Serum Erythropoietin Level and Mortality in Kidney Transplant Recipients

Miklos Z. Molnar,§* Adam G. Tabak,§§ Ahsan Alam,⁎ Maria E. Czira,† Anna Rudas,† Akos Ujszaszi,⁎ Gabriella Beko,** Marta Novak,†† Kamyar Kalantar-Zadeh,‡‡ Csaba P. Kovesdy,¶¶ and Istvan Mucsi†¶

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: HR1SD 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: HR1SD 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.


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 deficiency) (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...
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, menopausal 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 reintentions 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 (±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-naive 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 m² (2), 60% were men, and 21% were diabetic subjects, and mean hemoglobin was 137 g/L.

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 (HR1 SD increase = 1.28; 95% CI: 1.02 to 1.62). Moreover, adjustment for blood hemoglobin did not attenuate this association (HR1 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 generally 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 (HR1 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 (HR1 SD increase = 1.22; 95% CI: 1.12 to 1.32). This association remained significant after multivariable adjustments (HR1 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>
<thead>
<tr>
<th>Table 1. Patients’ baseline characteristics</th>
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<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>serum EPO (U/L)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender, female (%)</td>
</tr>
<tr>
<td>Time since last transplant (month)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Previous time on dialysis (month)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total ESRD time (month)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>eGFR (MDRD) (ml/min per 1.73 m&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Charlson Comorbidity index&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Presence of hypertension (%)</td>
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<td>Presence of diabetes (%)</td>
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<td>Presence of coronary heart disease (%)</td>
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<td>Smoking (%)</td>
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<tr>
<td>Hb (g/L)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Serum albumin (g/L)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>CRP (mg/L)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ln-CRP (mg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Use of ACE inhibitor or ARB (%)</td>
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<tr>
<td>Primary cause of ESRD (%)</td>
</tr>
<tr>
<td>chronic GN</td>
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<tr>
<td>chronic TIN</td>
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<tr>
<td>PKD</td>
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<tr>
<td>diabetic nephropathy</td>
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<tr>
<td>hypertensive nephropathy</td>
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<tr>
<td>others or unknown</td>
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<tr>
<td>Panel reactive antibody titer (%)&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Cold ischemic time (minute)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>History of delayed graft function (%)</td>
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<tr>
<td>History of acute rejection (%)</td>
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<td>HLA mismatches (%)</td>
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EPO, serum erythropoietin; N/A, not available; eGFR, estimated GFR; MDRD, Modification of Diet in Renal Disease; Hb, hemoglobin; ln-CRP, log-transformed C-reactive protein; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; GN, glomerulonephritis; TIN, tubulo-interstitialis nephritis; PKD, polycystic kidney disease.

<sup>a</sup> Values are median with interquartile range in parentheses.
<sup>b</sup> Values are mean ± SD.
<sup>c</sup> Values are median with minimum to maximum in parentheses.
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 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

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

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 In-CRP, Charlson Comorbidity index, average systolic BP, total time in ESRD, history of delayed graft function, smoking status, and history of acute rejection.
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.

Acknowledgments

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Disclosures

None.

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