am Soc Nephrol* 14: 462–468, 2003
10. Rigatto C, Parfrey P, Foley R, Negrijn C, Tribula C, Jeffery J: Congestive heart failure in renal transplant recipients: Risk factors, outcomes, and relationship with ischemic heart disease. *J Am Soc Nephrol* 13: 1084–1090, 2002
11. Messana JM, Chuang CC, Turenne M, Wheeler J, Turner J, Sleeman K, Tedeschi P, Hirth R: Association of quarterly average achieved hematocrit with mortality in dialysis patients: A time-dependent comorbidity-adjusted model. *Am J Kidney Dis* 53: 503–512, 2009
12. Rossert J, Gassmann-Mayer C, Frei D, McClellan W: Prevalence and predictors of epoetin hyporesponsiveness in chronic kidney disease patients. *Nephrol Dial Transplant* 22: 794–800, 2007
13. Lopez-Gomez JM, Portoles JM, Aljama P: Factors that condition the response to erythropoietin in patients on hemodialysis and their relation to mortality. *Kidney Int* December: S75–S81, 2008
14. Kausz AT, Solid C, Pereira BJ, Collins AJ, St Peter W: Intractable anemia among hemodialysis patients: A sign of suboptimal management or a marker of disease? *Am J Kidney Dis* 45: 136–147, 2005
15. Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, Patel UD, Singh AK: Secondary analysis of the

- CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. *Kidney Int* 74: 791–798, 2008
16. Kaysen GA, Muller HG, Ding J, Chertow GM: Challenging the validity of the EPO index. *Am J Kidney Dis* 47: 166, 2006
 17. Zhang Y, Thamer M, Stefanik K, Kaufman J, Cotter DJ: Epoetin requirements predict mortality in hemodialysis patients. *Am J Kidney Dis* 44: 866–876, 2004
 18. Solomon SD, Uno H, Lewis EF, Eckardt KU, Lin J, Burdman EA, de Zeeuw D, Ivanovich P, Levey AS, Parfrey P, Remuzzi G, Singh AK, Toto R, Huang F, Rossert J, McMurray JJ, Pfeffer MA: Erythropoietic response and outcomes in kidney disease and type 2 diabetes. *N Engl J Med* 363: 1146–1155, 2010
 19. Bradbury BD, Brookhart MA, Winkelmayer WC, Critchlow CW, Kilpatrick RD, Joffe MM, Feldman HI, Acquavella JF, Wang O, Rothman KJ: Evolving statistical methods to facilitate evaluation of the causal association between erythropoiesis-stimulating agent dose and mortality in nonexperimental research: strengths and limitations. *Am J Kidney Dis* 54: 554–560, 2009
 20. Streja E, Kovesdy CP, Greenland S, Kopple JD, McAllister CJ, Nissenson AR, Kalantar-Zadeh K: Erythropoietin, iron depletion, and relative thrombocytosis: A possible explanation for hemoglobin-survival paradox in hemodialysis. *Am J Kidney Dis* 52: 727–736, 2008
 21. 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: 1573–1579, 2011
 22. den Elzen WP, Willems JM, Westendorp RG, de Craen AJ, Blauw GJ, Ferrucci L, Assendelft WJ, Gussekloo J: Effect of erythropoietin levels on mortality in old age: The Leiden 85-plus Study. *CMAJ* 182: 1953–1958, 2010
 23. Sinnamon KT, Courtney AE, Maxwell AP, McNamee PT, Savage G, Fogarty DG: Level of renal function and serum erythropoietin levels independently predict anaemia post-renal transplantation. *Nephrol Dial Transplant* 22: 1969–1973, 2007
 24. Avkarogullari M, Bozkurt A, Akpınar O, Donmez Y, Demirtas M: The relation between serum erythropoietin level and severity of disease and mortality in patients with chronic heart failure. *Acta Cardiol* 63: 297–302, 2008
 25. van der Meer P, Lok DJ, Januzzi JL, de la Porte PW, Lipsic E, van Wijngaarden J, Voors AA, van Gilst WH, van Veldhuisen DJ: Adequacy of endogenous erythropoietin levels and mortality in anaemic heart failure patients. *Eur Heart J* 29: 1510–1515, 2008
 26. Kovesdy CP, Molnar MZ, Czira ME, Rudas A, Ujaszasi A, Rosivall L, Szathmari M, Covic A, Keszei A, Beko G, Lakatos P, Kosa J, Mucsi I: Associations between serum leptin level and bone turnover in kidney transplant recipients. *Clin J Am Soc Nephrol* 5: 2297–2304, 2010
 27. Kovesdy CP, Czira ME, Rudas A, Ujaszasi A, Rosivall L, Novak M, Kalantar-Zadeh K, Molnar MZ, Mucsi I: Body mass index, waist circumference and mortality in kidney transplant recipients. *Am J Transplant* 10: 2644–2651, 2010
 28. Molnar MZ, Czira ME, Rudas A, Ujaszasi A, Haromszeki B, Kosa JP, Lakatos P, Beko G, Sarvary E, Varga M, Fornadi K, Novak M, Rosivall L, Kiss I, Rempert A, Goldsmith DJ, Kovesdy CP, Mucsi I: Association between the malnutrition-inflammation score and post-transplant anaemia. *Nephrol Dial Transplant* 26: 2000–2006, 2011
 29. Molnar MZ, Czira ME, Rudas A, Ujaszasi A, Lindner A, Fornadi K, Kiss I, Rempert A, Novak M, Kennedy SH, Rosivall L, Kovesdy CP, Mucsi I: Association of the malnutrition-inflammation score with clinical outcomes in kidney transplant recipients. *Am J Kidney Dis* 58: 101–108, 2011
 30. Molnar MZ, Keszei A, Czira ME, Rudas A, Ujaszasi A, Haromszeki B, Kosa JP, Lakatos P, Sarvary E, Beko G, Fornadi K, Kiss I, Rempert A, Novak M, Kalantar-Zadeh K, Kovesdy CP, Mucsi I: Evaluation of the malnutrition-inflammation score in kidney transplant recipients. *Am J Kidney Dis* 56: 102–111, 2010
 31. Jassal SV, Schaubel DE, Fenton SS: Baseline comorbidity in kidney transplant recipients: A comparison of comorbidity indices. *Am J Kidney Dis* 46: 136–142, 2005
 32. Levey A, Greene T, Kusek J, Beck G, Group MS: A simplified equation to predict glomerular filtration rate from serum creatinine [Abstract]. *J Am Soc Nephrol* 11: 155A, 2000
 33. Belonje AM, Voors AA, van der Meer P, van Gilst WH, Jaarsma T, van Veldhuisen DJ: Endogenous erythropoietin and outcome in heart failure. *Circulation* 121: 245–251, 2010
 34. Brookhart MA, Schneeweiss S, Avorn J, Bradbury BD, Liu J, Winkelmayer WC: Comparative mortality risk of anemia management practices in incident hemodialysis patients. *JAMA* 303: 857–864, 2010
 35. Sherwood JB, Carmichael LD, Goldwasser E: The heterogeneity of circulating human serum erythropoietin. *Endocrinology* 122: 1472–1477, 1988
 36. Conlon PJ, Albers F, Butterly D, Schwab SJ: ACE inhibitors do not affect erythropoietin efficacy in haemodialysis patients. *Nephrol Dial Transplant* 9: 1358, 1994
 37. Kalantar-Zadeh K, McAllister CJ, Lehn RS, Lee GH, Nissenson AR, Kopple JD: Effect of malnutrition-inflammation complex syndrome on EPO hyporesponsiveness in maintenance hemodialysis patients. *Am J Kidney Dis* 42: 761–773, 2003
 38. Brines M, Cerami A: Erythropoietin-mediated tissue protection: Reducing collateral damage from the primary injury response. *J Intern Med* 264: 405–432, 2008
 39. Smith KJ, Bleyer AJ, Little WC, Sane DC: The cardiovascular effects of erythropoietin. *Cardiovasc Res* 59: 538–548, 2003
 40. George J, Patal S, Wexler D, Abashidze A, Shmilovich H, Barak T, Sheps D, Keren G: Circulating erythropoietin levels and prognosis in patients with congestive heart failure: Comparison with neurohormonal and inflammatory markers. *Arch Intern Med* 165: 1304–1309, 2005
 41. Pasqualetti P, Casale R: Circadian rhythm of serum erythropoietin in healthy subjects. *Riv Eur Sci Med Farmacol* 18: 91–93, 1996
 42. Roberts D, Smith DJ: Erythropoietin does not demonstrate circadian rhythm in healthy men. *J Appl Physiol* 80: 847–851, 1996
 43. Khosroshahi HT, Asghari A, Estakhr R, Baiaz B, Ardalan MR, Shoja MM: Effects of azathioprine and mycophenolate mofetil-immunosuppressive regimens on the erythropoietic system of renal transplant recipients. *Transplant Proc* 38: 2077–2079, 2006
 44. Al-Uzri A, Yorgin PD, Kling PJ: Anemia in children after transplantation: Etiology and the effect of immunosuppressive therapy on erythropoiesis. *Pediatr Transplant* 7: 253–264, 2003
 45. Singh AK, Szczeczek L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D: Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med* 355: 2085–2098, 2006
 46. Drueke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, Burger HU, Scherhag A: Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *N Engl J Med* 355: 2071–2084, 2006
 47. Pfeffer MA, Burdman EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, Feyzi JM, Ivanovich P, Kewalramani R, Levey AS, Lewis EF, McGill JB, McMurray JJ, Parfrey P, Parving HH, Remuzzi G, Singh AK, Solomon SD, Toto R: A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med* 361: 2019–2032, 2009
 48. Brookhart MA, Schneeweiss S, Avorn J, Bradbury BD, Rothman KJ, Fischer M, Mehta J, Winkelmayer WC: The effect of altitude on dosing and response to erythropoietin in ESRD. *J Am Soc Nephrol* 19: 1389–1395, 2008

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